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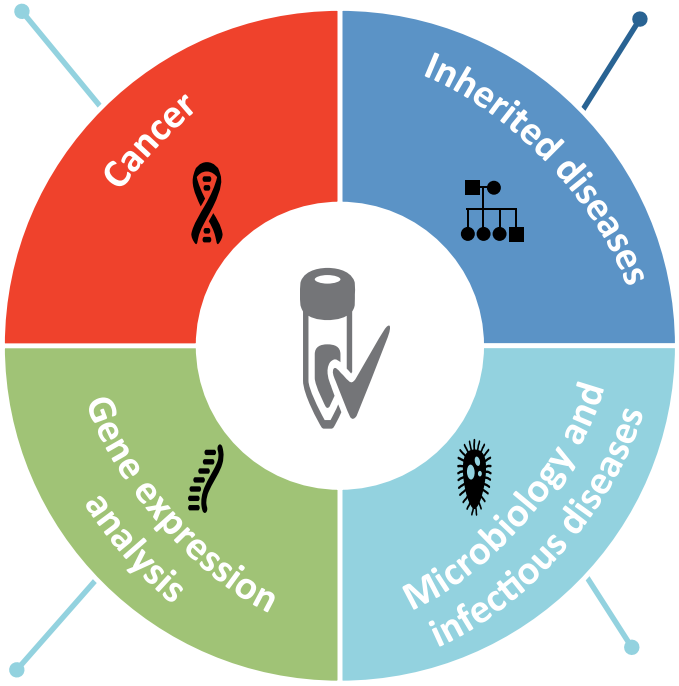

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His Highness Sheikh

Nawaf Al-Ahmad Al-Jaber Al-Sabah

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Sitting from L to R: *Dr Rajendra C.Mishra; Dr Surendra Nayak; Dr Abhay Patwari; (President)*
Dr (Mrs) Renu Gupta; Dr Syed M. Rahman Dr Radhakrishna Panicker;

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بسم الله الرحمن الرحيم

Minister of Al Diwan Al Amiri Affairs

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I am extremely happy to learn that the Indian Doctors Forum – Kuwait is celebrating their annual cultural extravaganza 'DOCFEST–2018' on Friday, 2nd February, 2018 at The Regency Hotel. On this occasion, they will be releasing the 14th volume of their popular annual health guide titled 'Laboratory Medicine–in Health and Disease'.

Laboratory Medicine forms an integral part of modern medicine. In fact, doctors depend upon results from the laboratory to make their diagnosis and monitor their treatment. However, there are many misgivings and myths among the general public regarding laboratory values, reports and their interpretation leading to unnecessary anxiety. IDF has already released a health guide on 'Imaging Sciences'. It is very apt that they are now completing the circle by bringing out this very informative and enlightening volume on laboratory medicine. I congratulate the editors and authors for their hard work.

Indian doctors in Kuwait have contributed immensely and positively towards the development of medical services in the State of Kuwait over the past 50 years. As they celebrate their annual 'DOCFEST–2018', I congratulate all members of the IDF and wish them success in all their endeavors.

I am very pleased to release this volume of the IDF Health Guide 2018 on 'Laboratory Science–in Health and Disease'.

Ali Jarrah Al-Sabah

Minister of Al-Diwan Al-Amiri Affairs

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AMBASSADOR OF INDIA
KUWAIT

29 January 2018

Message

I am glad to learn that Indian Doctors Forum, Kuwait, is holding its annual event "DOCFEST-2018" 'Bollywood - Down Memory Lane' and releasing its annual Health Guide.

The contribution of Indian Doctors in the field of healthcare in Kuwait is creditable and significant.

The Embassy of India extends its warm greetings to Dr. Abhay Patwari, President and all members of IDF and convey its best wishes for the success of the forthcoming "DOCFEST-2018".

(K. Jeeva Sagar)

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Kuwait Medical Association

Ref : KMA/22/2018

Date : 22/1/2018

Message

The Kuwait Medical Association has for the past 14 years provided an affiliation to the Indian Doctors Forum and we are proud of the work being done by them. They serve the underprivileged members of the expatriate community by conducting many health screening camps.

I am happy to learn that they will be celebrating their annual cultural extravaganza **"DOCFEST – 2018 – Bollywood - Down Memory Lane"** based on the changes in culture and style that Indian cinema has undergone over the decades, on 2nd February 2018 at the Regency Hotel. As is their tradition, they will be publishing their 14th volume of their popular health guide series. This year they have chosen to highlight the role of Laboratory Medicine in Health and Disease. Doctors depend so much on laboratory investigations and reports to make accurate diagnosis and monitor treatment. However, people have a lot of misunderstanding about laboratory procedures and interpretation of results causing unnecessary anxiety. This health guide will help them understand the intricacies of laboratory science. I congratulate the editors and contributors for their effort.

I am told that many other activities are planned this year to further benefit people in need of medical help. I wish the IDF all the best in its endeavors. Kuwait Medical Association will always be there to assist them whenever needed.

I wish all members of the IDF a very happy, healthy and prosperous New Year 2018.

Dr. Mohammed Al Mutairi

President, KMA

Chairman, Department of Cardiology

Chest Diseases Hospital, Kuwait

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MESSAGE

I am happy to know that the Indian Doctors Forum is celebrating its fourteenth anniversary at their annual event, the 'DOCFEST – 2018' on 2nd of February, 2018.

This association of Indian Doctors working in Kuwait has grown from strength to strength from a modest beginning in 2004 under the leadership of Late Dr. Narayanan Nampoory.

This year, the health guide focuses of an important issue for the society, namely Laboratory Medicine in Health and Disease'. People are always very anxious about laboratory investigations and interpretation of their results. It is important that people get authentic scientific information on the subject in simple language. I am sure this health guide will serve that purpose.

I would like to repeat my statement of last year. The Indian doctors have been a very vital part of medical services in Kuwait for the last 50 years. With Kuwait expanding its hospital bed strength, the need for manpower is increasing and we hope India can contribute in this regard.

It is my pleasure to be a part of the DOCFEST celebrations and I wish the IDF all the best in their endeavors.

Dr. Ibrahim Hadi
Secretary General
Kuwait Institute for Medical Specialization,
MOH, Kuwait

Dr. Ibrahim A. Hadi
Secretary General
Kuwait Institute for Medical Specializations

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Indian doctors in Kuwait have contributed immensely and positively towards the development of medical services in the State of Kuwait over the past 50 years. IDF has been doing great work towards community health and health education over the past 14 years and is one of the most important and premier Indian associations in Kuwait. As they celebrate their annual 'DOCFEST – 2018', I congratulate all members of the IDF and wish them success in all their endeavors.

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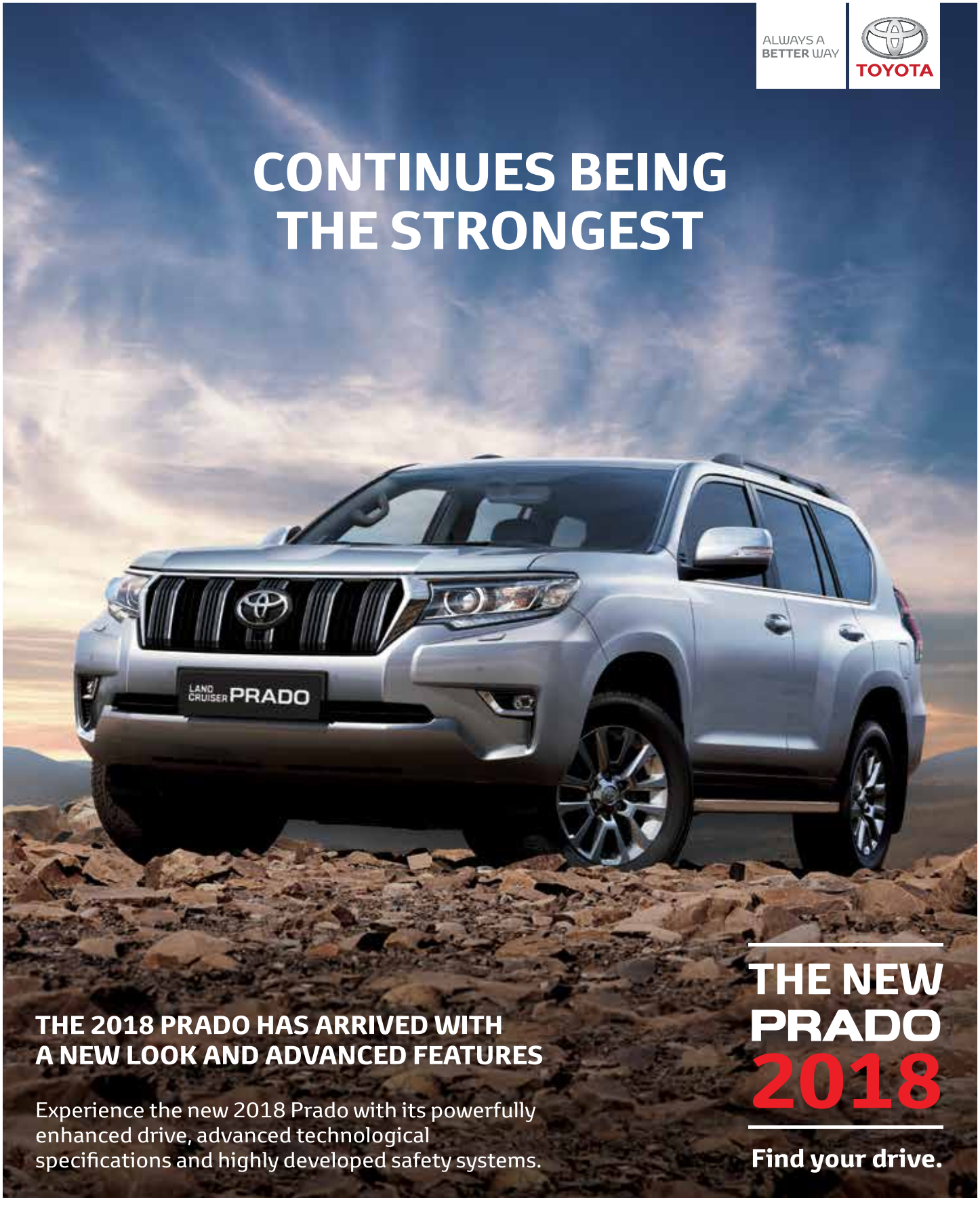

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24 January 2018

MESSAGE

IT GIVES ME IMMENSE PLEASURE TO KNOW THAT THE INDIAN DOCTORS FORUM (IDF), AFFILIATED TO THE KUWAIT MEDICAL ASSOCIATION, HAS SCHEDULED TO HOLD, LIKE EVERY YEAR, THE MEGA EVENT "IDF DOCFEST 2018" ON 02ND FEBRUARY 2018.

AS IS WELL-KNOWN NOW, IDF KUWAIT IS AN EMINENT ORGANISATION OF SELF-LESS, DEDICATED & COMMITTED HEALTHCARE PROFESSIONALS AND THEIR PRICELESS CONTRIBUTIONS TO KUWAIT'S MEDICAL SERVICES SYSTEM AND ALSO THEIR NOBLE COMMUNITY SERVICES, ARE PRAISEWORTHY AND LAUDABLE INDEED.

I WISH THIS FORUM OF EMINENT, SERVICE-ORIENTED PROFESSIONALS CONTINUED SUCCESS IN ALL THEIR EFFORTS TOWARDS SERVING THE COMMUNITY.

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President's Message

It gives me great pleasure to welcome you all to the 14th DOCFEST today. Indian Doctors Forum (IDF), a socio-cultural organization of Indian doctors working in Kuwait, both in the Government and private sector was formed in 2004 under the patronage of H. H. Sheikh Sabah Al Ahmad Al Jaber Al Sabah, the Amir of the State of Kuwait and the Kuwait Medical Association.

The main objectives of the IDF are to spread health awareness among the lay people by conducting regular health screening camps in collaboration with the other Indian associations in Kuwait and the publication of an annual Health Guide which provides authentic scientific information in simple English on a chosen theme. Keeping in mind the increasing anxiety of the society in Laboratory investigations and reports, this year we have chosen the subject 'Laboratory Medicine in Health and Disease' for our health guide. I would like to thank our chief editor Dr. Girish Yadav and his team for working so hard to bring out this 14th volume of the Health Guide on time. With this information we hope patients can make an informed decision regarding their specific health needs. We are very grateful to our chief patron H. E. Sheikh Ali Al-Jarrah Al-Sabah for gracing this occasion and consenting to release this 14th volume today.

IDF also provides a platform for all its members and their families to fulfill their social and cultural aspirations as you will witness during the cultural extravaganza. All participants are IDF members and they have toiled hard over the past 3 months under the guidance of professional choreographers to put on this dazzling show. My thanks are due to my cultural secretaries Dr. Prashant Purohit and Dr. Pooja Chodankar for conceiving and executing this project.

In addition, IDF tries to resolve the many difficulties that the Indian doctors encounter in Kuwait regarding their career and life. We, as a lobbying group, try to strengthen the long-standing friendly relationship between the peoples of Kuwait and India. IDF also organizes the biannual 'KMA-IDF oration to showcase Indian medical talent in order to encourage medical tourism to India and the possible training of Kuwaiti doctors in Indian medical centers of excellence. We warmly welcome our new ambassador H.E. Shri. K. Jeeva Sagar and hope the embassy will continue its support to IDF in the years to come.

This year, IDF plans to launch its IDF Patient Assistance Fund to further help underprivileged patients needing medical treatment, now that new charges are applicable in ministry hospital and clinics.

I would like to thank H.H. the Amir of the State of Kuwait, Al Diwan Al Amiri affairs and H.E. Sheikh Ali Al Jarrah Al Sabah for their kind patronage of all IDF activities. My thanks are also due to the Kuwait Medical Association and its President Dr. Mohamad Al-Mutairi for helping us to fulfill our social responsibility. I am grateful for the many Kuwaiti and Indian business houses that provide us with resources to do our work. I appreciate the hard work put in by IDF members and their families to present today's dazzling cultural extravaganza. The members are our true strength. I want to give a clarion call to all Indian doctors working in Kuwait to become members of the Forum. I thank the members of IDF for reposing their faith in me and for giving me the opportunity to serve as their President. I hope I can fulfill their aspirations. Last but not the least I thank my family for inspiring me to do my best and supporting me wholeheartedly.

I wish one and all a very happy, healthy and prosperous New Year 2018. I hope to see all of you and many more at the DOCFEST 2019.

Dr Abhay Patwari,
PRESIDENT- IDF

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Secretary's message

At the outset let me wish you all ahealthy and happy new year.

We,the Indian doctors working in Kuwait for theMinistry of Health and private sector ,are serving the citizens of Kuwait and the expatriate community. Our associationis one of the largest sociocultural associationsof professionals in Kuwait and has been rewarded with the Pravasi Bharath Samman award for ourservice to the community in the year 2013. IDF has been growing from strength to strength since its inception in the year 2004 with nonstop activities for its members and the community at large. We are working under the umbrella of theKuwait Medical A ssociation and with the patronage of his highness the Amir of Kuwait and the Indian embassy ofKuwait. We respect and abide by the laws of the land.

Over allthese years we have been serving the community through free health screening camps, health education, seminars in schools and other healthawareness and promotion activities.We also conduct an annual Inter school health quiz to encourage students from various Indian schools in Kuwaitwith an interest inthe medical sciences. We encourage our members to showcase their cultural, creative and athletic talents through various activities such as sports days, picnics, musical events, dramas, skits etc.

Our annual health guide will bereleased during DOCFEST 2018.The current issue (XIV'th),titled «Laboratory medicine«,is a very useful handbook to have in the home library. We are thankful to the chief editor Dr.Y Girish,the editorial board,andthe contributors for all their hard work. We are sure this health guide will be a helpfulmanual to understandlaboratory medicine and its importancein patient management. Modern advances in molecular biology, immuno-histochemistry, flow cytometry, automation in blood value estimations and tissues processing have made laboratory results more reliable and accurate .The application of quality control and accreditations of the laboratory services have strengthened the validity of laboratory results. As Louis Pastuer once said «Without Laboratories men of science are soldiers with out arms».

Our sponsors are the backbone of all our activities without whose whole hearted generous financial support we wouldn›t have been able to conductour activities. We are thankful to theAmiri Diwan and the Kuwaiti and Indian business community for their constant support over the years.

Our own members and their families will entertain you today through their dance skills by taking you through the world of Hindi cinema under the theme «Bollywood -Down memory lane» .We are sure you will enjoy their performances.

Long Live IDF.....
DrSurendra Nayak Kapadi.
General Secretary IDF

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From the Editors' Desk

Dear Readers,

It is a matter of great pleasure and pride for me to present the 14th volume of the health guide which is an annual publication of Indian Doctors Forum. The theme of this year's health guide is "Laboratory Medicine in Health and Disease".

Laboratory services are an integral part of modern medicine and make up one of the main pillars of diagnostic medicine. This health guide is dedicated to brief the readers about various aspects of lab specialty –its subdivisions, details of investigations, their interpretation and usefulness for monitoring health status as well as for diagnosis and monitoring of diseases. In this health guide, the details are presented in 40 articles from different specialties of laboratory medicine including Biochemistry, Hematology, Microbiology, Histopathology and Cytology. We have tried our best to cover the entire spectrum of common clinical laboratory investigations ranging from newborn screening to the current developments in genome sequencing. The articles have been written by the experts in the respective fields andare aimed at answering the common man's FAQ's. I am sure that the readers will find it very informative, useful and interesting.

The advancements in the field of medicine, like any other field of science, have resulted in super specialization so that a specialist of one discipline of medicine cannot remain fully aware about the happenings in other disciplines. During my career of more than 40 years in this profession in Kuwait, I have witnessed a phenomenal advancement from handheld test tube and a few chemicals to fully automated systems and most sophisticatedanalysis such as mass spectrometry for identifying metabolites and microorganisms, immunohistochemistry, cell markers by flow cytometry, cytogenetics, PCR and other molecular techniques. For highly specialized busy doctors it is not possible to keep abreast with advancements in all the medical fields. So, this health guide will also be useful to our fellow physicians.

I am thankful to all the authors for taking interest and writing the articles. I am glad that some of the articles are written by our Kuwaiti colleagues.

I was fortunate to have an excellent editorial committee which comprised Dr Aravinda Rao, Dr Arvind Raina, Dr Ramesh Pandita, Prof Dilip Das and Dr Prashant Purohit. I would like to express my thankfulness to them. Thanks also to Dr Vinod Grover, Dr Arun Joshi and Dr Arijit Chattopadhyay for their useful suggestions.

Finally, thanks to the President Dr Abhay Patwari, the office bearers and the Executive Committee of Indian Doctors Forum for giving me opportunity to serve the community by preparing this health guide.

Dr Y. Girish
Chief Editor
IDF – Health Guide 2018

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INTRODUCTION TO LABORATORY MEDICINE
ROLE OF MEDICAL LABORATORY IN HEALTH & DISEASE

Dr Y. Girish
Biochemistry Unit, Department of Laboratory
Ibn Sina Hospital

Dr Aravinda Rao
Biochemistry Unit, Department of Laboratory
Al Sabah Hospital

Laboratories serving us for our health management can be in a hospital or polyclinic or be can be an independent establishment. The size of a medical laboratory (the lab) can range from a small lab just doing basic tests to a multispecialty hospital lab or to an industry like establishment called "reference laboratory" capable of performing all possible sophisticated tests.

In general, a medical laboratory consists of divisions such as Biochemistry, Hematology, Histopathology-Cy topathology, Microbiology and more. Each specialty is manned by specialized staff including te chnologists and doctors. In a hospital setup the laboratory is connected within by Laboratory Information System (LIS) and to outpatient clinics, wards, intensive care units, operation theaters by Hospital Information system (HIS). Laboratory testing service can be divided into a pre-analytical phase, an analytical phase and a post-analytical phase. For any special test, patients are given instructions for preparation and specimen collection. When a specimen from a patient arrives at the laboratory through a messenger or through a pneumatic tube system from any of the hospital departments, the specimen is registered and then transferred to particular lab unit (section) where it is processed as required.

The analysis is performed by automated analyzers or manually or by a mix of both. All steps of analysis ar e monitored by quality control system and supervised and checked by well trained specialized staff. Reports are verified by supervisory staff and released to the clinician, either in physical form or electronically.

The final phase of the process is incorporation of the information carried in the lab report into patient management and this is the responsibility of the lab expert who gives his interpretive comment and advice on further testing and the treating physician who will come to an impression putting together clinical details and results of lab and other investigations.

In reality, Laboratory services make up one of the three main pi llars of diagnostic medicine, the others being imaging studies and clinical examination.

The lab tests carried out on body fluids like blood, urine etc reflect the state of functioning of organs or metabolic systems in the body. These tests detect increased or decreased concentrations of various ch emical substances, metabolites, enzymes and hormones which are abnormal during diseases. Many times it happens that one or more of these substances become abnormal before the underlying disease become apparent. When a doctor requests 'check-up' tests on an apparently healthy person,

the following tests are particularly of importance. Many of these are discussed in detail in the other sections of this health guide.

Following is a brief overview :

1. Blood Glucose

Blood glucose ("sugar" of blood) levels change throughout the day. After eating, levels rise and then settle down after about two hours. They are at their lowest point before the first meal of the day, which is normally breakfast. For blood glucose test, a fasting or 2 hours after meal blood specimen is collected. Elevated blood glucose is a sign that your body either isn't making enough insulin—the hormone that moves glucose into the cells to be used for energy—or isn't using insulin efficiently. High blood glucose levels can indicate that you have diabetes or prediabetes. Blood glucose can be moderately high without any specific symptoms. If the level is high, the physician requests various other tests such as HbA1c and oral glucose tolerance test to confirm or rule out diabetes. Early detection definitely helps the patient to change life style and prevent diabetes.

Blood glucose check up should be done after the age of 35 to 40 years once a year.

2. Cholesterol and other Lipids

Cholesterol and its subdivisions LDL (bad) and HDL (good) cholesterol and triglycerides (fat) are the lipids of which we have learnt to be worried or even scared, as they can raise person's risk of heart attack and stroke. They must be measured once around the age of twenty to ensure that there is no risk of a familial cholesterol problem. If no such risk exists, the lipids may be measured around 35 to 40 years and if there is no pointer to increased risk, every 5 years thereafter. You should get the blood drawn after 12-hour fast in the morning.

3. Creatinine

Creatinine is a waste product of muscle metabolism, is excreted by the kidneys and it accumulates in blood when kidneys begin to fail. It is very useful blood test for kidney function and should be done along with other checkup tests. A high level of creatinine is not a direct cause of symptoms, and someone with above-normal levels may not notice any change. Some people may have an incidental finding of severe kidney disease and elevated creatinine on routine blood work without having any symptoms. So, checkup for blood levels of creatinine is important in apparently healthy person or person with high risk of kidney failure such as high blood pressure, diabetes, patient taking drugs which are damaging to kidneys. Test for creatinine can be done on a blood specimen together with other tests in a biochemistry laboratory.

4. Calcium

Calcium changes are rare, but high calcium due to parathyroid gland over-function can silently cause kidney stones and bone damage. Testing Calcium once in a few years may help in early detection of hypercalcemia and treatment could be started in time. Test for calcium can be done on a blood specimen together with other tests in a biochemistry laboratory.

5. Liver Tests

Liver profile or Liver function tests, especially ALT (Alanine Transaminase), can be high without symptoms due to minor effect of some drugs or mild infection. GGT (Gamma-glutamyl transpeptidase) can be high in alcoholics or because of certain medications. These tests can be done on a blood specimen along with other check-up tests such as for glucose and lipids.

6. Vit D

Testing for vitamin D is not recommended as routine check-up, but may be advised for people who are home-bound or who are not exposed to sunlight or people at high risk such as low blood calcium-phosphate, a past history of low trauma fracture or bone pain.

7. Urine routine

Urine (usually first morning or spot urine specimen) is checked qualitatively for glucose, albumin, cells, RBCs and microscopy for casts. Generally, this does not contribute much over blood tests done as 'check-up'.

8. Prostate Specific Antigen (PSA)

Prostate cancer is the leading cause of cancer death in man. PSA may be done as a screening test for men in the 40-70 years age group years particularly for high risk men with a family history and belonging to black race. Prostate cancer is a slow growing disease and repeat testing is to be done in consultation with the urologist.

9. Complete Blood Count (CBC)

This test requires whole blood specimen. CBC includes Hemoglobin (Hb), white (WBC), red (RBC) and platelet cell counts and different type of white blood cells. Many times, slight low or high Hb does not give any symptoms. Early detection helps to treat before specific symptoms and signs appear. In an apparently healthy person, possibility of certain type of abnormal hemoglobin can be suspected by this test which require further tests to confirm.

10. PAP smear test

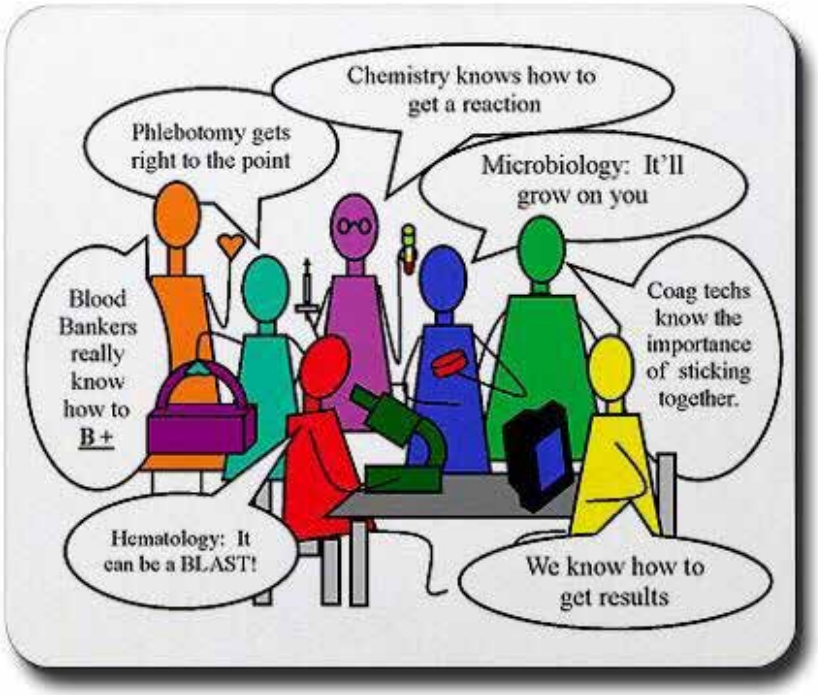
This procedure, which detects the presence of precancerous or cancerous cells in the cervix, is recommended for women for early detection of cervical cancer. Women of age 21-29 years should have PAP smear every 3 years. Between 30 to 65 years, they may continue with PAP test 3 yearly or PAP and human papilloma virus testing every 5 years. After 3 negative tests in a row, the screening may be discontinued.

11. Newborn screening

There are many inherited metabolic and other diseases which, if detected early after birth, can be treated resulting in near normal life for the baby. Without treatment such children would have debilitating permanent damage. Many countries, including Kuwait, have newborn screening programs of varying range and all parents should make best use of such service.

Role of Laboratory in Disease

When health deteriorates the patient approaches a doctor. Doctor examines the patient and tries to reach a correct diagnosis, so that proper treatment can be given. Laboratory investigations become the first line of this effort. Various imaging studies are used in tandem. These two investigative modalities make up the sheet anchor of diagnostic pursuit. Many times it happens that a final diagnosis can only be achieved by laboratory services. Moreover, during the course of treatment, laboratory plays an important role in follow up of the effectiveness of treatment. In the following pages, you will see how common laboratory investigations are used in health service and how you could do well knowing about them.



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SCREENING PROGRAM FOR THE NEWBORN

Dr Aravinda Rao
Biochemistry Unit, Department of Laboratory
Al Sabah Hospital

There are a number of diseases that are passed on to the children from the parents through their genes. Genes are carried in 46 chromosomes which are in the nucleus of our cells and are responsible for synthesizing all proteins in the body, on which the structure and the function of the body depend. The genes are made up of a long rope of DNA kept tightly coiled in the chromosomes. The DNA rope itself is made by joining together only 4 types of units called nucleotides. The DNA serially carries 3-nucleotide codes, each code for one amino acid of a protein, which is the final product of the gene. This may be called 'quaternary coding' much like the 'binary coding' in computer languages. Even if one of these units in the chain is missing or changed from one to other (a mutation), the quality of the gene, and thus of the protein, may be affected, many times deleteriously, causing a disease.

We inherit one copy of a gene from each parent. One of these may be affected by a mutation. If the child inherits disease causing mutation from each of the parents, he will suffer from the disease due to the defective gene-product. Some diseases are caused by only one copy of the mutated gene and then we call the inheritance as 'dominant', in contrast to a disease requiring both copies of the abnormal gene ('recessive inheritance'). Most of the genetic diseases follow recessive inheritance. Some may be carried on the 'sex chromosome' and cause sex-linked inheritance, which causes sexual bias in inheritance.

Inherited or genetic diseases can be diagnosed by (a) the abnormalities observed in body structure or function, (b) analyzing tissues or body fluids for abnormal substances which accumulate, (c) analyzing the abnormal protein produced by the defective gene and finally (d) by analyzing the defective gene itself.

Genetic diseases may become obvious at any time after birth: in the newborn period, in infancy, in childhood or later in adult life. They may affect normal life to varying degrees- minimally to severely. Untreated severe diseases may cause early death. Many of these diseases have no treatment that can decrease the suffering. Many diseases can be managed such that the quality of life can be improved substantially or even cured. Because of this, most developed countries have adopted a newborn screening program in which every baby born is investigated within the first two days for a pre-determined set of diseases. The type and number of diseases may vary from place to place

depending upon the prevalence of the disease, facilities available and the budget allocation. More and more countries have started to establish such programs and in many countries private laboratories have made the service available on payment.

A genetic disease is chosen to be screened on the basis of these criteria:

- Normal appearance in the newborn period.
- Progression of the disease well known to be serious and can be halted if treated early in the newborn period.
- A simple, specific and sensitive test is available and is fast and suitable for large numbers.
- If identified, can help the family plan future children.
- Cost of screening is not high when compared to the cost of taking care of late diagnosed child for a life time.

Untreated, the infants grow to have irreversible damage, resulting in mental and physical retardation and disabilities, hormonal abnormalities or blood diseases having extensive ill-effects. Early detection and treatment prevents the evolution of disease to a large extent.

Phenylketonuria (PKU) was the first disease for which newborn screening was introduced by an American bacteriologist Robert Guthrie in 1962. PKU is caused by deficiency of an enzyme Phenylalanine hydroxylase which breaks down phenylalanine (an important amino acid – building block of proteins in the body). Drops of blood are obtained by pricking the skin of the heel with a needle and placed on a filter paper and then allowed to dry. Such sample of blood is called dried blood spot (DBS). This sample is stable over weeks. Guthrie used the ability of phenylalanine in the blood spot to stimulate growth of certain bacteria, which have an obligatory requirement for phenylalanine for survival. In PKU, phenylalanine accumulates in the blood and tissues because of the block in its breakdown pathway and when the blood is incubated with the Guthrie test system, causes florid growth of the bacteria, which suggests PKU.

Since the Guthrie test started being used widely, there have been many developments in the screening service. Many more diseases were included and more sophisticated tests were introduced. Now many countries have mandatory newborn screening service, mandatory because of the heavy burden placed by requirement of medical care and social needs of the undiagnosed and untreated patients on the government exchequer.

In the US, most of the about 4,000,000 newborn are screened in a year and 1/30 turn out to be positive for one of the screened diseases. In the order of importance, the diseases screened for are: hearing loss, congenital hypothyroidism, cystic fibrosis, sickle cell disease, medium chain acyl-CoA dehydrogenase deficiency (MCAD), galactosemia, PKU, congenital adrenal hyperplasia (CAH) etc. The pattern is not the same in all the countries. Cystic fibrosis and MCAD are rare in Kuwait but common in the western countries. Cystic fibrosis is not uncommon in India but MCAD may be rare.

In India, no state has organized systematic neonatal screening as yet. There have been pilot studies. Amino acidurias, organic acidurias, fatty acid oxidation defects (FAOD's), congenital hypothyroidism have all been reported. In a study of 98,256 infants in Karnataka State, a higher prevalence of homocystinura and hyperglycinemia were observed rather unexpectedly.

Introduction of the screening program is largely based on the methods available. Guthrie tests are not used any more. Automatic analyzers are employed. Analyzers meant for the routine laboratory can be adapted for many tests. Most important development is the advent of Liquid chromatography-tandem mass spectrometry ('Tandem mass' or LC-MS/MS). One 3 mm circle of blood spot can be analyzed for

screening a number of amino acid defects, organic acidurias and FAOD's : in all over 30 diseases. It is an expensive equipment, but cheap to run.

Kuwait introduced the service a few years ago and it is free of cost for all nationalities up to the stage of confirmation and covers deliveries in private hospitals too. Blood is collected by heel prick on filter papers (in 4 x 1-cm circles) 24-48 hours after the child is born in the Newborn Screening Offices located in the district hospitals. Collection before 24 hours or from a pre-term infant may cause false positive results and in such cases a repeat testing is desirable after a week to two weeks. The samples are analyzed in the Kuwait Medical Genetic Centre. The analysis is done on automatic analyzers and tandem-mass analyzer and the results are transferred to the Screening Offices. If a result is positive, the parents are contacted immediately and a set of confirmatory tests is carried out. If confirmed, the infant is referred to a metabolic specialist and is put on the treatment regimen without delay.

Blood spot specimen collection

Filter paper for blood spot
specimen collection used in Kuwait

Some diseases become evident in the first few days of birth, showing lethargy, feeding difficulty, vomiting, flabby muscles or even seizures and loss of consciousness. They need to be treated intensively. Treatment involves modalities to reduce the offending accumulating substance, including special diets and supplements and replacing the missing factor like thyroid hormone or steroid hormone.

It is important for parents to participate actively in the Newborn Screening Program and benefit from it. Hypothyroidism, adrenal hyperplasia (defective synthesis of steroid hormone), biotinidase deficiency, galactosemia, amino acidurias, organic acidurias and fatty acid oxidation defects are screened for in the state of the art Kuwait program. It is the duty of every resident to participate in the program not just to prevent possible disease in her child, but also to reduce the burden on government healthcare service.

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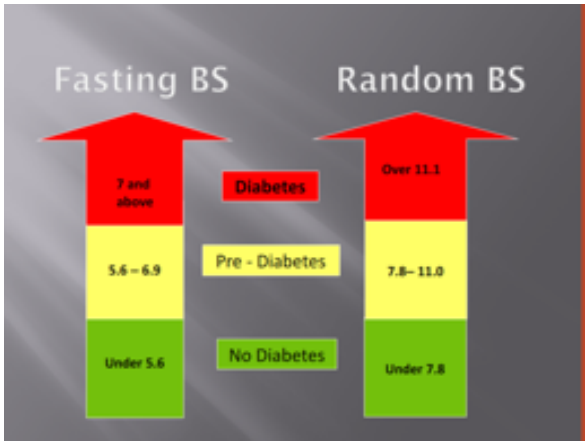
ROLE OF LAB TESTS IN THE DETECTION AND MANAGEMENT OF DIABETES

Dr Noble Zachariah
Dar Al Shifa Hospital & Clinic

Diabetes is a very common disease which can damage almost all organs in the body and it is necessary that it is detected early and managed properly. Though there are other abnormalities associated with it, high blood glucose (sugar) is the easiest to be detected. In majority of the common type (Type 2) diabetes, there is hardly any symptom and unless one is screened for diabetes, it may go undetected for many years. As per WHO, 50% of the people with diabetes do not know that they have diabetes and therefore screening of those at risk is the need of the hour.

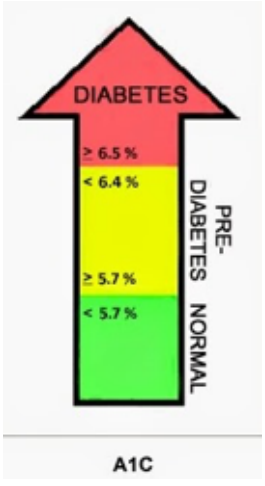
Who should be screened and what are the screening tests ?

Anyone who is overweight (BMI more than 25), with family history of diabetes, sedentary life, risk factors like high blood pressure, cholesterol abnormalities, heart disease, stressful life, fatty liver, polycystic ovary syndrome, history of delivering large babies or diabetes during pregnancy should be screened irrespective of age.



All individuals who are above 45 years of age are to be screened even when there are no risk factors.

Fasting blood sugar less than 5.6 mmol/l and post prandial(2 hours after a carbohydrate rich meal)less than 7.8 mmol/l are normal. Fasting blood sugar above 7 and any time blood sugar above 11.1 is diabetes. In between is the condition of pre-diabetes. The Glucose Tolerance Test (GTT) which was often used in the past is done only when the above blood sugars don't give a clear picture. When possible the blood test HbA1c is to be done. This test measures the change in blood hemoglobin depending on the average blood sugar over the past 3 months and the test does not require fasting. HbA1c of less than 5.6% is normal, 5.6% to 6.4% is pre-diabetes and 6.5% and above is diabetic.



In the natural history of diabetes, pre-diabetes becomes diabetes unless corrective action through proper diet, lifestyle and exercise are undertaken. Diabetes is preventable in the majority with corrective actions in the pre-diabetes stage.

Gestational Diabetes: Some pregnant women develop diabetes due to the hormonal changes that occur in pregnancy and the blood sugars usually revert to normal after delivery. It is important to detect and manage this well for the sake of the health of the mother and baby. In a person with no known risk

factor, the screening is done in the 24th to 28th week of pregnancy. Those with high risk factors may be screened at the first ante natal visit itself. If the 1 hour (after drinking 50 g glucose drink) blood sugar is above 7.8mmol/l, the pregnant woman should undergo a 3 hour glucose tolerance test after overnight fasting of 8 hours with 100 grams of glucose. Diabetes is diagnosed if two of the results are higher than:

Fasting: 5.3 mmol/L

1 hour: 10 mmol/L

2 hour: 8.6 mmol/L

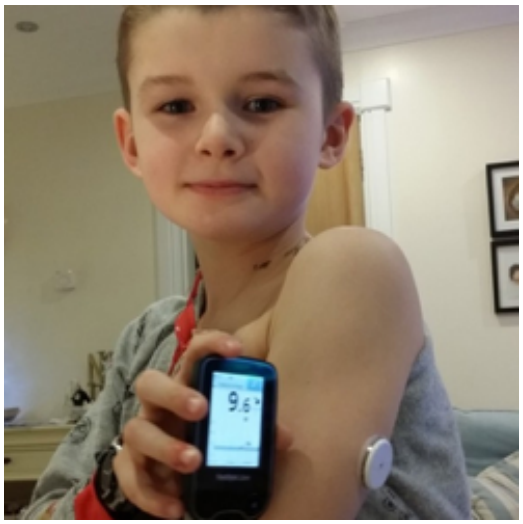
However, the patient is advised to maintain her sugar levels below targets given in the accompanying table.

Blood tests for people with diabetes

People with diabetes should empower themselves to maintain their blood sugar as normal as possible. This way they can reduce the risk of complications. The best way would be to Self-Monitoring of Blood Glucose (SMBG) at home.

They should learn the way food, exercise, emotions and infections affect the blood sugar control. The best times to do the tests would be fasting and 2 hours after lunch as these are shown to correlate best with HbA1c in Type 2 diabetes. In Type 1 diabetes (where there is no insulin production in the pancreas) more frequent tests will have to be done to adjust the dose of insulin. The same goes for people with Type 2 diabetes requiring insulin Continuous Glucose monitoring without the need for finger prick is very helpful in such people with Type 1 diabetes need to test their urine for ketones when the blood sugar is high.

Periodic lab tests by the physician should include HbA1c, urine microalbumin and Lipid profile. Kidney functions and liver functions may need to be monitored if abnormal.



Special tests for diabetes

These tests need to be done in special situations and not routinely.

When Type1 diabetes starts in childhood, it can be diagnosed with the typical history, symptoms, high blood sugar, ketones in the urine and blood. But it may be difficult in Latent Autoimmune Diabetes of Adult (LADA) which is Type 1 diabetes often masquerading as Type 2. For this one need to test antibodies like:

- Glutamic Acid Decarboxylase Autoantibodies (GADA or Anti-GAD)—This test looks for antibodies built against a specific enzyme in the pancreatic beta cells that produce insulin.
- Insulin Autoantibodies (IAA)
- Insulinoma-Associated-2 Autoantibodies (IA-2A)
- Islet Cell Cytoplasmic Autoantibodies (ICA
- Zinc Transporter 8 (ZnT8Ab) antibodies

C Peptide is related to the insulin production in the pancreas. If the C Peptide level is low, the person requires insulin injection as treatment.

Many Type 2 patients are prematurely put on insulin injection without correcting the correctable factors. These people may be managed without insulin injections if their C Peptide level is normal. This however has to be attempted under the supervision of an experienced physician with frequent monitoring of blood sugars.

Maturity Onset Diabetes of the Young (MODY) is genetically determined diabetes in the young people below the age of 25 years and is often confused with Type 1 diabetes. There is pancreatic beta cell dysfunction but no auto antibodies as in Type 1. Ketoacidosis does not occur. Genetic testing is required to define the sub types as the management and prognosis vary with the sub type.

So lab tests play an important role in detecting pre-diabetes and diabetes; type of diabetes, management of diabetes and its associated conditions properly, thereby avoiding complications and ensuring longevity with good quality of life.

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CHOLESTEROL AND OTHER LIPIDS

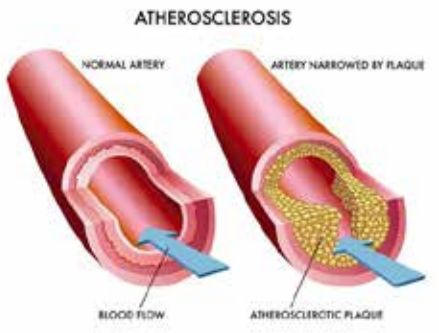
Dr Ahmad Al Sarraf
Biochemistry Unit, Department of Laboratory
Kuwait Cancer Control Center

Introduction

Cholesterol is a soft, waxy fat-like substance that is a natural component of all the cells in the body. We need it to build cells and synthesis of hormones. But too much can cause a problem.

Cholesterol comes from two sources. Your body (specifically your liver) makes 70 - 80% of all the cholesterol you need and the rest you get from foods of animal source.

There are actually two types of cholesterol: "bad" and "good." LDL cholesterol is the bad kind. HDL is the good kind. Too much of the bad kind increases the chances that cholesterol will start to slowly build up in the inner walls of arteries that feed the heart and brain. To make it simple, LDL cholesterol is like a delivery person who carries stuff all through the house and drops it along the way. HDL cholesterol is like someone who picks up the dropped stuff and puts it away. Together with other substances, cholesterol can form a thick, hard deposit that can narrow the arteries and make them less flexible. This condition is known as atherosclerosis. If a clot forms and blocks a narrowed artery, a heart attack or stroke can result. High cholesterol is one of the major controllable risk factors for coronary heart disease, heart disease, heart attack and stroke. Other risk factors such as smoking, high blood pressure or diabetes increase the risk even more.



Causes

In some cases, high cholesterol levels may be inherited, your liver may make too much cholesterol, or your body may not remove LDL from your blood efficiently. High cholesterol and elevated triglycerides (fat /oil) can also be associated with other diseases, such as diabetes, liver disease and hypothyroidism. But eating foods high in saturated fat and not getting enough exercise cause most often high cholesterol. High cholesterol is more common in people who are overweight or obese.

The most important risk factors for high cholesterol are:

- Being overweight or obese
- Eating a diet high in saturated fat and trans fat (rich in artificial butter/ margarine)
- Not getting enough exercise
- Family history of heart disease
- High blood pressure
- Smoking
- Diabetes

Laboratory testing - "Lipid Profile"

When testing is required, the doctor generally asks for 'Lipid Profile', which includes all the lipid (fat) components in the blood. Blood is drawn after an overnight (12-14 hours) fast. Serum (the cell free part) is separated and Cholesterol (Total), HDL cholesterol and triglycerides are analyzed. Total cholesterol is carried in blood mainly in particles VLDL, LDL and HDL. Triglycerides represent the oil in the blood and most of it is in VLDL, which also contains some cholesterol. From the triglycerides result VLDL cholesterol is calculated. LDL cholesterol is calculated from other cholesterol results.

Testing for lipids is only a part of the assessment of the risk of heart disease or stroke which may result from hardening of arteries from cholesterol deposition. It is important to consider other contributors including: family history, high blood pressure, diabetes, smoking history and age. The doctors compute the risk of developing heart disease in 10 years' time and we can easily do it on-line by entering the parameters in the risk calculator (ATP III protocol). The calculator gives the level of target LDL cholesterol for reduction. Doctors also look for presence of 'Metabolic Syndrome' which is a related condition which is diagnosed with additional inclusion of obesity and blood triglycerides in profiling. This only highlights, that though individual parameters may not look very abnormal, they can combine to put the patient in high risk.

Desirable Blood Levels of Lipds in mmol/L (mg/dL)

	Cholesterol	LDL-chol, low risk	LDL-chol, medium risk	LDL-chol, high risk	HDL-chol	Triglycerides
Male	<5.2 (200)	<4.1 (160)	<3.3 (130)	<2.6 (100)	> 1.05 (40)	<1.7 (150)
Female	<5.2 (200)	<4.1 (160)	<3.3 (130)	<2.6 (100)	>1.5 (60)	<1.7 (150)

Management

The TLC (therapeutic lifestyle changes) diet is recommended for people who have high cholesterol. With the TLC diet, less than 7% of your daily total calories should come from saturated fat, and only 25% to 35% of your daily calories should come from fat, overall. Sodium should be limited to 2,400 mg per day.

- A healthy diet can help you lose weight. Losing just 5 kilograms may help lower your cholesterol
- Cut down on saturated fats and trans fats. No more than 10% of your daily calories should come from saturated fat
- Eat whole grains, whole wheat bread, oatmeal, oat bran, and brown rice.
- Eat more fruits and vegetables, which are high in fiber and can help lower cholesterol levels.
- Eat fish at least 2 servings of fish each week.
- Eat phytosterols and stanols found in nuts, seeds, and vegetable oils

The American Heart Association (AHA) has developed dietary guidelines that help lower fat and cholesterol intake and reduce the risk of heart disease. The AHA does not recommend very low-fat diets, because new research shows that people benefit from unsaturated ("good") fats, such as those found in olive oil, avocados, and nuts.

Losing Weight

Being overweight increases the risk of high cholesterol and heart disease. Even 5 kilograms weight loss can lower LDL twice as much as diet alone. Weight loss often results in lower triglyceride levels and increased HDL, too. To maintain a healthy diet, you should aim for a gradual, weekly weight loss of 1/2 to 1 Kilogram.

Getting Exercise

Regular exercise reduces the risk of death from heart disease and helps lower LDL cholesterol levels, especially when combined with a healthy diet. Just 30 minutes of moderate exercise 5 times per week can help you lose weight or maintain a proper weight, reduce LDL and triglyceride levels, and increase levels of HDL. Studies show that every 10 minutes of added exercise per session is associated with increase in HDL cholesterol. Exercise may also lower blood pressure.

Medications

Lowering your cholesterol level reduces your risk of heart disease and stroke. Studies show that for every 1% reduction in cholesterol levels there is a 2% reduction in the rate of heart disease. People who already have heart disease or are at higher risk benefit most from lowering their cholesterol. Changes in lifestyle, improved diet and more exercise, are the most effective means of both preventing and, in less severe cases, treating high LDL cholesterol levels. In addition to recommending lifestyle changes, doctors often prescribe specific cholesterol-lowering medications.

If your LDL cholesterol remains high, after changing your diet and exercise habits, your doctor may prescribe medications to lower it. If your cholesterol is very high, you may start drug therapy at the same time you improve your diet and exercise habits. Drugs commonly used to treat high cholesterol include:

- Statins: These are usually the drugs of choice as they are easy to take and have few interactions with other drugs. Side effects may include muscle pain and stomach upset.
- Bile acid sequestrants: These are used to treat high levels of LDL. Common side effects include bloating, constipation, heartburn, and elevated triglycerides. People who have high levels of triglycerides (fats in the blood) should not take bile acid sequestrants.
- Ezetimibe (cholesterol absorption inhibitors): This medication limits how much LDL cholesterol can be absorbed in the small intestine. Side effects include headaches, nausea and muscle weakness.
- Fibrates: These medicines are effective at lowering triglyceride levels, and moderately effective at lowering LDL. They are used to treat high triglycerides and low HDL. Side effects include muscle pain, stomach upset, sun sensitivity, gallstones and irregular heartbeat.

Prognosis and Complications

Several complications may occur if high cholesterol is left untreated. These include:

- Heart disease: High cholesterol levels more than double the risk of heart attack. Lowering cholesterol by 1% reduces the risk of coronary artery disease by 2%.

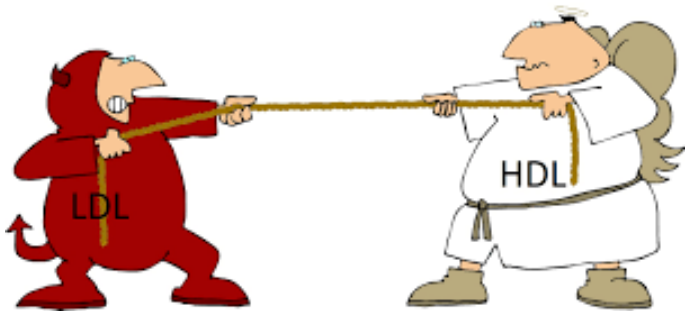
- Stroke: Low levels of HDL ("good") cholesterol have been associated with an increased risk of stroke.
- Insulin resistance: 80% of people with low HDL and high triglycerides may develop insulin resistance which leads to high blood sugar levels and develop diabetes.

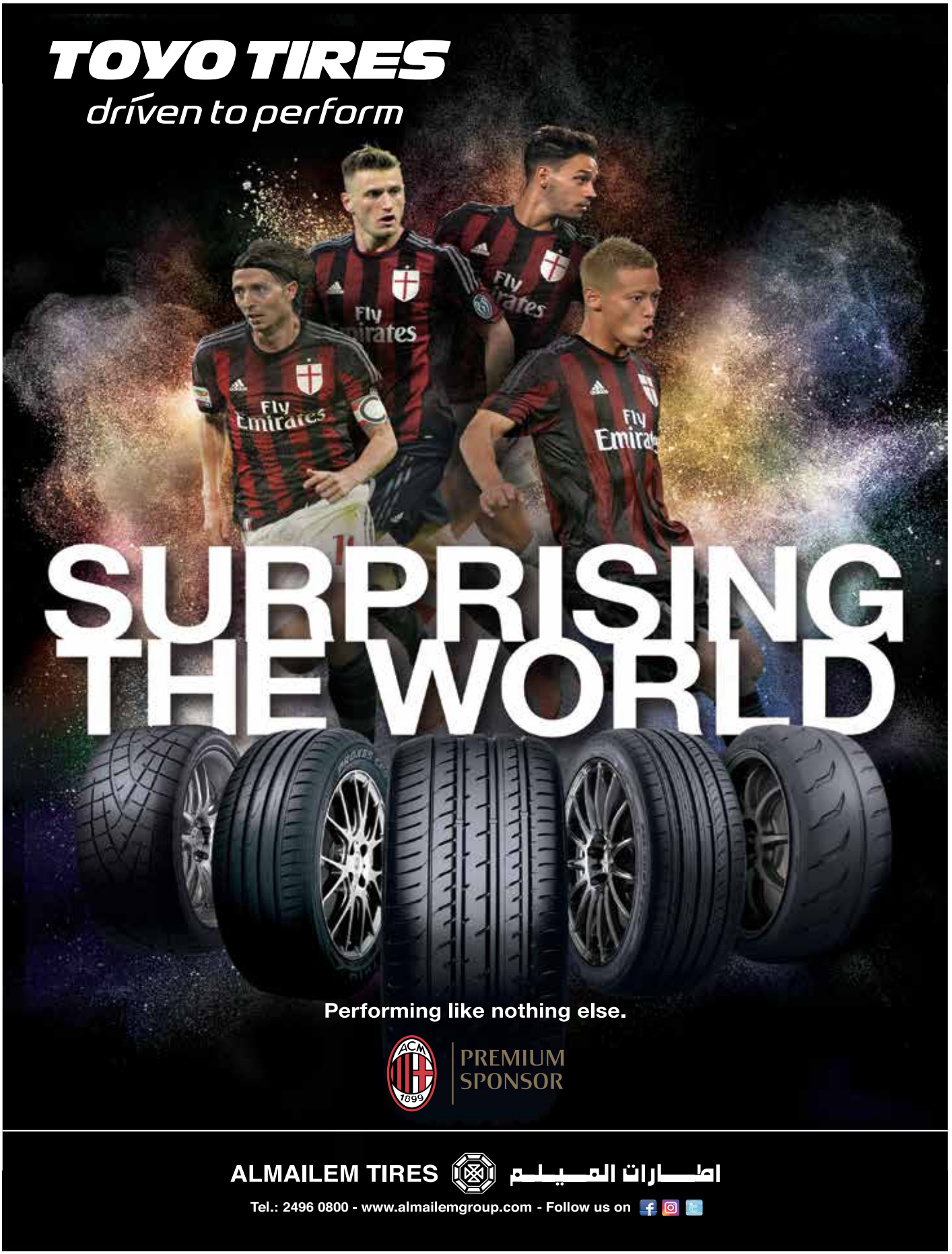
Conclusion

Maintaining a proper weight, eating a diet low in saturated fat, and exercising can lower cholesterol levels and improve long-term prognosis.

Further Reading

National Cholesterol Education Program. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA. 2001;285(19):2486-2497.

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Road way to success...

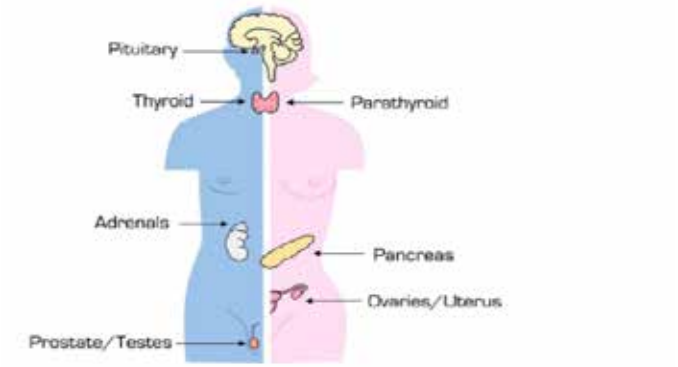
Laboratory evaluations of Hormonal (Endocrine) Diseases

Dr Arijit Chattopadhyay
Endocrinology Unit, Department of Medicine
Al Sabah Hospital

Hormonal tests are integral part of laboratory evaluations of many endocrine diseases like underactive thyroid (hypothyroid), overactive thyroid (hyperthyroid), growth and puberty related issues of children, infertility, polycystic ovary disease, obesity, diabetes etc. Doctors rely heavily on laboratory testing in the diagnosis of hormonal (endocrine) diseases and its response to treatment. In order to avoid misdiagnosis and over-treatment, laboratory testing should be regarded as adjunct to good clinical diagnosis and management.

What are the important laboratory tests for thyroid diseases?

Thyroid function test (TFT) measuring total T4/ FT4 and TSH is the basis to identify whether the gland is underactive (high TSH, low T4), or overactive (low TSH, high T4). Additional tests useful in the diagnosis include thyroid autoantibodies (anti-TPO and anti-Tg), Ultrasonography of thyroid, thyroid gland FNAC and in case of overactive thyroid, radio-iodine scan (RAIU). In treated patients, TFT kept at mid-normal range, TSH 0.5-2.5 mIU/L, performed at 4-6 month interval to monitor response to treatment.



Why is diagnosing thyroid diseases important in pregnancy?

Hypothyroidism in pregnancy increases demand of thyroxine, therefore treatment must be adjusted and patients require more frequent laboratory investigation at 1-2 month intervals. Patients are advised regular treatments to avoid untoward side effects to mother and developing baby.

Doctor, my child is not growing, what should be done?

Pituitary gland, so called 'master gland' is responsible for growth and transformation of a child into adulthood a process called 'puberty'. There is a need to assess growth hormone (GH/ IGF1), pituitary hormones (LH, FSH, Estrogen/Testosterone) if a child is not growing properly. Various other tests performed for growth assessment include bone age (X-ray to check bone development), routine laboratory profile (CBC, LFT, TFT), and MRI pituitary in selected cases.

Please remember that age of pubertal development is different for boys and girls, and there are individual variations, therefore these tests must be performed judiciously.

What is polycystic ovary disease (PCOD)?

Many females during reproductive ages (15-45 Years) develop irregular menstruation, unwanted facial and body hairs, frequent obesity and infertility related to ovarian hormone imbalance. Laboratory Investigation at early menstrual phase (Day 2- Day 5) is crucial for diagnosis; LH, FSH, Estrogen, Testosterone, Insulin, ultrasound (USG) of pelvis and adrenal. Patients require targeted treatment; LASER treatment for unwanted hairs, hormone treatment for menstruation and hairs, lifestyle measures for obesity and ovulation induction treatment for subfertility/ infertility. Hormone evaluations are integral part of management and follow up. PCOD patients should also be investigated for prediabetes/ insulin resistance.

How does a laboratory investigation help to manage infertility?

Infertility/ subfertility is growing concern in modern society due to various reasons; increased age of marriage, professional career goal, obesity, qualitative changes of semen.

Various tests employed for infertility include; A) Male factors:- semen analysis (normal values; total count • 20 million, motility • 50 %, progressive motility •25 %, normal viscosity, healthy morphology), USG scrotum and testes, hormones LH, FSH, Testosterone, prolactin, B) Female factors:- USG of pelvis, hormones TFT, LH, FSH, Estrogen, Prolactin, Progesterone, TORCH agents testing, Hystero-salpingogram (HSG) to look for uterus, fallopian tube and selected cases hysteroscopy.

How to approach obesity?

Obesity and diabetes are modern day epidemic. Major causes of obesity are combination of familial and environmental factors: increased calorie intake, sedentary life style, decreased physical activity -and not an endocrine cause. However patients must be evaluated for cholesterol (Total Ch, LDL-Ch, HDL-Ch), triglyceride (Tg), Fasting blood glucose (FBG), Insulin, cardio-metabolic assessment (ECG, ECHO, exercise ECG, nuclear scan of heart) etc. Some patients require additional hormone testing like thyroid function test (TFT), cortisol, ACTH etc.

Conclusion

- Endocrine testing helps clinician to diagnose and follow-up patients effectively.
- Basic understanding and knowledge of endocrine testing are important.
- Readers are requested not to get pre-occupied by borderline laboratory results.

Renal Stones : Why do we analyze ?

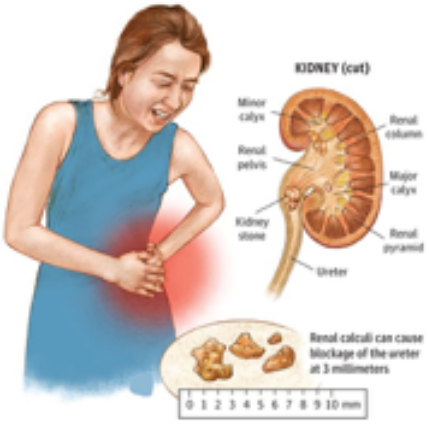
Dr Anwar Al-Anjeri
Biochemistry Unit, Department of Laboratory
Mubarak Al Kabeer Hospital

Mankind has always suffered from calculi in the efferent urinary tract. For example, urinary calculus was found in the pelvic area of a young man in a tomb dating back to 4800 BC near El Amrah, Egypt.

Renal or kidney or urinary stones (renal lithiasis, nephrolithiasis) are hard deposits made of minerals and salts that form inside your kidneys. These are common cause of hospital admission via the accident and emergency (A&E) department. In United States, the life-time risk for men is 12% and for women 5% with estimated annual incidences for men ranging from 100 to 300 per 100,000. They are 2-4 times commoner in men than in women and their prevalence has risen over the last 40 years. There is a high recurrence rate without medical treatment, estimated at around 25% in 10 years for a first time stone former and 75% for recurrent stone formers.

Symptoms

Renal stones occur in the renal pelvis, the ureter or the bladder. Calcification (deposition of calcium) can also occur scattered throughout the renal parenchyma (nephrocalcinosis). A renal stone may not cause symptoms until it moves around within kidney or passes into ureter, the tube connecting the kidney and bladder. At that point, patient may experience these signs and symptoms:

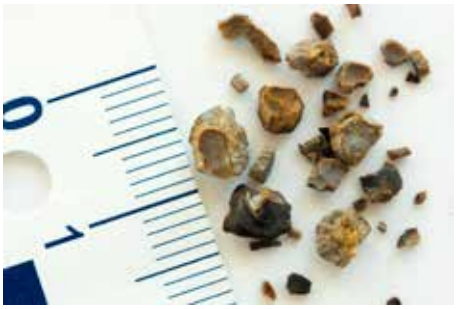


- Severe pain in the side and back, below the ribs
- Pain that radiates to the lower abdomen and groin
- Pain that comes in waves and fluctuates in intensity
- Pain on urination
- Pink, red or brown urine
- Cloudy or foul-smelling urine
- Nausea and vomiting
- Persistent need to urinate
- Urinating more often than usual
- Fever and chills if an infection is present
- Urinating small amounts

Pain caused by a kidney stone may change, for instance, shifting to a different location or increasing in intensity, as the stone moves through your urinary tract. The unpredictability of recurrent episodes may affect the quality of life and career choice. The costs of medical and surgical procedures plus costs from loss of work are considerable. Therefore, there are strong incentives to prevent recurrence.

Types of renal stones

The majority of stones (around 75 – 80%) contain calcium salts, generally calcium oxalate often with calcium phosphate. In total, 5 –10% of stones are uric acid and there are rare inherited stones.



Risk factors

Many factors contribute to stone formation and often more than one is involved in individual patients. These include abnormally high excretion of stone constituents, poor fluid intake, alteration in urine pH and decreases in protective urinary components. Around 20% of stone formers have a family history of stones, probably indicative of a genetic predisposition.

Certain occupations are more at risk of stone formation when fluid intake is reduced during the working day because there are few opportunities to pass urine (lorry drivers, teachers, production workers) or working conditions are very hot and there is excessive sweating (miners, engineers or workers in tropical countries). Eating a diet that's high in protein, sodium (salt) and sugar may increase your risk of some types of kidney stones. This is especially true with a high-sodium diet. Too much salt in your diet increases the amount of calcium your kidneys must filter and significantly increases your risk of kidney stones. High body mass index (BMI), large waist size and weight gain have been linked to an increased risk of kidney stones. Gastric bypass surgery, inflammatory bowel disease or chronic diarrhea can cause changes in the digestive process that affect your absorption of calcium and water, increasing the levels of stone-forming substances in your urine.

Diseases and conditions that may increase your risk of kidney stones include renal tubular acidosis (RTA), cystinuria, hyperparathyroidism, certain medications and some urinary tract infections. Distal RTA is a condition, either familial or acquired, in which the kidneys lose their ability to produce urine with a pH of 5.5 or less. Distal RTA is associated with renal stone formation. There is often an accompanying low serum bicarbonate and/or potassium and low urine citrate. The increased amounts of phosphate excreted in urine are available for calcium phosphate crystallization. Cystinuria, an autosomal recessive disorder, is the cause of 1-2% of renal stones. The pure stones are radiolucent (invisible) on X-ray imaging, but cystine may also be mixed with calcium oxalate crystals, which makes the stones radio-opaque. The stones are frequently large and can cause scarring, obstruction and, frequently, renal failure. The presence of infection invalidates the pH value and causes low urine citrate levels. Direct questions should be asked about medication, because frusemide, for instance, increases urine calcium excretion. Many patients take vitamin supplements, some of which contain calcium and vitamin D. Some patients who suffer gastric symptoms take over-the counter preparations high in calcium; for example, some antacid tablets, which contain 680 mg of calcium per tablet.

Citrate in urine inhibits the formation of calcium salts by forming soluble complexes with calcium. It is taken in the diet in many citrus fruits. Urinary citrate levels are lowered in the presence of infection and antibiotics. Magnesium is another inhibitor of calcium salt formation. Low urine magnesium concentrations will encourage salt crystallization. Urate is known to potentiate salt crystal formation.

Diagnosis

General investigations include abdominal X-rays, intravenous urogram (IVU) or Spiral computed tomography (CT) scanning.

The hospital laboratory can provide important diagnostic usefulness of information regarding the **chemical composition of renal stones.** It has been recognized since the 1950s and has improved so that it is now possible to correlate the results of an analysis with the appropriate diagnosis and therapeutic regimen.

If doctor suspects stone in urinary tract, various diagnostic tests and procedures are carried out. **Blood test** for serum creatinine (as a measure of renal function), calcium, sodium, potassium, phosphate, urate and parathyroid hormone.

Urine tests includes analysis of mid-stream urine to exclude infection and detect haematuria. Determining urinary pH also helps. A urine pH greater than 7 suggests infection with bacteria which split urea in the urine to ammonia and predisposing to magnesium ammonium phosphate stones. A urine pH less than 5 suggests uric acid stones.
24 hour urine is checked for calcium, phosphate, uric acid, oxalate and citrate.

Stone analysis :

Methods which have been used include wet or dry chemical analysis, polarisation microscopy, infrared spectroscopy etc.

If the patient passes stone, it is collected in a special bottle and sent to lab for analysis. In Kuwait laboratories, infrared spectrometry technique is used (automated FT-IR analyzer) Lab analysis of the passed stone will reveal the chemical composition of stones such as calcium oxalate, calcium phosphate, Uric acid and cystine. Most stones are of mixed composition. About 80% are made of a mixture of calcium oxalate (CaOx) and calcium phosphate (CaP) in various proportions.

The doctor uses this information to determine what's causing kidney stones and to form a plan to prevent more kidney stones.

Treatment

Depending on your situation, you may need nothing more than to take pain medication and drink lots of water to pass a kidney stone. In other instances, for example, if stones between 5 mm and 2 cm in diameter, lithotripsy is an option. Larger stones need to be removed surgically.

Kidney stones that can't be treated with conservative measures, either because they're too large to pass on their own or because they cause bleeding, kidney damage or ongoing urinary tract infections, may require more-extensive treatment. Procedures may include extra-corporeal shock wave lithotripsy (ESWL).

In addition, some calcium phosphate stones are caused by overactive parathyroid glands, which are located on the four corners of your thyroid gland. When these glands produce too much parathyroid hormone (hyperparathyroidism), the calcium levels can become too high and kidney stones may form as a result.

Prevention

The doctor may recommend preventive treatment to reduce the risk of recurrent kidney stones if the patient is at increased risk of developing them again.

- **Drink more fluid**

This is probably of greatest importance. You should aim to produce at least 2 L of urine every day; you will need to drink about 3 L of fluid during the day to achieve this. Suitable drinks are tap water, fruit squashes, fizzy drinks (try low calorie ones) and herbal teas. You may include some ordinary tea but no more than two to three cups daily. Coffee may be included but no more than six cups daily.

- **Avoid oxalate-rich foods**

Some foods are particularly rich in oxalate and it is important to avoid excessive amounts of these. Common oxalate-rich foods are chocolate, cocoa, nuts, peanut butter, strawberries, rhubarb, beetroot, spinach and parsley. Tea should be limited as mentioned earlier.

- **Reduce your salt intake**

You will do this by using less salt in cooking and by adding less salt to food at the table. You should try to avoid particularly salty foods such as Marmite, Bovril, Oxo, tinned and packet soups, tinned meats and meat pastes, salted crisps, nuts and smoked foods. You may use pepper, mixed herbs, garlic, spices or vinegar as alternative flavorings.

- **Continue eating calcium-rich foods, but use caution with calcium supplements.**

Calcium in food doesn't have an effect on your risk of kidney stones. Continue eating calcium-rich foods unless your doctor advises otherwise.

- **Aim to use high-fibre foods**

Medications

Medications can control the amount of minerals and salts in urine and may be helpful in people who form certain kinds of stones. The type of medication prescribed will depend on the kind of kidney stones. Thiazide diuretic or a phosphate-containing preparation help prevent calcium stones from forming. Allopurinol and an alkalizing agent may dissolve the uric acid stones.

Conclusion

Investigation of patients with renal stones can best be managed from the laboratory as there is a tendency for surgeons to concentrate on treating the stone and not the underlying cause.

An agreed and consistent approach between urologists, the laboratory, the stone clinic and urology outpatients is an effective way to screen for risk factors and to manage the patients successfully.

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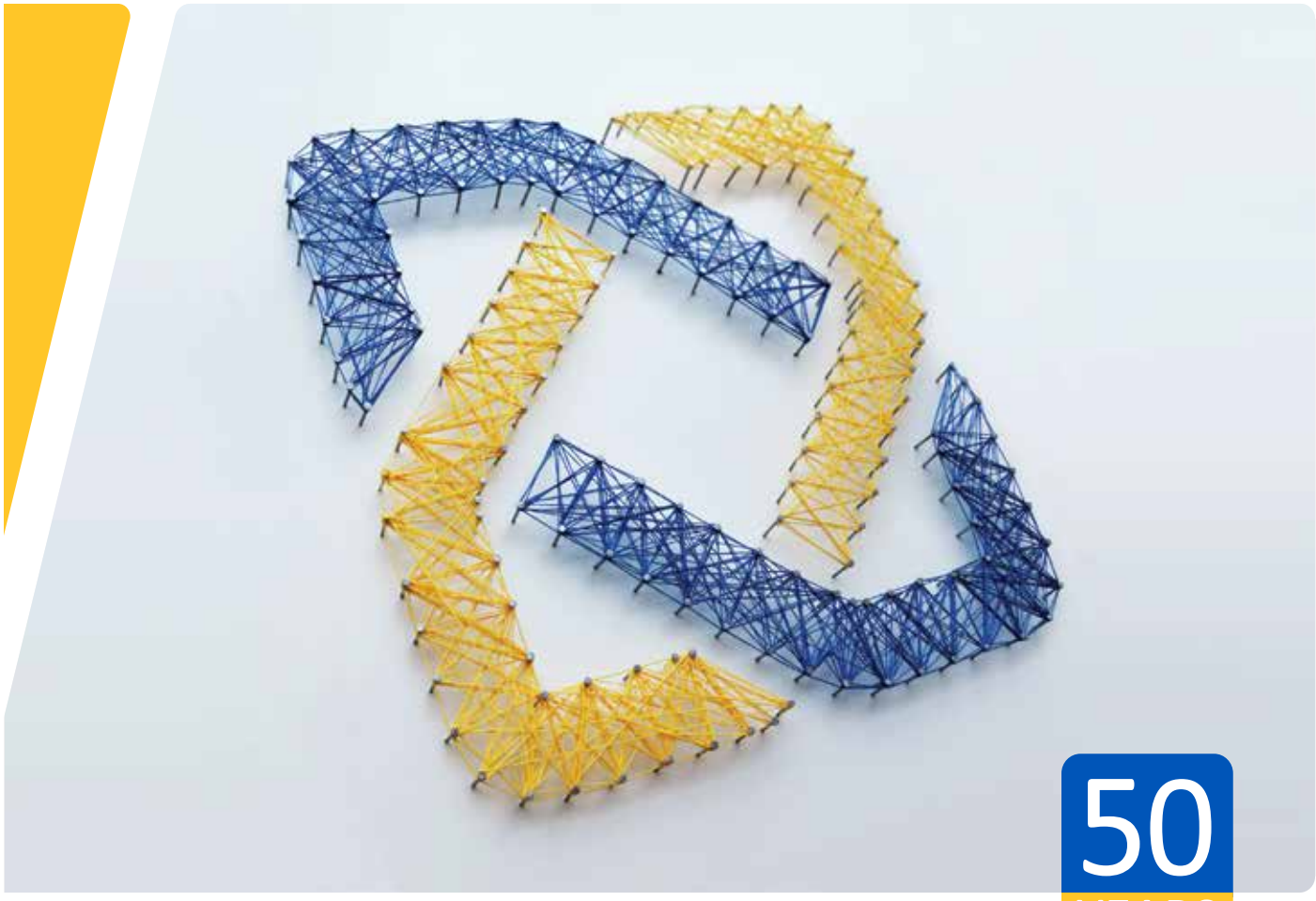

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VITAMIN D TESTING: HYPE or HOPE

Dr Jasem Khalifa
Biochemistry Unit, Department of Laboratory
Al Sabah & Psychiatry Hospital

INTRODUCTION

Vitamin D plays an important role in many places throughout the body, including the development and calcification of the bones.

Adequate exposure to sunlight and the use of dairy products with vitamin D have significantly reduced the incidence of vitamin D deficiency. However, vitamin D deficiency is still a common problem in many populations, particularly in older adults.

WHAT IS VITAMIN D?

Vitamin D is an oil-soluble vitamin that has several important functions in the body:

- It helps to absorb dietary calcium and phosphorus from the intestines.
- It suppresses the release of parathyroid hormone, a hormone that causes bone resorption (breakdown).

Through these actions, vitamin D keeps the calcium and phosphate levels in the blood normal, thereby promoting bone health. Vitamin D may have other benefits, such as improving muscle and immune function, but these areas are not well established.

Natural sources of vitamin D

Vitamin D is made in the skin under the influence of sunlight. The amount of sunlight needed to synthesize adequate amounts of vitamin D varies, depending upon the person's age, skin color, sun exposure, and underlying medical problems.

The production of vitamin D from the skin decreases with age. In addition, people who have darker skin need more sun exposure to produce adequate amounts of vitamin D, especially during the winter months.

Another important source of vitamin D is foods, where it may occur naturally (in fatty fish, cod-liver oil, and [to a lesser extent] eggs). In the United States, commercially fortified cow's milk is the largest source of dietary vitamin D, containing approximately 100 international units of vitamin D per 8 ounces. Vitamin D intake can be estimated by multiplying the number of cups of milk consumed per day by 100 (two cups milk = 200 international units vitamin D). In other parts of the world, cereals and bread products are often fortified with vitamin D.

Although vitamin D is found in cod liver oil, some fish oils also contain high doses of vitamin A. Excessive vitamin A intake can be associated with side effects, including liver damage and fractures.

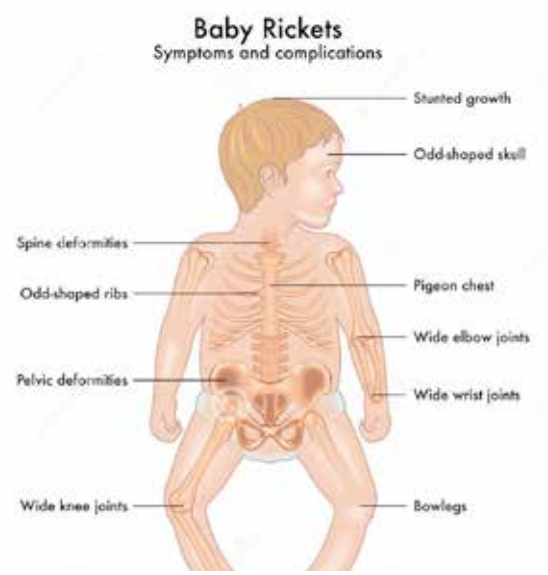
CAUSES OF VITAMIN D DEFICIENCY

The main reasons for low levels of vitamin D are:

- Inadequate intake — Infants, children, and older adults are at risk for low vitamin D levels because of inadequate vitamin D intake. Human breast milk contains low levels of vitamin D and most infant formulas do not contain adequate vitamin D. Older adults often do not consume enough vitamin D rich foods, and even when they do, absorption may be limited.
- Inadequate sun exposure — Parents of infants and children are often advised to keep their child out of the sun, which reduces vitamin D synthesis from the skin. Exposure to the sun is not recommended as a source of vitamin D for infants and children due to the potential long-term risks of skin cancer. Adults who have limited sun exposure are also at increased risk of vitamin D deficiency, especially if their skin is dark. In addition, reduced amounts of vitamin D are made in the skin and stored in the body as we age. This is especially true in the winter months in cold countries (like in some northern areas of USA, such as Boston, Massachusetts and Edmonton, Alberta) where the skin virtually ceases to produce vitamin D between October and April. In the summer months, the use of sunscreen limits vitamin D synthesis.
- Diseases or surgery that affect fat absorption — certain diseases affect the body's ability to absorb adequate amounts of vitamin D through the intestinal tract. Examples of these include celiac disease, Crohn's disease, and cystic fibrosis. Surgery that removes or bypasses portions of the stomach or intestines can also lead to low vitamin D levels. An example of this type of surgery is gastric bypass.
- Kidney and liver disease —Liver and kidney have important enzymes that change vitamin D from sun-exposed skin or food to the biologically active form of vitamin D. People with chronic kidney and liver disease are at increased risk of low active vitamin D levels because they have decreased levels of these enzymes.
- Less common causes of vitamin D deficiency include familial diseases that impair the enzymes in the liver or kidney that create the biologically active form of the vitamin. This results in inadequate amounts of active vitamin D.

POTENTIAL COMPLICATIONS OF VITAMIN D DEFICIENCY

The most serious complications of vitamin D deficiency are low blood calcium (hypocalcemia), low blood phosphate (hypophosphatemia), rickets (softening of the bones during childhood), and osteomalacia (softening of the bones in adults). Mother's milk is not sufficient in Vitamin D particularly if she is deficient and rickets results in reduced growth, bow legs, bossy skull, beads on the ribs and widened wrists. Osteomalacia is common in women not exposed to sunlight and eating food with excessive fiber which prevents calcium absorption and causes bone pain . However, these complications have become less common over time because many foods and drinks have added vitamin D.



Subclinical vitamin D deficiency or vitamin D insufficiency is common, and is defined as a lower than normal vitamin D

level that has no visible signs or symptoms. However, vitamin D insufficiency is often associated with reduced bone density (osteopenia or osteoporosis), and in some cases a mild decrease of the blood calcium level, elevated parathyroid hormone (which accelerates bone resorption), an increased risk of falls, and possibly fractures, all of which can seriously affect a person's quality of life.

Thus, identifying and treating vitamin D insufficiency or deficiency is important to maintain bone strength. Treatment may even improve the health of other body systems, such as the immune, muscular, and cardiovascular systems, although more research is needed in these areas.

DIAGNOSIS OF VITAMIN D DEFICIENCY

A low vitamin D level can be diagnosed with a blood test called 25 hydroxyvitamin D or 25(OH)D (OH = hydroxy D = vitamin D). Although there is no formal definition of vitamin D deficiency, some groups use the following values in adults:

- A normal level of vitamin D is defined as a 25(OH)D concentration greater than 75 nmol/L.
- Vitamin D insufficiency is defined as a 25(OH)D concentration of 50 to 75 nmol/L.
- Vitamin D deficiency is defined as a 25(OH)D level less than 50 nmol/L.
- Vitamin D toxicity is defined as a 25(OH)D level greater than 250 nmol/L.

WHO NEEDS TESTING FOR VITAMIN D?

Testing for vitamin D deficiency or insufficiency is not recommended for everyone, but may be advised for people who are home-bound or in a long-term care facility (e.g. nursing home), if the person has a medical condition that increases the risk of vitamin D deficiency or insufficiency, and for anyone with osteoporosis or a past history of a low-trauma fracture (e.g., fracture after fall from standing), low blood calcium (hypocalcaemia) or phosphate (hypophosphatemia).

Because vitamin D supplementation in the general population is safe, it is reasonable to advise supplementation without testing. Routine testing of Vitamin D is medically not necessary prior to or after starting vitamin D supplementation.

TREATMENT OF VITAMIN D DEFICIENCY

Vitamin D supplements — There are many types of vitamin D preparations available for the treatment of vitamin D deficiency or insufficiency. The two commonly available forms of vitamin D supplements are ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). We suggest vitamin D3 when possible, rather than vitamin D2, because vitamin D3 is the naturally occurring form of the vitamin and it may raise vitamin D levels more effectively.

Dosing — The recommended dose of vitamin D depends upon the nature and severity of the vitamin D deficiency.

In people whose vitamin D level is normal 75 nmol/L, a dose of 800 IU of vitamin D per day is usually recommended.

In people whose vitamin D level is <50 nmol/L, treatment usually includes 50,000 IU of vitamin D2 or D3 by mouth once or more per week for six to eight weeks, and then 800 to 1000 (or more)

international units of vitamin D3 daily thereafter.

In people whose vitamin D level is 50 to 75 nmol/L, treatment usually includes 800 to 1000 IU of vitamin D3 by mouth daily, usually for a three-month period. However, many individuals will need higher doses. Once a normal level is achieved, continued therapy with 800 IU of vitamin D per day is usually recommended.

In infants and children whose vitamin D level is <50 nmol/L, treatment usually includes 1000 to 5000 international units of vitamin D2 by mouth per day (depending on the age of the child) for two to three months.

People who have diseases or conditions that prevent them from absorbing vitamin D normally (eg, kidney or liver disease), the recommended dose of vitamin D will be determined on an individual basis.

Do I need other vitamins or minerals?

During treatment for vitamin D deficiency, it is important to consume at least 1000 mg of calcium per day for premenopausal women and men/ 1200 mg per day for postmenopausal women.

A blood test is recommended to monitor blood levels of 25(OH)D at least 12 months after beginning treatment. The dose of vitamin D may need to be adjusted based on these results and subsequent blood levels of 25(OH)D obtained to assure that normal levels result from the adjusted dose.

Side effects of vitamin D are uncommon unless the 25(OH)D level becomes very elevated (>250 nmol/L) and the person is taking high dose calcium supplements. However, it is important to follow dosing instructions closely and to avoid taking multiple products that contain vitamin D (e.g. multivitamin and vitamin D).

If 25(OH)D levels do become very elevated, complications such as high blood calcium levels or kidney stones can develop, but this happens extremely rarely.

PREVENTION OF VITAMIN D DEFICIENCY

As mentioned previously, the amount of vitamin D you need per day to maintain a normal level of 25 hydroxyvitamin D (25[OH]D) depends upon your skin color, sun exposure, diet, and underlying medical conditions.

In general, adults are advised to take a supplement containing 800 international units of vitamin D per day to maintain a normal vitamin D level. Older people who are confined indoors may have vitamin D deficiency even at this intake level.

All infants and children are advised to take a vitamin D supplement containing 400 international units of vitamin D, starting within days of birth. For infants and children, vitamin D is included in most nonprescription infant multivitamin drops. In some countries, it is possible to buy infant drops that contain only vitamin D.

Exposure to the sun or tanning beds is not recommended as a source of vitamin D because of the risk of skin cancer.

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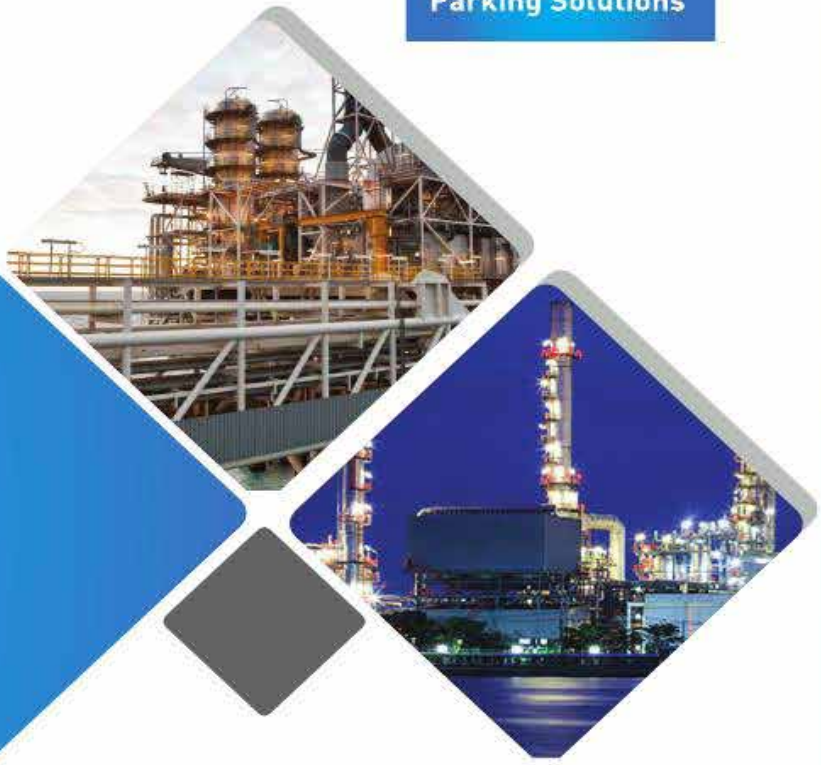

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TUMOR MARKERS : LABORATORY TESTS FOR CANCER

Dr Y. Girish
Biochemistry Unit, Department of Laboratory
Ibn Sina Hospital

What are tumor markers?

Tumor markers are substances that are produced by cancer or by other cells of the body in response to cancer or certain benign (noncancerous) conditions. Most tumor markers are made by normal cells as well as by cancer cells; however, they are produced at much higher levels in cancerous conditions. These substances can be found in the blood, urine, stool, tumor tissue, or other tissues or bodily fluids of some patients with cancer. Most tumor markers are proteins. However, more recently, patterns of gene expression and changes to DNA have also begun to be used as tumor markers.

Many different tumor markers have been characterized and are in clinical use. Some are associated with only one type of cancer, whereas others are associated with two or more cancer types. No "universal" tumor marker that can detect any type of cancer has been found.

There are some limitations to the use of tumor markers. Sometimes, noncancerous conditions can cause the levels of certain tumor markers to increase. In addition, not everyone with a particular type of cancer will have a higher level of a tumor marker associated with that cancer. Moreover, tumor markers have not been identified for every type of cancer.

How are tumor markers used in cancer care?

Tumor markers are used to help detect, diagnose, and manage some types of cancer. Although an elevated level of a tumor marker may suggest the presence of cancer, this alone is not enough to diagnose cancer. Therefore, measurements of tumor markers are usually combined with other tests, such as biopsies, to diagnose cancer.

Tumor marker levels may be measured before treatment to help doctors plan the appropriate therapy. In some types of cancer, the level of a tumor marker reflects the stage (extent) of the disease and/or the patient's prognosis (likely outcome or course of disease).

Tumor markers may also be measured periodically during cancer therapy. A decrease in the level of a tumor marker or a return to the marker's normal level may indicate that the cancer is responding to treatment, whereas no change or an increase may indicate that the cancer is not responding. Tumor markers may also be measured after treatment has ended to check for recurrence.

How are tumor markers measured?

A sample of blood, tumor tissue or body fluid is collected from a patient and sent to laboratory, where various methods are used to measure the level of the required tumor marker. Immunoassay methods, which are used in laboratories in Kuwait, are very specific and sensitive.

If the tumor marker is being used to determine whether treatment is working or whether there is

a recurrence, the marker's level will be measured in samples taken over time. Usually these "serial measurements," which show whether the level of a marker is increasing, staying the same, or decreasing, are more meaningful than a single measurement.

What tumor markers are currently being used ?

A number of tumor markers are currently being used for a wide range of cancer types. Tumor markers that are currently in common use are listed below :

MARKER	CANCER TYPE	SPECIMEN/ TISSUE	HOW USED
Alpha-fetoprotein (AFP)	Liver cancer and Germ cell tumors	Blood	To help diagnose liver cancer and follow response to treatment; to assess stage, prognosis, and response to treatment of germ cell tumors
Beta-2-microglobulin (B_2M)	Multiple myeloma, chronic lymphocytic leukemia, and some lymphomas	Blood	To determine prognosis and follow response to treatment
Beta-human chorionic gonadotropin (Beta-hCG)	Choriocarcinoma and Germ cell tumors	blood or urine	To assess stage, prognosis, and response to treatment
CA15-3	Breast cancer	Blood	To assess whether treatment is working or disease has recurred
CA19-9	Pancreatic cancer, Gallbladder cancer, bile duct cancer, and gastric cancer	Blood	To assess whether treatment is working
CA-125	Ovarian cancer	Blood	To help in diagnosis, assessment of response to treatment, and evaluation of recurrence
Calcitonin	Medullary thyroid cancer	Blood	To aid in diagnosis, check whether treatment is working, and assess recurrence
Carcino-embryonic antigen (CEA)	Colorectal cancer and some other cancers	Blood	To keep track of how well cancer treatments are working or check if cancer has come back
Chromogranin A (CgA)	Neuroendocrine tumors	Blood	To help in diagnosis, assessment of treatment response, and evaluation of recurrence

Estrogen / Progesterone Receptor (ER, PR)	Breast cancer	Tumor tissue	To determine whether treatment with hormone therapy and some targeted therapies is appropriate
Immunoglobulins	Multiple myeloma and Waldenströmmacroglo	Blood and urine	To help diagnose disease, assess response to treatment, and look for recurrence
Neuron-specific Enolase (NSE)	Small cell lung cancer and neuroblastoma	Blood	To help in diagnosis and to assess response to treatment
Prostate-specific antigen (PSA)	Prostate cancer	Blood	To help in diagnosis, assess response to treatment, and look for recurrence
Thyroglobulin	Thyroid cancer	Blood	To evaluate response to treatment and look for recurrence

Can tumor markers be used in cancer screening?

For a screening test to be useful, it should have very high sensitivity (ability to correctly identify people who have the disease) and specificity (ability to correctly identify people who do not have the disease). If a test is highly sensitive, it will identify most people with the disease—that is, it will result in very few false-negative results. If a test is highly specific, only a small number of people will test positive for the disease who do not have it—in other words, it will result in very few false-positive results.

Although tumor markers are extremely useful in determining whether a tumor is responding to treatment or assessing whether it has recurred, no tumor marker identified to date is sufficiently sensitive or specific to be used on its own to screen for cancer.

Is Prostate-Specific Antigen (PSA) recommended for diagnosis/screening for Prostate cancer ?

Prostate cancer is the most important cause of cancer deaths in men and many men enquire about doing this test for screening for cancer.

Prostate-specific antigen, or PSA, is a protein produced by cells of the prostate gland. The PSA test measures the level of PSA in a man's blood. The blood level of PSA is often elevated in men with prostate cancer. PSA test was originally approved by the FDA in 1986 to monitor the progression of prostate cancer in men who had already been diagnosed with the disease. In 1994, the FDA approved the use of the PSA test in conjunction with a digital rectal exam (DRE) to test asymptomatic men for prostate cancer. Men who report prostate symptoms often undergo PSA testing (along with a DRE) to help doctors determine the nature of the problem.

However, many conditions other than prostate cancer cause an elevation in PSA level like prostatitis (inflammation of the prostate) and benign prostatic hyperplasia (BPH) (enlargement of the prostate) and these are not pre-cancerous. Urinary tract infection, rectal examination of prostate, prostate biopsies and prostate surgery also increase PSA level. Conversely, some drugs—including finasteride and dutasteride, which are used to treat BPH—lower a man's PSA level. Also, some men with PSA levels below 4.0 ng/mL have prostate cancer, just as many men with higher levels do not have prostate cancer.

Until about 2008, many doctors and professional organizations encouraged yearly PSA screening for men beginning at age 50. Particularly, men who are at higher risk of prostate cancer (African American men and men whose father or brother had prostate cancer), are recommended to begin screening at age 40 or 45.

In general, however, the higher a man's PSA level which is rising over time, the more likely it is that he has prostate cancer. A confirmed (by repeat test) high level of PSA requires further tests to rule out or rule in cancer: trans-rectal ultrasound, biopsy, cystoscopy, x-rays etc.

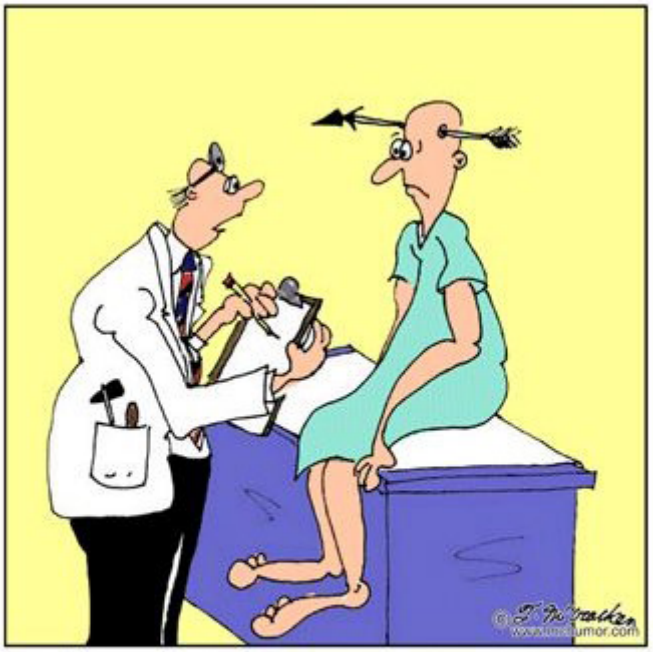
What kind of research is under way to develop more accurate tumor markers ?

Cancer researchers are turning to proteomics (the study of protein structure, function, and patterns of expression) in hopes of developing new biomarkers that can be used to identify disease in its early stages, to predict the effectiveness of treatment, or to predict the chance of cancer recurrence after treatment has ended.

Scientists are also evaluating patterns of gene expression for their ability to help determine a patient's prognosis or response to therapy.

Further Reading

1. Clinical practice guideline by the American Society of Clinical Oncology (ASCO) on tumor markers for breast cancer, colorectal cancer, lung cancer, and others.
2. Use of Tumor Markers in Clinical Practice, Laboratory medicine practice guidelines by the National Academy of Clinical Biochemistry



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LABORATORY DIAGNOSIS OF ALLERGIC DISEASES

Dr Radhakrishna Panikkar
Al Rashid Allergic Diseases Hospital

What is allergy?

Allergies, also known as allergic diseases, are a number of conditions caused by abnormal sensitivity of the immune system to something in the environment that usually causes little or no problem in most people.

Our immune system comprises different proteins known as Immunoglobulins - IgG, IgE, IgA, IgM and IgD . Immunoglobulin E (IgE) plays an important role in allergy reactions and is therefore often called the "allergy antibody".

If you are allergic to a particular substance (known as allergen), the immune system mistakenly believes that this, normally harmless substance, e.g. pollen, is harmful. When you are exposed to this particular substance for the first time, the immune system starts the production of IgE as an attempt to protect you. This IgE antibodies remain in your body and next time when you are exposed to the same allergenic substance, an allergic reaction and or allergic disease may occur. Sometimes these allergic diseases with high IgE is otherwise called as "atopy". Those causing allergies include pollens, certain foods, chemicals, insect stings, and some medicines.

The allergic diseases include nose allergies (allergic rhinitis, sinusitis), urticaria or hives, atopic dermatitis or eczema, allergic asthma, and sometimes fatal reactions known as anaphylaxis.

What are the tests used to identify allergy?

There are various methods to identify the agents responsible for these allergies.

These are skin prick tests, skin patch tests and blood test. Skin prick test and patch tests cannot be performed when the patient has skin diseases, when he/she is on medications like anti-histamines and in conditions like Diabetes, Heart diseases, Pregnancy etc. In such situations blood testing is the only tool to diagnose the allergy cause. A sample of blood is drawn and sent to the laboratory with a request to assay allergen specific IgE's. When a large numbers of allergens are suspected by the doctor, a few tubes of blood may be required.

Advantages of blood testing

Blood test can be performed irrespective of age, skin condition, medications, symptoms, allergic disease activity, and even in pregnancy. Adults and children of any age can take an allergy blood test. IgE antibodies against multiple allergens can be detected with a single blood sample. Allergy blood tests are very safe, since the person is not exposed to any allergens during the testing procedure.

What is the blood test used now? What Does the Test Measure?

Enzyme immunoassay (EIA) is employed in the KCCC immunology laboratory for quantitative total IgE and allergen specific IgE for inhalant, food, occupational and environmental allergens. The technique used is FDA approved and current International ISO Standards guidelines compliant. ImmunoCAP test is the alternative to the above and isused in Allergic Diseases Hospital Lab.ImmunoCAP test is an immunoassay with immunofluorescent technique. Both these techniques are vast improvement over earlier radioimmunoassay technique such as radioallergosorbent test (RAST).

These tests measure the concentration of specific IgE antibodies in the blood, i.e. IgE against the substance that is allergic to the person. A person who has an allergy has increased blood levels of IgE. A rule of thumb is that the higher the IgE antibody value, the greater the likelihood of symptoms. Allergen-specific IgE's found at low levels in asymptomatic individual can nevertheless help predict future symptom development. The results cannot just help determine what allergens a patient is allergic, but also help predict and follow the disease development, estimate the risk of a severe reaction, and explain cross-reactivity.

A low total IgE level is not adequate to rule out allergy to commonly inhaled allergens. The patients with a high total IgE have a high probability of allergic sensitization, but further investigation with allergy tests for specific IgE antibodies is often warranted.

ImmunoCAP assay can be performed on hundreds of allergens such as

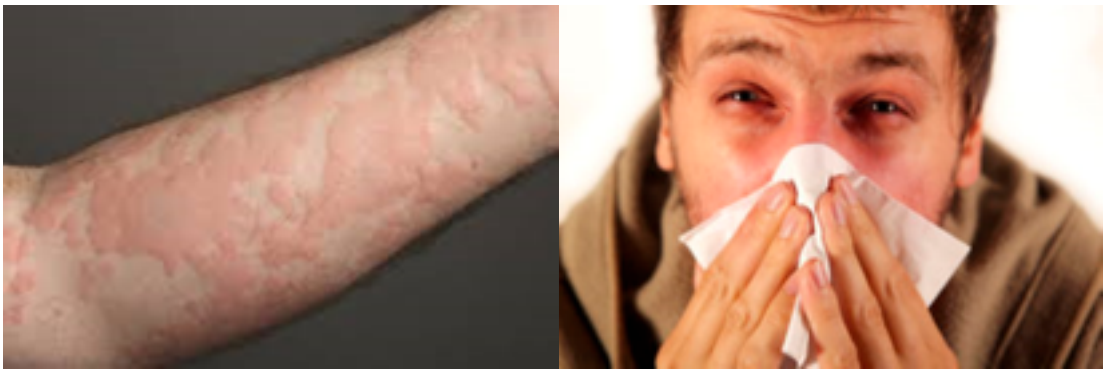
- Grass, weed and tree pollens
- Microorganisms
- Mites, mold
- Cats, dogs and other furred animals
- Insects
- Food allergens

in a single blood sample. Specific IgE blood test can definitively rule in or rule out atopic disease as the cause of or contributing factor to these symptoms.

Specific IgE results can help your doctor determine the individual allergy profile. He or she can then use this profile to work with you to create an individualized treatment plan. This might include medications to alleviate suffering and tips to help you reduce exposure to your specific allergic triggers, including rhinitis triggers, asthma triggers, and food allergy triggers. The doctor can also decide to treat with allergen immunotherapy in which small quantities of the offending allergen(s) may be administered to trigger production of protective antibodies, which prevent the allergens from binding the offending specific IgE antibodies (which is the cause of allergy reactions). Even in the case of a patient having high specific IgE's to several allergens (polysensitization), immunotherapy using a couple of select allergens may benefit the patient.

Who Should Be Tested?

Specific IgE blood test should be strongly considered for patients with recurrent or chronic rhinitis, sinusitis, allergic-rhinitis seasonal or perennial allergylike symptoms recurrent otitis media, asthma, food allergy, urticaria and other conditions in which IgE mediation is suspected.



Urticaria Allergic rhinitis

Interpretation of ImmunoCAP Test

	Specific igE is undetectable <0.35 Ku/L	**Specific igE is elevated >0.35 Ku/L**
Total IgE Normal Lesss than 100 IU/L	Symptoms not attributed to allergy	Does not rule out allergy if specific IgEs are elevated
Total IgE Elevated More than 100 IU/L	May indicate high risk of allergy. Further investigations warranted	Symptoms attributed to allergy

Management Options according to Specific IgE

Specific IgE (K/L)	**Level**	**Management Options**
<0.35	Absent/undetectable	Consider causes other than allergy
0.35-0.70	Low	Consider avoidance of allergens Consider treatment with medicine
0.71-3.50	Moderate	Treatment with medicines
3.51-17.5	High	If no improvement with medical treatment, refer to allergy specialist for specific allergen immunotherapy
17.6-50	Very high	
51-100	Very high	
>101	Very high	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LABORATORY DIAGNOSIS OF AUTOIMMUNE DISEASES

Prof. Raj Raghupathy
Department of Immunology
Faculty of Medicine & Mubarak Al Kabeer Hospital

What is Immunity?

A person resistant to a certain disease or protected from a disease is considered "immune" to it, and the state of being specifically resistant to a particular disease is described by the word "immunity". Immunity relies predominantly on our lymphocytes and develops specifically in response to an infection or vaccination, after which our immune system eliminates the invading bacteria or viruses. Millions of different, unique antigens (molecules) are recognized by our remarkable immune system. Immunity is mainly mediated by antibodies and T lymphocytes; antibodies specifically recognize and bind to bacteria and viruses thus preventing them from causing infection, while T cells kill cells that are infected by viruses thus eliminating the infection.

What is Autoimmunity ?

Our immune system attacks foreign invaders such as bacteria, viruses and parasites. Generally, the immune system does not attack "self", that is, our own molecules and cells. Unfortunately, in some people the immune system makes immune reactions against their "self" molecules, cells or organs. This condition called "autoimmunity" (the word "auto" means "self"). In simple terms, autoimmunity is immunity against "self", i.e. against our own cells and organs.

What are Autoimmune Diseases ?

When autoimmune reactions result in damage to our cells and tissues, autoimmune diseases may result. Autoimmune diseases are a result of destruction of a person's cells and organs by that person's own antibodies (i.e. autoantibodies) and/or autoimmune T cells. Some autoimmune diseases such as Hashimoto's thyroiditis are caused by autoantibodies that destroy the thyroid gland, due to which thyroid hormone production decreases. Some autoimmune diseases are caused by autoimmune T cells; for example, rheumatoid arthritis results from autoimmune T cells that attack the tissues in joints. About 3% to 9 % of people suffer from one autoimmune disease or another. Almost all organs are victims of autoimmune diseases. For example, Type 1 diabetes results from autoimmunity to the pancreas, myasthenia gravis affects the muscles, multiple sclerosis results from autoimmune damage to the brain, and ankylosing spondylitis affects the vertebrae.

How are Autoimmune Diseases Diagnosed ?

The physician's diagnosis of autoimmunity is based on the patient's history, clinical examination of signs and symptoms, initial or basic laboratory tests and specific immunological tests.

A. Basic Laboratory Tests

Autoimmune diseases often manifest with abnormal laboratory parameters. Some autoimmune diseases bring about decreased levels of red blood cells (anemia), platelets (thrombocytopenia) and white blood cells (leukopenia). For example, the autoimmune disease called Systemic Lupus Erythomatosus (SLE), caused by antibodies to DNA, red blood cells (RBC) and platelets leads to decreased numbers of platelets and white blood cells (WBC). This is tested in the laboratory by performing a differential blood count. Laboratory tests are also done to measure serum levels of specific organ enzymes; for example, autoimmune hepatitis manifests with elevations of transaminases, bilirubin, and serum proteins, while autoimmune inflammatory damage to muscle tissues which is seen in diseases like dermatomyositis and polymyositis results in increased levels of muscle enzymes such as creatinine kinase, ALT and AST. The diagnosis the autoimmune disease glomerulonephritis which involves injury to the kidneys is aided by tests for urine levels of proteins (proteinuria) and RBC (hematuria).

Inflammatory Markers:

Autoimmune diseases are generally "inflammatory diseases"; in response to ongoing inflammation, levels of several proteins are altered in the blood; these are called inflammatory markers. Some markers such as C-Reactive Protein (CRP), fibrinogen and haptoglobin are increased, while serum levels of molecules like albumin are decreased. CRP, a serum protein that activates some aspects of our immune system is elevated during inflammation, and is thus routinely used as a measure of current inflammation. Inflammation also causes an elevation in the Erythrocyte Sedimentation Rate (ESR) which is a measure of the speed with which RBC settle down at the bottom of a tube, and depends on levels of serum proteins and the interaction of RBC with these proteins. The severity of inflammation is reflected by the level of CRP. Levels of albumin tend to decrease in autoimmune conditions like glomerulonephritis or autoimmune inflammatory bowel disease. We should be aware, however, that inflammatory markers are not diagnostic of inflammation; they do not provide information on where the inflammation is and what has caused it. They only reflect abnormalities that are seen in autoimmune diseases.

B. Laboratory Testing for Autoantibodies

A large panel of autoantibodies is typically tested in a Clinical Immunology Laboratory using a range of immunological tests. The two most commonly used immunological tests for autoantibodies are ELISA and immunofluorescence. ELISA is a sensitive and specific test for autoantibodies. It involves adding the patient's blood serum to plastic wells coated with the relevant molecule (auto-antigen) and measuring the amount of autoantibodies using enzyme-labeled anti-antibodies. The immunofluorescence test involves adding the patient's serum to sections (or slices) of the relevant tissue (e.g, thyroid, kidney, liver etc) and then visualizing the autoantibodies bound to the tissue section under an ultraviolet microscope. This test allows the visualization of the precise cells to which the autoantibodies bind.

A few examples of commonly tested autoantibodies can be mentioned here (and presented in the Table). About 70% of patients with rheumatoid arthritis have an autoantibody against their own antibodies; this autoantibody, called Rheumatoid Factor, is indicative of progressive arthritis, but is also seen in other autoimmune conditions such as Sjogren's syndrome and systemic lupus erythematosus. Recently, a new marker for rheumatoid arthritis has been described; antibodies to Cyclic Citrullinated Peptide (CCP) are found in 95% of patients with rheumatoid arthritis. Some autoimmune diseases like SLE involve the production of autoantibodies against the person's own nuclear antigens such

as nucleic acid, histone, chromatin etc. These autoantibodies are measured in the lab using ELISA or immunofluorescence but are not specific to SLE, as they are also present in patients with Hashimoto's thyroiditis, autoimmune hepatitis etc. A much more specific antibody in SLE are autoantibodies to double-stranded DNA; levels of this antibody help in the diagnosis and monitoring of this disease. Several other autoantibodies are used in immunodiagnosis such as those against "extractable nuclear antigens" in SLE, anti-neutrophil cytoplasmic antibodies that contribute to the diagnosis of vasculitis and inflammatory bowel disease and antibodies that help diagnose autoimmune inflammatory myopathies. Autontibodies to the basement membranes of the lung and kidney cause the tissue damage seen in Goodpasture's syndrome, while antibodies to RBC membrane proteins are responsible for the destruction of RBC seen autoimmune hemolytic anemia.

The presence of an autoantibody in a patient helps in the diagnosis as it is more "specific" than other parameters, but does not assure a diagnosis of an autoimmune disease. A positive test for an autoantibody along with appropriate signs and symptoms helps the physician diagnose the condition. Some healthy individuals and people with other non-autoimmune diseases and infections may have low levels of some autoantibodies, so interpretation of these tests must be done with care. However, laboratory tests for autoimmune diseases play a vital role in helping the physician diagnose the disease, as well as the severity and response to medication.

Summarized in the table below are the autoimmune diseases, affected organs/ tissues and the auto-antibodies tested for the purpose of diagnosis.

Autoimmune Disease	Target	Autoantibodies to
Type 1 diabetes	Pancreas	Insulin, other pancreatic antigens
Hashimoto's thyroiditis	Thyroid gland	Thyroid proteins
Rheumatoid arthritis	Tissues in joints	Antibody molecules
Systemic lupus erythematosus	Skin, kidney etc	DNA, RBC, platelets
Goodpasture's syndrome	Lungs, kidneys	Basement membranes of lungs, kidneys
Autoimmune hemolytic anemia	RBC	RBC surface proteins
Myasthenia gravis	Muscles	Receptor proteins on muscles
Infertility	Sperm	Sperm proteins

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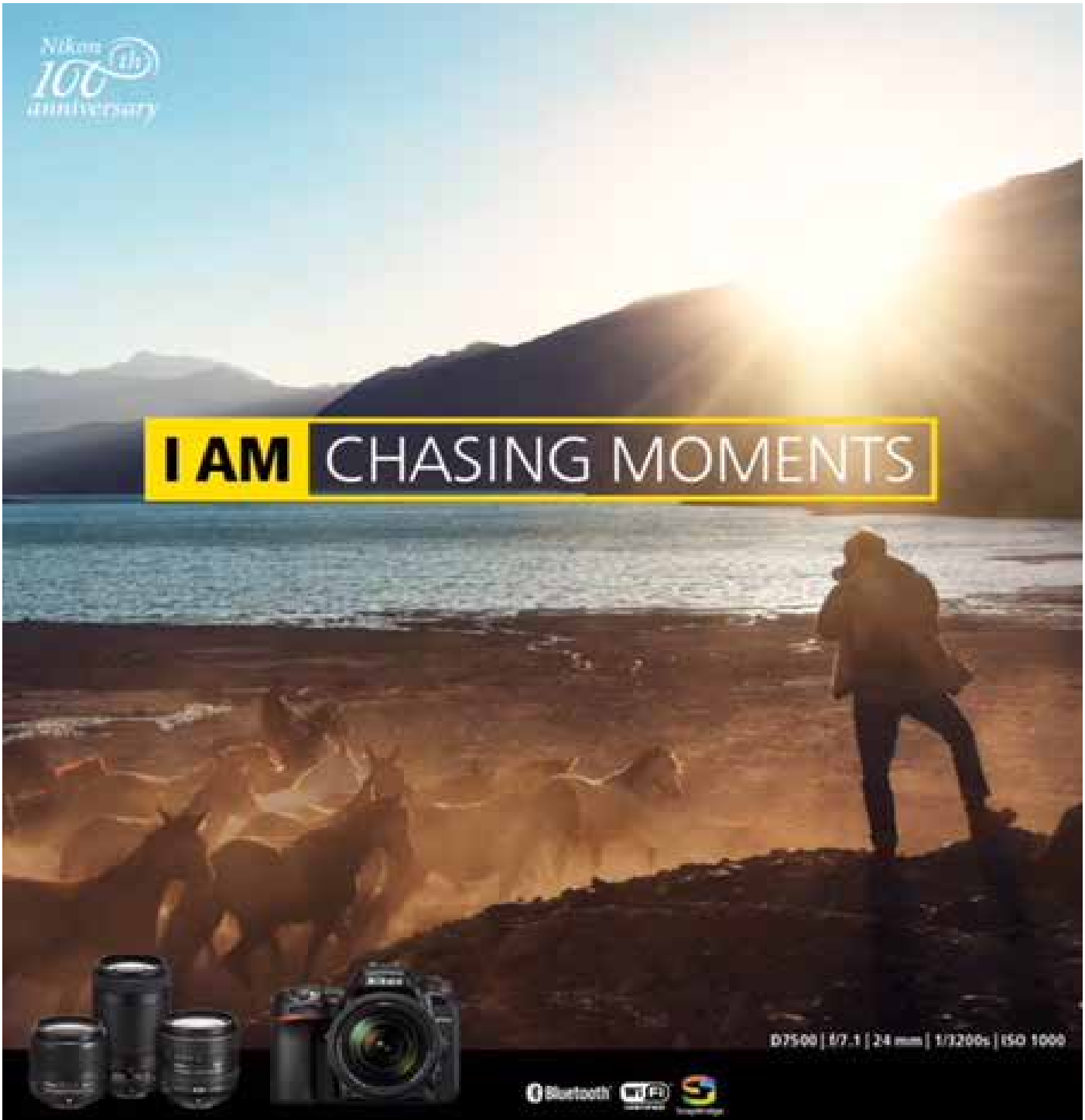

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CLINICAL MICROBIOLOGY: WHAT IS DONE HERE ?

Dr Prashant Purohit
Microbiology Unit, Department of Laboratory
Al Sabah Hospital

What is Clinical Microbiology?

Microbiology (Micro + Bio + Logos) is a branch of science which deals with the study of small organisms, which are difficult to be seen without using a microscope (microorganisms), and may cause disease after entering the human body, known as infection.

In a hospital, what is the role of a Clinical Microbiology laboratory?

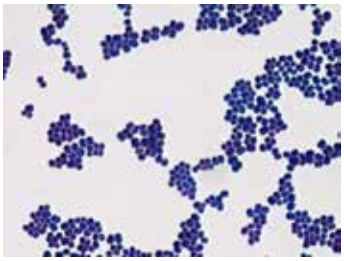
Here, the microorganism causing the infection is grown in culture, identified, and then it is tested that it could be killed with which drug, i.e. antibiotic (sensitivity testing). Depending on their target organisms, the drugs are labeled as antibacterial, antifungal, antiviral, or anti-parasitic agents. Collectively, these are called antimicrobial agents (AMA). The treatment thus targeted more effectively cures the patients.

The work seems quite complex. What are the different sections in a CM laboratory?

Following are the different sections of a Clinical Microbiology laboratory:

1. Bacteriology: For cultivation and sensitivity testing of bacteria from patients' sample.
2. Mycology: Growing, identification & sensitivity testing of different fungi.
3. Parasitology: Identification of parasite from blood, stool and urine samples.
4. Virology: Identification of different viruses from various samples, and detection of antiviral resistance if required, using culture &/or molecular methods.
5. Serology: Different infections induce antibodies against themselves in the body, which are detected in this section for diagnostic as well as prognostic purposes.

How are the bacteria grown?

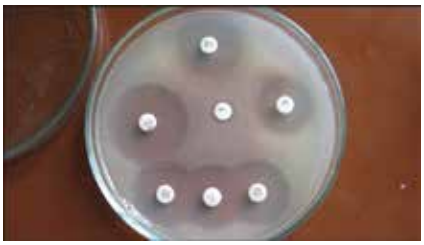


Samples are collected according to the suspected site of infection, and in the laboratory these are segregated and handed over to the appropriate section. In Bacteriology the sample is planted on different culture media depending on the most commonly implicated microorganism in that infection. Culture media are artificially created substances which contain all the necessary ingredients for the growth of different bacteria. After plantation the culture plates are kept inside an incubator with temperature same as that of human body (35-37°C). After an incubation of 24-48 hours the bacteria grow (many may take 3-10 days) on the surface of the culture medium in 'colonies'.

Each colony is made up of hundreds of thousands of bacteria, which can only be visible when a colony is smeared on a slide, stained and examined under the microscope.

Many samples like sputum and stool contain the normal bacteria which live in human body without causing any harm (commensals), along with the disease-causing (pathogenic) bacteria. The pathogenic bacteria have to be isolated out from the other commensals growing on the culture plate. They are picked up and planted on another culture plate- and incubated again the same way for another 24-48 hours.

How the antimicrobial susceptibility test (antibiotic sensitivity test) is done?



From the pure growth of the pathogenic bacteria, various identification and AMA-sensitivity tests are put up. This process takes another 24-48 hours' time.

For sensitivity testing, the isolated pure colonies of the pathogenic bacteria are spread on the surface of the agar-plate. On the surface of the plate, different discs containing antibiotics in a standardized concentration are placed. The plates are incubated for 18-24 hours. The effective antibiotics will show a 'zone of inhibition' around them.

Such a zone shows that the drug is effective against that bacterium. These zones are measured and compared with the standard zone sizes provided by different international guidelines like Clinical Laboratory Standards Institute (CLSI) of the USA, and EUCAST from the European Zone. Based on which, the bacteria are decided to be susceptible (S), resistant (R), or intermediate susceptible (I) to the antibiotic. No zone around an antibiotic disc means the bacteria are totally resistant to it. Overall, the entire culture and sensitivity test may take around 72 to 96 hours to be finalized.

That means, anybody can start the antibiotic treatment after seeing the report?

No. A typical culture & sensitivity report contains several antibiotics but it is the treating doctor who chooses the correct choice depending on many factors like the actual source/site of infection, its severity, pregnancy status, patient's other parameters like kidney & liver functions etc., as many drugs may be harmful for some patients.

Any other goals which may be achieved by culture & sensitivity tests?

Yes. There are several. A few most important ones are as following:

1. **Modification of reporting:** Some antibiotics may look effective in routine testing but may not be effective in patient's body. For example, the deformation of the big zone around the clindamycin disc (CC) into a 'D' shape opposite its neighboring erythromycin (E) disc shows inducible resistance to clindamycin. In such a case, the treatment with clindamycin may fail, hence it is reported as 'R' despite the big zone of inhibition around the CC disc. Similarly, several different mechanisms of resistance are detected during the test for several organism-antibiotic combinations.
2. **Antagonism/synergism:** If two drugs are antagonistic, they nullify each-other's effect, while synergy indicates that the two antibiotics will enhance each-other's effect if used together. This also can be detected during the routine sensitivity test.

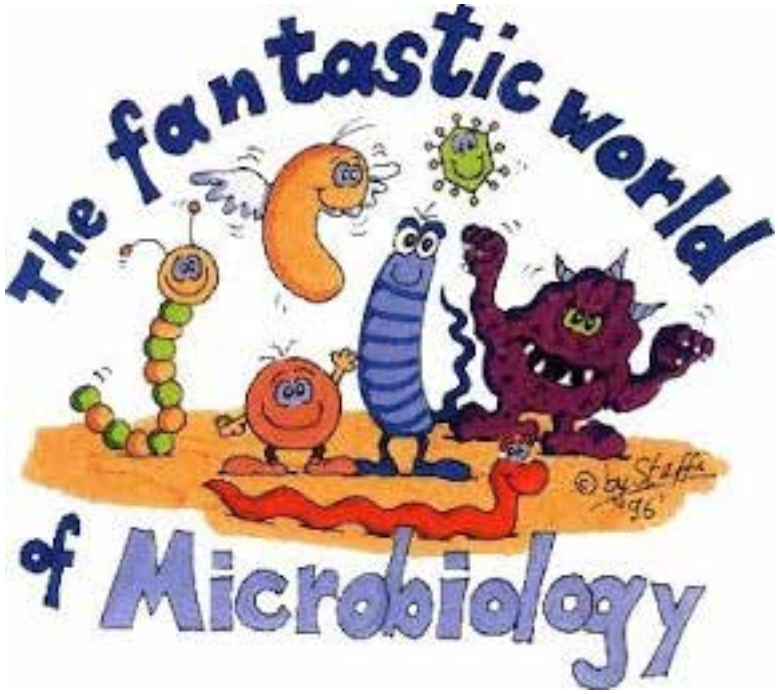
3. **Antibiotic Stewardship:** In the present era, the biggest problem is increasing resistance of bacteria to most of the antibiotics. We need to use the antibiotics judiciously, for example not to use an antibiotic with broad spectrum of coverage of bacteria, when the bacterium in question is susceptible to a narrow-spectrum antibiotic. The laboratory does not release the broad spectrum antibiotics if the narrower spectrum antibiotics are effective. This is known as antibiotic stewardship.

Microbiology laboratories are open 24 X 7. Why?

There are many life-threatening infections, in which early reporting is life saving. For example blood culture and CSF reporting. Emergency reporting is also important for infection-control purpose, to prevent the spread an infection to the other patients in the hospital, thus preventing hospital outbreaks. These include diarrhea-causing Clostridium difficile, multi-drug-resistant organisms like carbapenems-resistant enterobacteriaceae group bacteria, Acinetobacter baumannii, Pseudomonas aeruginosa, methicillin-resistant Staphylococcus aureus (MRSA) etc. are reported on telephone, as well as in writing.

Further Reading

Koneman's Color Atlas and Textbook of Diagnostic Microbiology (Seventh ed.). Procop GW, et al (editors). Philadelphia: Wolters Kluwer health 2017.



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SPUTUM CULTURE AND THROAT SWAB CULTURE

Dr Komal Tak
Microbiology Unit, Department of Laboratory
Chest Disease Hospital

SPUTUM CULTURE AND ANALYSIS

What is Sputum?

When you have a respiratory tract infection or a lung-related disorder, your lungs produce a thick substance known as sputum. This sputum can make it hard to breathe, cause coughing, and harbor bacteria. It contains mucus, microorganisms (bacteria, yeast etc.), cellular fragments & sometimes blood.

What is the purpose of the test?

Your doctor may order sputum culture if you have symptoms like cough, fever, chills, difficulty in breathing, chest pain, fatigue, confusion, muscle aches etc. Culture of sputum can help your doctor diagnose the cause of your symptoms, which may be any of these conditions: bronchitis, lung abscess, pneumonia, tuberculosis, chronic obstructive pulmonary disease, or several other infections. Certain harmful bacteria, viruses, or fungi can cause respiratory conditions. By determining what may be causing your symptoms, your doctor can find the best medication to cure the infection.

In some instances, your doctor may order a complete blood count to determine if white blood cells are elevated. This increase in white blood cells can indicate an infection.

What are the benefits of the test?

Providing a sample of sputum is noninvasive, means it does not require any instrument entering your body, and it requires little time. This can help them determine an appropriate diagnosis & treatment plan and further if your course of treatment for a respiratory infection is working.

If you are elderly or you have a suppressed immune system, lung damage, or a lung condition, such as chronic obstructive pulmonary disease or cystic fibrosis, it is important to get early diagnosis and treatment to avoid serious consequences.

What are the risks of the test?

Providing a sample of sputum by coughing is safe. If you have a respiratory infection, the coughing required to produce the sample might cause some discomfort.

How should you prepare for the test?

Ask your doctor if you should temporarily stop taking any medication before giving a sputum sample. Antibiotics, anti-inflammatory drugs, and steroids can affect the results of a sputum culture.

How is the sample collected?

You can give a sputum sample in your doctor's office, a lab testing site, or a hospital. In some cases, your doctor may ask you to collect your specimen at home. After collecting a sputum sample at home, take it to a laboratory as quickly as possible to help it stay fresh.

You will get the best results if you provide a sample first thing in the morning, before you have anything to eat or drink. For a patient with dentures, remove the dentures first. This will help you produce a sample of sputum from the deepest part of your chest containing pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated. Twenty-four hour collections should be discouraged because there is not only a greater chance of contamination but bacterial pathogens may potentially become diluted.

Sterile container for sputum specimen collection

Before providing the sample, your doctor will ask you to rinse out your mouth with water or saline solution (avoid using proprietary mouth washes or gargles that may contain antibacterial substances). This helps clear microorganisms from your mouth. Then, your doctor will ask you to breathe deeply and cough deeply. As you cough up sputum, you will deposit it into a sterile collection cup (sterile wide mouthed jar with tightly fitted screw cap lid). Carefully and tightly replace the lid.

For the best results, it is important to provide a sample that includes sputum, not just saliva. Saliva is watery. Sputum is usually yellow and thick. If you have an infection, your sputum may also be green or spotted with blood. Saliva will contain microorganisms found in your mouth, while sputum will contain microorganisms from your lungs. They may not be the same types of microorganisms.

If you cannot produce a sample of sputum on your own, your doctor or lab technician can help you provide a sample. They may ask you to inhale sterile saline solution (avoid the use of "Saline for injection", many preparation of which contain antibacterial substance). This will help loosen the sputum deep in your lungs.

How is the sample analyzed?

A Clinical Microbiologist will analyze your sputum sample using a variety of methods:

Gram's stain

They will use what is called a "Gram's stain" to learn if the sample is adequate and contains enough bacterial cells to proceed. The presence of 25 or more pus cells per 100X field indicates excellent specimen.

Sputum culture

If your sputum sample is adequate, the technician will process it to identify the bacteria or fungus. A bacterial infection may require up to 48 hours to grow. It may take a week or more for fungi to reproduce. They will also determine the antibiotic which may work to treat your infection.

How are the results interpreted?

It is normal and healthy for sputum to have certain types of bacteria growing in your airways without causing illness. Laboratory will work to tell the difference between bacterial that make you sick and those that keep you well. If your results are "Negative" or "Normal", the lab found no evidence of disease-causing bacteria or fungi in your sputum sample. If your symptoms persist, you may have an infection due to a virus or other microorganism that was not identified in your sputum sample. Some types of organisms cannot be grown and identified using a sputum culture. Additional testing may be necessary.

If your results are "Positive" or "Abnormal", the lab found evidence of disease-causing bacteria or fungi in your sample. Your doctor will use the results of the analysis to choose which treatment they recommend.

THROAT SWAB CULTURE

What is a Throat Swab Culture?

A throat swab culture is routinely used to test for bacterial infections like strep throat or tonsillitis.

This procedure is performed in the doctor's office. Your doctor will swab your throat to take a throat culture.

A throat swab culture, or throat culture, is a test commonly used to diagnose bacterial infections in the throat. These infections can include strep throat (group A streptococcus bacteria, Streptococcus pyogenes), pneumonia, tonsillitis, whooping cough, and meningitis.

What Is the Purpose of a Throat Swab Culture?

Most sore throats are caused by a virus. Many sore throats go away within a few days without any specific treatment.

Your doctor will generally order a throat culture test if you have symptoms that suggest strep throat or another infection. Redness, swelling, and white streaks or pus on the tonsils as well as red spots in the roof of the mouth are signs of infections. These signs do not indicate whether the infection is viral or bacterial, so a throat swab culture is necessary. Strep throat is very contagious, so it is important that it is diagnosed early. Exclusion of bacterial cause of infection will avoid unnecessary intake of antibiotics, which work only against bacteria, not against viruses. These antibiotics alter our normal, helpful bacteria living in our intestines. These normal flora save us from several infection causing organisms. The antibiotics given injudiciously make us prone to the infections resistant to these antibiotics.

How Can I Prepare for a Throat Swab Culture?

Antiseptic mouthwash should be avoided before this test. You should also tell your doctor if you have been taking any antibiotics because this could affect the test results.

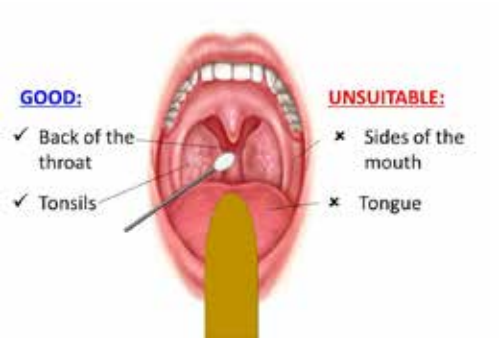
If your child is undergoing the examination, you should ask them to remain still. You may need to help gently restrain them.

How Is a Throat Swab Culture Performed?

Your doctor will ask you to open your mouth and tilt your head back. If necessary, your doctor may use a tongue depressor. This can help your doctor have a better view of the back of your throat. Having the patient phonate long "ah" serves to lift the uvula and helps prevent gagging. They will then rub a sterile cotton swab across the back of your throat, your tonsils, and any other sore areas for a few seconds. The swab will collect a sample of the secretions being produced in the back of your throat.

The sample your doctor collects is taken to a laboratory. It usually takes a few days to culture the bacteria.

There are no risks or complications associated with a throat swab culture. The test may cause momentary gagging because the back of the throat is a sensitive area, but it should not be painful.



What Can I Expect After the Test?

A 'Negative' throat swab culture means that no infectious bacteria are present in your throat. The infection is most likely caused by a virus, and does not require any antibiotics.

A 'Positive' test indicates the presence of streptococci (the bacterium that causes the 'strep throat') or a few other bacteria. If the test result is positive, the results can be used to determine which bacteria are causing the infection. Once your doctor knows what is causing the infection, he can figure out a diagnosis and treatment plan.

In order to treat a bacterial infection, your doctor will prescribe an antibiotic, which is indicated in your 'culture & sensitivity' report.

Your doctor may suggest over-the-counter drugs, to help relieve throat pain or reduce fever. Most people start to feel better after a day or two, but if symptoms continue to persist after 48 hours you may need to contact your doctor again.

Rapid Antigen detection test (RADT) or Strept Screen

This test is available in some clinics and result is available within 10 to 20 minutes. A positive test may indicate immediate start of antibiotic-treatment without further testing. A negative test indicates further confirmation by Throat Swab Culture.

Suggested Reading

1. Koneman's Color Atlas and Textbook of Diagnostic Microbiology Sixth Edition

2. Bailey & Scott's Diagnostic Microbiology Twelfth Edition

BLOOD AND CSF CULTURE

Dr Siddiq M. Khan
Microbiology Unit, Department of Laboratory
Chest Diseases Hospital

BLOOD CULTURE

What is blood culture ?

Blood culture is a gold standard test for detection of infection of blood (septicaemia) by different microorganisms like bacteria and fungus, identifying the type of organisms and their sensitivity to antibiotics.

What is it used for ?

It is used in the diagnosis of infection of heart valves (endocarditis), infection of covering of brain (meningitis), infection of lung (pneumonia), infection of bones (osteomyelitis), infection of skin and the tissue under it etc., in which the infecting organism is circulating in the blood stream.

How to collect blood cultures ?

Blood should be collected under strict aseptic conditions to reduce contamination. It should be performed only by physician, qualified nurse, or phlebotomist.

How many blood culture sets should be collected ?

For majority of patients, two sets of blood cultures are recommended. The second or third set is taken from a different site. This not only increases the yield but also recognises if any contamination has occurred.

When should it be collected ?

Samples should be taken as soon as possible following a spike of fever and ideally prior to antimicrobial treatment. If a patient is already on antibiotic, blood should be collected just before the next dose is due (when the concentration of antibiotic is low).

Pediatric and Adult (aerobic & anaerobic) blood culture BACTEC bottles

What is the volume of blood to be collected ?

Adults: 8-10 ml in each bottle; collected in two bottles, one for the organisms growing in the presence of oxygen (aerobes), and the other one for the organisms growing in absence of oxygen (anaerobes).

Infants: 1-3 ml (because the concentration of microorganisms during bacteremia is higher than adults).

After the collection, the bottles are sent to the Microbiology laboratory, where these are kept in the machine for incubation under human body-like temperature (35-37°C).

An automated blood culture incubation machine

How many days are recommended for incubation ?

Most bacteria can grow in 2-3 days but some can take 10 days or more. Fungus can take up to 14 days for growth. On the growth of any organism, the oxygen inside the blood culture bottle is utilized and carbon dioxide is generated by the organism. When the level of carbon dioxide reaches higher than a preset threshold level, it is sensed by the sensor in the incubation machine which gives an alarm.

Suggested reading

1. http://whqlibdoc.who.int/publications/ 2010/9789241599221_eng.pdf
2. https://en.wikipedia.org/wiki/Blood_culture
3. BACTEC manual

CSF CULTURE

What is CSF ?

Cerebrospinal fluid (CSF) is a fluid that is present between the membranes which cover the brain and spinal cord.

Why is CSF culture done ?

It is done to diagnose meningitis (infection of covering of brain) and in cases of unexplained fever (pyrexia of unknown origin PUO)

How is CSF collected ?

It is collected by lumbar puncture by well trained doctor under aseptic conditions. If possible, three tubes (1ml each) should be collected for microbiology, chemistry and cytology.


Lumbar puncture


Collection of sample

What is the incubation period for CSF culture ?

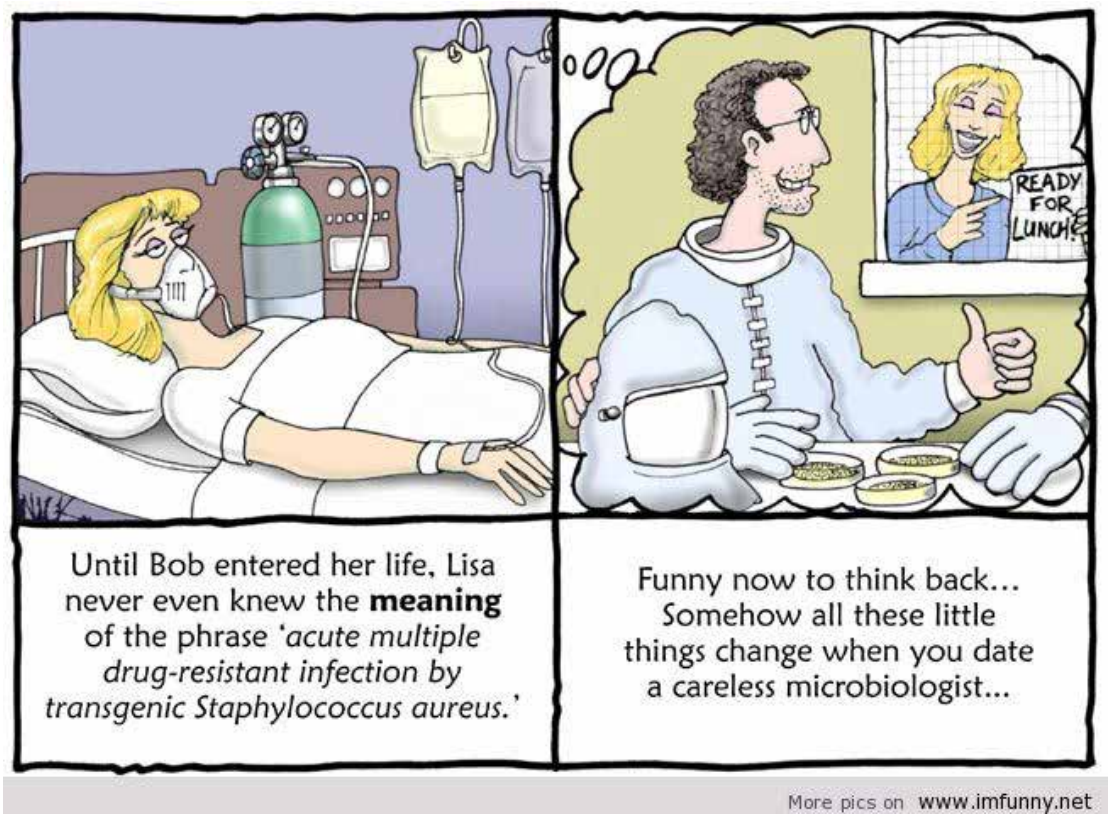
All the culture media are incubated for 1 week.

How is the culture reported ?

Report directly detection of bacterial antigen by appropriate test (agglutination) and most probable antibiotic as early as possible for starting the treatment without delay. All the microorganisms grown in culture are identified, and antimicrobial susceptibility testing is done. All positive CSF culture are notified to preventive medicine especially meningococcus and H.influenzae to prevent spread of infection, by giving the family contacts antibacterial prophylaxis.

Suggested reading

1. http://www.cdc.gov/meningitis/labmanual/chap04-biosafety.html
2. http://health.ucsd.edu/labref/p541.html



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- Soap Foam Dispensers with Cartridge.

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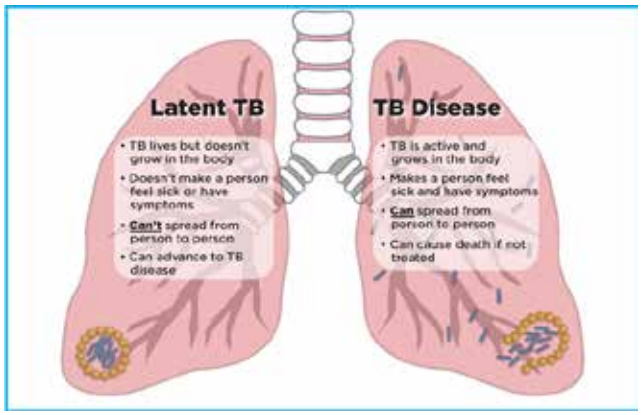
INTERFERON-GAMMA RELEASE ASSAYS (IGRAS) : BLOOD TESTS FOR DETECTION OF TB INFECTION

Dr Susan Malayil Joseph
Microbiologist
TB Control Unit, Tuberculosis Reference Laboratory

What are IGRAs ?

Interferon-Gamma Release Assays (IGRAs) are whole-blood tests that can aid in diagnosing infection with the bacterium causing tuberculosis *(Mycobacterium tuberculosis)*. They do not help differentiate latent tuberculosis infection (LTBI) from tuberculosis disease



Two IGRAs that have been approved by the U.S. Food and Drug Administration (FDA) are:

- QuantiFERON®-TB Gold In-Tube test (QFT-GIT)
- T-SPOT®.TB test (T-Spot)

How do IGRAs work ?

IGRAs measure a person's immune reactivity to M. tuberculosis. White blood cells from most persons that have been infected with M. tuberculosis will release interferon-gamma (IFN-g) when mixed with antigens (substances that can produce an immune response) derived from *M.tuberculosis*.

To conduct the tests, fresh blood samples are mixed with antigens and controls. The antigens, testing methods, and interpretation criteria for the two types of IGRAs differ.

What are the advantages of IGRAs ?

- Requires a single patient visit to conduct the test.
- Results can be available within 24 hours.
- Do not boost responses measured by subsequent tests.
- Prior BCG (Bacille Calmette-Guérin) vaccination does not cause a false-positive IGRA test result.

What are the disadvantages and limitations of IGRAs ?

- Blood samples must be processed within 8-30 hours after collection while white blood cells are still viable.
- Errors in collecting or transporting blood specimens or in running and interpreting the assay can decrease the accuracy of IGRAs.

- Limited data on the use of IGRAs to predict who will progress to TB disease in the future.
- Limited data on the use of IGRAs for:
  a. Children younger than 5 years of age;
  b. Persons recently exposed to M. tuberculosis;
  c. Immunocompromised persons; and
  d. Serial testing.
- Tests may be expensive.

TST	IGRA
Good for serial testing	Not as good for serial testing
Inexpensive	More expensive
Universally accessible	Skill, equipment and timeframe needed limit accessibility
Low specificity in certain populations (BCG-60%)	High specificity in all populations
Two visits	One visit
Variability in test interpretation by reader *****	Low variability in test interpretation by reader

How do you interpret IGRA test results ?

IGRA interpretations are based on the amount of IFN-g that is released or on the number of cells that release IFN-g. They are reported as positive, negative, or indeterminate.

As with the tuberculin skin tests (TSTs, popularly known as Mantoux Test or MT), IGRAs should be used as an aid in diagnosing infection with *M.tuberculosis*. A positive test result suggests that M. tuberculosis infection is likely; a negative result suggests that infection is unlikely. An indeterminate result indicates an uncertain likelihood of *M.tuberculosis* infection.

A diagnosis of LTBI requires that TB disease be excluded by medical evaluation. This should include checking for signs and symptoms suggestive of TB disease, a chest radiograph, and, when indicated, examination of sputum or other clinical samples for the presence of *M.tuberculosis*. Decisions about a diagnosis of M. tuberculosis infection should also include epidemiological and clinical history.

Recommendations on when to use IGRA tests

- IGRAs can be used in place of (but not in addition to) TST in contact investigations, testing during pregnancy, and screening of health care workers and others undergoing serial evaluation for M. tuberculosis infection.
- Populations in which IGRAs are preferred for testing:
  a. Persons who have received BCG (either as a vaccine or for cancer therapy); and
  b. Persons from groups that historically have poor rates of return for TST reading.
- TST is preferred over IGRAs for testing children less than 5 years of age.
- As with TST, IGRAs generally should not be used for testing persons who have a low risk of infection and a low risk of disease due to *M.tuberculosis*.
- The availability and benefits of IGRAs are evaluated on individual basis to prioritize in their use.
- Routine testing with both TST and IGRA is not recommended. However, results from both tests might be useful in the following situations:
  a. When the initial test is negative and:
    - The risk for infection, the risk for progression to disease, and the risk for a poor outcome are high (e.g., HIV infected persons or children under 5 years of age who are exposed to a person with infectious TB).

- There is clinical suspicion for TB disease (e.g., signs, symptoms, and/or radiographic evidence suggestive of TB disease) and confirmation of M. tuberculosis infection is desired.
- Taking a positive result from a second test as evidence of infection increases detection sensitivity.

b. When the initial test is positive and:

- Additional evidence of infection is required to encourage acceptance and adherence (e.g., foreign-born healthcare workers who believe their positive TST is due to BCG). A positive IGRA might prompt greater acceptance of treatment for LTBI as compared with a positive TST alone.
- The person has a low risk of both infection and progression from infection to TB disease. Requiring a positive result from the second test as evidence of infection increases the likelihood that the test reflects infection.

c. When the initial IGRA result is indeterminate/borderline/invalid and a reason for testing persists.

Multiple negative results from any combination of these tests cannot exclude *M.tuberculosis* infection. Selection of the most suitable test or combination of tests for detection of *M.tuberculosis* infection is based on the reasons and the context for testing, test availability, and overall cost of testing.

IGRAs in Children

Children are at high risk of developing active TB disease, if infected. Furthermore, diagnosis of TB is a persistent challenge with young children, who are often unable to produce sputum and for whom conventional microbiological tests have low sensitivity. Available data suggest that TST and IGRAs have similar accuracies for the detection of TB infection or the diagnosis of disease in children. Subgroup analysis suggested a lower sensitivity for all tests in young (<5 years of age) or HIV-infected children. Both TST and IGRAs had similar correlations with the exposure gradient in children. However, the ability of either TST or IGRA alone was suboptimal to rule in or rule out active TB. Thus, in children with suspected active TB, every effort should be made to collect appropriate clinical specimens for microbiological and molecular testing, and IGRAs should be used with other clinical data (e.g., TST results, chest X-ray findings, and history of contact) to support a diagnosis of active TB.

IGRAs in HIV-Infected Persons

In HIV-infected persons with active TB, sensitivity of T SPOT.TB assay is higher than QFT assay in low- and middle-income countries. However, neither IGRA was consistently more sensitive than the TST in head-to-head comparisons. IGRAs, in particular the T-SPOT.TB assay, may be less affected by the degree of immunosuppression, but results differed across geographical settings.

Thus, current evidence suggests that IGRAs perform similarly to the TST in identifying HIV-infected individuals with presumed LTBI. Both TST and IGRAs have suboptimal sensitivity for active TB, suggesting a potential role for using both tests, especially in severely immunocompromised individuals.

Conclusions

Both TST and IGRAs are acceptable. IGRAs offer some improvements over the TST. There are situations where neither test is appropriate (e.g. active TB diagnosis in adults) and situations where both tests may be necessary to detect *M.tuberculosis* infection (e.g., immunocompromised populations), and there are situations where one test may be preferable to another. For example, IGRAs may be preferable to the TST in populations where BCG is given after infancy or given multiple times. In contrast, TST may be preferable to the IGRAs for serial testing of health care workers.

The primary goal of IGRAs is to identify those who will benefit from LTBI therapy. Unfortunately, IGRAs (and TST) are limited in this regard, for reasons including the low absolute risk of progression to disease, inability to distinguish reactivation from reinfection, reduced accuracy in immunocompromised patients, and inability to discriminate the various stages within the spectrum of LTBI. LTBI screening should be reserved only for those who are at sufficiently high risk of progressing to disease.

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DIAGNOSIS OF FUNGAL INFECTIONS: CLINICAL MYCOLOGY

Dr Aarti Chadha
Microbiology Unit, Department of Laboratory
Mubarak Al Kabeer Hospital

Introduction

The branch of biology which deals with the study of fungi ,is known as Mycology. The term is derived from mykes, a greek word for mushrooms. The systemic study of fungi is about 200 years old, however, their clinical manifestations have been known since antiquity. The knowledge of mycology has increased exponentially over the years to the level of an independent biomedical speciality.

The fungi are widely found in the environment and most of these are harmless commensals, contaminants or non pathogenic agents. Some of the fungi are even useful to the mankind in several ways. Only a small number of organisms are causing diseases among humans, animals and plants. Most of the fungi exist as moulds but there are number of pathogenic yeast and many are dimorphic as well. The dimorphic fungi assume mould form in nature and parasitic yeast form when causing infection to the host. These are now recognised as significant cause of morbidity and mortality among human host. Such infections today are among the most difficult to manage.

Some fungi can cause diseases in healthy people, but most of the fungal infections occurs in individuals already experiencing serious illness and those who are immunocompromised (whose immunity is reduced because of some disease process or some medication they are taking).

Table 1: Predisposing factors for fungal infections

1. More aggressive treatment modalities for cancer patients.
2. Increased number of organ and stem cell transplant procedures.
3. Increased number of AIDS cases with longer survival
4. Widespread use of broad spectrum antibiotics.
5. Increased number of intravenous drug abusers.
6. Prosthetic devices and catheter borne infections.

Common fungal infections

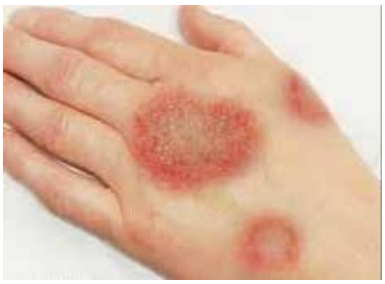
Clinically fungal infections can be categorised into 3 categories: Superficial/Cutaneous/Muco-cutaneous fungal infections, Subcutaneous fungal infections, and Systemic fungal infections.

Superficial/Cutaneous/Muco-cutaneous fungal infections: infections of the outer layers of skin, hair, nail or mucous membranes. Examples – Ringworm (dermatophytosis), candida skin infection, oral candidiasis, vaginal candidiasis.

Dermatophytosis: This is commonly known as ringworm infection. It is caused by a special type of fungi grouped together as Dermatophytes. It can involve any part of the skin :

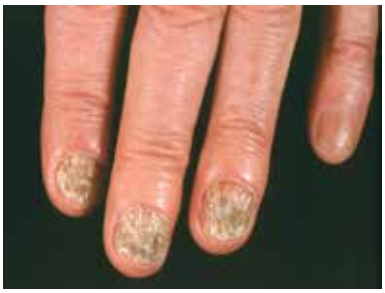
Tinea corporis, T. pedis/athlete's foot, Tineal cruris (Jock itch), hair (Tinea capitis) or nail (onychomycosis).

Superficial Candida infection: It is infection of skin or mucosa with a fungus called Candida. Its examples include nappy rash , oral candidiasis (oral thrush, curd-like patches on tongue & palate), and vaginal candidiasis (white, itchy vaginal discharge in sexually active females).

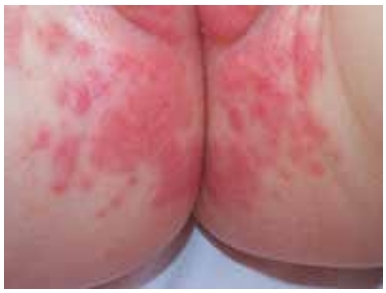


Tinea corporis

Tinea capitis



Onychomycosis



Nappy rash

Subcutaneous fungal infections : Usually seen after traumatic implantation of fungal elements into subcutaneous tissue (the tissue beneath the skin). Example : Sporotrichosis , occurs as a result of traumatic inoculation of thorns or splinters subcutaneously, leading to formation of nodules usually seen on the extremities of rose-gardeners.

Systemic fungal infections: These can be caused by true pathogenic fungi or opportunistic fungi. True pathogenic fungi can cause diseases in healthy population, generally confined to a particular endemic area. These are dimorphic fungi like histoplamosis, blastomycosis, coccidioidomycosis. These fungal infections usually present as chronic pulmonary disease or disseminated disease. Opportunistic fungi cause disease in immunicompromised host, for example systemic candidiasis, cryptococosis and aspergillosis. These can involve any organ. Cryptococcus is epidemiologically linked to pigeons and their excreta worldwide. Some species colonize Eucalyptus trees.

Laboratory diagnosis of fungal infections

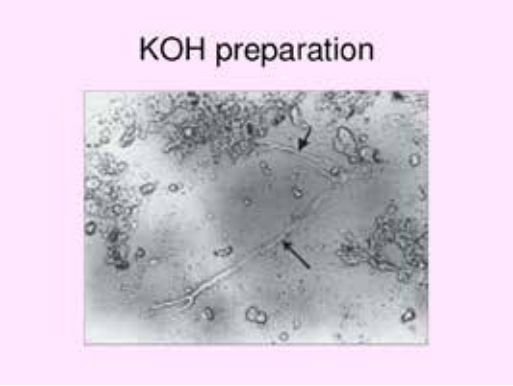
Lab diagnosis of fungal infections requires proper collection of specimen and timely transfer to the laboratory for recovery of fungi as sometimes contaminating bacteria may rapidly over grow. For superficial mycosis, the specimens can be skin scrapings, nail clippings and scraping from nail bed,

plucked hair, depending on the site of the lesion.

For systemic infections specimens are according to the organ involved, it may be biopsy, tissue, pus, urine, blood, cerebrospinal fluid (CSF) etc.

Direct Examination:

- Wet mount that is KOH (Potassium hydroxide, which dissolves the unwanted tissues sparing the fungus, thus improving the visibility of the fungus) preparation for characteristic appearance of fungal elements.
- Histopathology
- Newer techniques like fluorescent antibody staining



Fungal filaments in direct KOH preparation made with skin scrapings

Fungal culture: Fungi grow relatively slow and cultures should be retained for at least 4-6 weeks. Special media like Sabouraud's Dextrose Agar are needed to grow the fungi.

Interpretation of culture: For true pathogen, the isolation of fungi from any specimen is regarded as an evidence of infection. But opportunistic pathogenic fungi require repeated isolation and sometimes serological evidence also. Fungal culture is read in 2 parts as macroscopic and microscopic for identification.

Fungus grown on a Sabouraud's Dextros Agar plate

Antifungal susceptibility testing (AST): it provides information to the clinicians to select the appropriate antifungal agent for treatment. It is more important for the systemic mycosis which usually requires intra-venous (i.v) therapy.

Treatment: Superficial fungal infections can be treated by topical or oral antifungal agents which are usually available over the counter or on prescription eg dermatophytosis (duration can be long from 6-12 weeks for nail and hair infections). Systemic and subcutaneous infections require i.v therapy for required period usually according to the AST results. Common antifungal agents are azoles, amphotericin, terbinafin, griseofulvin and the newer echinocandins, each one of them have their own side effects and toxicity profiles.

Immunologically intact humans manifest robust defences against fungal diseases. Recent human social evolution has rendered a large population susceptible to infections with fungi. A concerted scientific and social effort is needed to meet these challenges.

Suggested Reading

1. Microbiology Society(2015). Fungal Diseases. Microbiology Today 43:1. www.microbiology.org/MTfungaldiseases.
2. Types of fungal infections /Fungal diseases/CDC. www.cdc.gov/fungal/diseases.

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PARASITIC DISEASES & THEIR DIAGNOSIS

Dr Padmaja Panjarathinam
Microbiology Unit, Department of Laboratory
Al Jahra Hospital

A parasite is an organism that lives in or on another organism known as its host. It is dependent on the host to live, grow and multiply. Parasites range in size from one-celled organisms called protozoa to worms that can be seen with naked eye.

Parasitic diseases are prevalent worldwide but are more common in tropical and sub-tropical regions. Environmental factors, overcrowding, poor sanitation and non-availability of safe drinking water contribute to the occurrence, distribution and transmission of parasitic diseases.

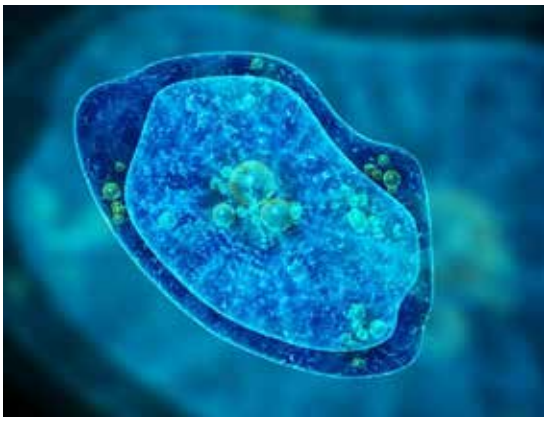
Food and water-borne parasitic diseases:

Numerous parasites can be transmitted through contaminated food and water. Some infections are transmitted through consumption of foods like under cooked meat, fish or raw vegetables. Symptoms of food-borne parasitic diseases vary greatly depending on the type of parasite.

Simple steps like hand washing, personal hygiene, washing of vegetables and fruits before consumption, avoiding under cooked meat or fish can prevent many of these infections.

PARASITE	**DISEASE CAUSED**
Entamoeba histolytica	Amoebiasis – dysentery, colitis, liver abscess
Giardia lamblia	Giardiasis – diarrhea, gas, abdominal cramps.
Ascaris lumbricoides (round worm)	Ascariasis – abdominal discomfort, intestinal obstruction.
Trichuris trichura (whip worm)	Trichuriasis – painful dysentery, rectal prolapse.
Enterobius vermicularis (pin worm)	Enterobiasis – perianal itching.
Echinococcus granulosus (dog tape worm)	Hydatid disease – cysts in liver, lungs, spleen, bone, kidneys, brain, eyes.
Taenia solium (pork tape worm)	Cysticercosis – Formation of tissue cysts in brain,muscle and other tissues.
Toxoplasma gondii	Toxoplasmosis – mild symptoms in immunocompetent adults, can affect the eyes (retinochoroiditis), infection acquired during pregnancy can cause congenital malformations

Diagnosis of most food-borne parasitic infections: This is mostly by microscopic examination of stool to demonstrate the various forms of parasites like trophozoites, cyst, larvae etc .



Entamoeba histolytica

Ascaris lumbricoides

Concentration of stool sample by sedimentation or floatation and the use of techniques like the saline/ iodine wet mount and special stains like Giemsa and modified Ziehl-Neelsen help in the identification of the parasite. Sometimes multiple samples may be needed as some parasites may be shed intermittently. In the case of enterobiasis a transparent adhesive tape is applied over the perianal region and subsequently examined under the microscope to identify the eggs which are stuck to the adhesive side of the tape. Serological tests to detect antibodies are used in the diagnosis of Toxoplasma, Echinococcus and Taenia solium. Histopathological examination of tissue lesions is diagnostic in Taenia solium and Echinococcus.

Vector-borne parasitic diseases:

Vectors are living organisms, usually blood sucking insects, which can transmit diseases between humans or from animals to humans. Mosquitoes are the best known disease vectors. Others include ticks, flies, sandflies, fleas, triatomine bugs and some fresh water aquatic snails. Malaria alone causes more than 400,000 deaths every year globally.

Parasite	**Vector**	**Disease caused**
Plasmodium Sp.	Anopheles Mosquito	Malaria
Wuchereria bancrofti, Brugia malayi	Culex, Anopheles, Aedes and Mansonia mosquitoes	Lymphatic filariasis
Leishmania donovani	Sand fly	Kala azar , Cutaneous Leishmaniasis
Trypanasoma brucei	Tsetse fly (Glossina)	African trypanosomiasis or sleeping sickness
Onchocerca volvulus	Black fly (Simulium)	African river blindness

Diagnosis of most vector-borne parasitic infections: Demonstration of the parasite in the peripheral blood smear is used in the diagnosis of malaria and filariasis. Histopathological examination is used in the diagnosis of leishmaniasis, trypanosomiasis and Onchocerca. Characteristic LD bodies in lymph nodes, liver, spleen, bonemarrow or skin specimens is diagnostic of Leishmania. The parasite can be demonstrated in lymph node aspirate in trypanosomiasis and in skin shavings in Onchocerciasis .

Parasitic infection through skin-penetration by parasitic larvae:

Parasite	Disease caused
Ankylostoma duodenale (Hook-worm)	Abdominal pain, diarrhea, anaemia.
Schistosoma haematobium/mansoni/ japonicum	Schistosomiasis – Acute and Chronic. Increased risk of bladder cancer.
Strongyloides stercoralis	Strongyloidiasis – Abdominal pain, diarrhea, skin rash.

Diagnosis: These parasites are diagnosed by detection of their ova on stool-examination. Schistosoma can also be detected in urine.

Conclusion:

Parasitic diseases are a major public health challenge faced by the world. Prompt diagnosis and treatment, surveillance and monitoring, implementation of appropriate preventive measures is essential for the control of these diseases.

Suggested Reading:

https://www.cdc.gov/parasites

http://www.who.int

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INTERPRETATION OF COMMON SEROLOGICAL TESTS

Dr Aneesa Ahmed Abdullah
Microbiology Unit, Department of Laboratory
Ibn Sina Hospital

Serological tests detect and measure the level of antibodies in blood as a result of exposure to foreign objects such as bacteria, viruses etc. These tests are done on a sample of serum (the clear liquid that separates from the blood when it is allowed to clot). An antibody (ab), also known as an immunoglobulin (Ig) is produced by B-cells to neutralize these foreign objects. When used correctly, serological tests play an important role in determining the immune status and in diagnosing acute infection. When the test is used as a tool to diagnose clinical disease, it is essential to test two samples, two weeks apart.

WIDAL TEST FOR THE DIAGNOSIS OF TYPHOID FEVER (ENTERIC FEVER)

The Widal test is done to detect *Salmonella* antibodies in patients with typhoid and paratyphoid fevers against the flagellar H antigen, somatic O antigen and capsular Vi antigen of *Salmonella typhi* and *Salmonella paratyphi A, B and C*. The antibodies develop at the end of the first week of fever and the titres keep rising till the 4th week, after which they slowly decrease. A single Widal test is not sufficient for diagnosis and at least two tests need to be done. O antigen titre of 100 or more and H antigen titre of 200 or more is diagnostic of active infection. A fourfold rise in the titre during the course of an infection, signifies active infection. Diagnosis of typhoid fever based on a positive Widal test alone is not recommended and hence isolation of *Salmonella* from blood, urine, stool and other body fluids by culture is confirmatory.

Reasons for a positive and negative Widal test are shown in table 1.

Table 1: Interpretation of Widal test

Widal Positive	Widal Negative
The patient has typhoid fever	Absence of infection by *S.typhi*
Previous immunisation with *Salmonella* antigen	The carrier state (individuals who excrete salmonella in stool even after treatment)
Cross-reaction with non-typhoidal *Salmonella*	Previous antibiotic treatment
Infection with malaria, dengue or other enterobacteriaceae	Widal test performed very early in the first week of fever

SYPHILIS SEROLOGY: VDRL AND TPHA

The etiological agent of syphilis is *Treponema pallidum* Antibodies become detectable at about 3-4 weeks following exposure and remain at detectable levels for long periods after treatment.

1) Non-treponemal antibody tests -RPR (Rapid Plasma Reagin) and VDRL (Venereal Desease Research Laboratory) detect antibodies that are not specifically directed against the *Treponema pallidum*. These tests are positive in syphilis and also in IV drug users, Lyme disease, malaria, tuberculosis etc.

2) Treponemal antibody tests - FTA-ABS (Flourescent treponemal antibody absorption) and TPHA (*T.pallidum* particle Haemagglutination assay) detect antibodies that are specific for *T.pallidum*. Non-treponemal antibodies typically disappear in an adequately treated person after about 3 years. Therefore, a positive treponemal screening result must be followed by a nontreponemal test (such as RPR) to differentiate between an active infection or reinfection and also one that occurred in the past and was successfully treated.

Table 2: Interpretation of serological tests for Syphilis

Treponemal tests (TPHA)	Non-treponemal test (VDRL)	Interpretation
Nonreactive	Nonreactive	Absence of syphilis or very early syphilis
Reactive	Nonreactive	Prior treated or untreated syphilis
Reactive	Reactive	Active syphilis
Nonreactive	Reactive	False positive non-treponemal test

BRUCELLOSIS

Brucellosis is caused by bacteria of the genus Brucella that affect humans and numerous animal species. CDC utilizes Brucella microagglutination test (BMAT), a modified version of the serum (tube) agglutination test (SAT), that can detect antibodies to Brucella. For appropriate diagnosis , two serum samples are required. The first serum sample should be taken within 7 days after onset of symptoms and the second should be drawn 2-4 weeks later to check for a rise in antibodies. A fourfold or greater rise in antibodies confirms brucellosis. Titres higher than 1:160 in conjunction with a compatible clinical presentation are considered highly suggestive of infection.

VARICELLA –ZOSTER INFECTION

Varicella –zoster virus (VZV) causes two distinct exanthematous (rash-associated) diseases: chickenpox (varicella) and shingles (herpes zoster). Serological screening for IgG-class antibodies to VZV will aid in identifying non-immune individuals. The presence of IgM class antibodies to VZV is suggestive of acute or recent infection. However results should be correlated with clinical presentation.

Serological screening for IgM and IgG class antibodies by Enzyme Immunoassays (EIA) to Varicella Zoster Virus (VZV) is shown in table 3.

Table 3: Interpretation of serological tests for Varicella zoster virus (VZV)

IgG IgM	+ +	Indicates acute or recent infection with VZV
IgG IgM	- +	Primary or reactivated infection
IgG IgM	+ -	Indicates previous vaccination with VZV. Protective immunity to reinfection
IgG IgM	- -	Indicates absence of prior exposure to VZV, Non immunity Negative results does not rule out a VZV infection. The specimen may have been drawn before appearance of antibodies therefore testing a new serum sample in 2 to 3 weeks should be done.

Suggested Reading

1. Lateef A, Aprileona L King. Widal agglutination test – 100 years later: still plagued by controversy. Postgrad Med J 2000;76:80-84.
2. 2015 Centers for disease control and prevention. Sexually Transmitted Diseases Treatment Guidelines.
3. Andres F, Steven C Johnson. Diagnostic tests for syphilis. Neurol clin pract. 2014apr;4(2):114-122.
4. Yankowitz J,Grose C. Congenital infections. In Essentials of Diagnostic Virology. New York 2000,pp 187-201.

I consider hospitals only as the entrance to scientif-ic medicine; they are the first field of observation which a physician enters; but the true sanctuary of medical science is a laboratory; only there can he seek explanations of life in the normal and pathologi-.cal states by means of experimental analysis

Claude Bernard —


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LABORATORY DIAGNOSIS OF BLOOD-BORNE VIRUSES

Dr Shama A. Shetty
Microbiologist Unit, Department of Laboratory
Ibn Sina Hospital

Blood-borne viruses (BBVs) are viruses that some people carry in their blood and can spread the virus from one person to another by blood and body fluids (vaginal secretions, semen and breast milk). The BBVs of concern are the Human immunodeficiency virus (HIV) – a virus which causes acquired immunodeficiency syndrome (AIDS) affecting the body's immune system and hepatitis B (HBV) and hepatitis C, affecting the liver.

Human immunodeficiency viruses (HIV-1 and HIV-2)

These viruses attacks the body's immune system, specifically the CD4 cells (T cells) , which help the immune system fight off infections. Untreated HIV reduces the number of CD4 cells in the body, making the person more likely to get other infections or infection related cancers. Over time, HIV can destroy so many of these cells that the body cannot fight the infections and the disease. Recommended laboratory testing for HIV is shown in table 1.

Laboratory Diagnosis of HIV

Test	Detects	Interpretation
Screening test: HIV-1/2 Antigen Antibody combination immunoassay	HIV-1 p24 Antigen and HIV-1and HIV-2 antibodies	**Non-reactive** no evidence of HIV infection. **Reactive** Do further diagnostic test after this test
Diagnostic test: HIV-1/HIV-2 Antibody differentiation immunoassay	HIV-1 and HIV-2 Antibodies	**Reactive** HIV-1 (+) HIV-2(+) = antibodies detected **Non-reactive** HIV-1 (-) and HIV-2(-) further confirmation required with confirmatory test
Confirmatory test: HIV-1 nucleic acid test(NAT)	RNA (Genetic material)	NAT (+) Acute HIV-1 NAT (-) Negative for HIV-1

Hepatitis B virus (HBV) causes liver infection which may lead to death due to cirrhosis and liver cancer. Laboratory diagnosis of hepatitis B involves measurement of several HBV specific antigens and antibodies. Serological tests are used to distinguish acute, self-limited infections from chronic HBV infections and to monitor vaccine-induced immunity. Hepatitis B vaccines are safe and upto 95% of vaccinated individuals form effective antibodies when they get the vaccine and are protected from hepatitis B. This vaccine is given in 3 doses. For persons who have been vaccinated, but with anti-HBs level <10m IU/ml, a booster dose is recommended. Hepatitis B serological test results interpretation is shown in Table 2.

Interpretation of different tests for HBV infection

	Test 1	Test 2	Test 3
Interpretation and recommmendation	HBsAg (surface Antigen)	HBsAb (ant-Hbs) (surface antibodies)	HbcAb (anti-Hbc) (Antibodies to core antigen)
Not immune Has not been infected, but is still at risk for future infection. **Needs the vaccine**	-	-	-
Immune Surface antibodies present due to infection. Recovered from prior hepatitis B infection. Cannot infect others **Vaccine not needed**	-	+	+
Immune May have been already vaccinated. Cannot infect others. **Vaccine not needed**	-	+	-
Hepatitis B infection Means hepatitis B virus is present. Can spread the virus to others	+	-	+
Unclear May be – recovering from acute infection/ having undetectable level of HBsAg with chronic infection/ immune with undetectable levels of HBsAb	-	-	+

Hepatitis C virus (HCV)

Hepatitis C, a common chronic blood borne infection is associated with serious morbidity and mortality. Every chronic hepatitis C infection starts with an acute phase. Acute hepatitis C usually goes undiagnosed because it rarely causes symptoms. When signs and symptoms are present they may include jaundice, along with fatigue, nausea, fever and muscle aches. Acute symptoms appear one to three

months after exposure to the virus and last for two weeks to three months. Long–term infection with HCV is known as chronic hepatitis C. Chronic hepatitis C is usually a "silent" infection for many years, until the virus damages the liver enough to cause the signs and symptoms of liver disease.

Interpretation of HCV diagnostic test results

Test outcome	Interpretation	Further actions
HCV antibody nonreactive	No HCV antibody detected	Sample can be reported as nonreactive for HCV antibody.no action required. if recent exposure in person tested is suspected, test for HCV RNA.
HCV antibody reactive	Presumptive HCV infection	Repeatedly reactive result indicates current infection or past infection which has resolved, or biologic false positivity. Hence test for HCV RNA
HCV antibody reactive,HCV RNA detected	Current HCV infection	Provide person tested with appropriate counselling and link person tested to care and treatment
HCV antibody reactive HCV RNA not detected	No current HCV infection	No further action required in most cases Test with another antibody assay and follow up with HCV RNA testing

Ebola Virus

Ebola is a severe and deadly disease caused by a virus. Symptoms include fever, diarrhea, vomiting, bleeding, and often death. Ebola can occur in humans and other primates (gorillas, monkeys and chimpanzees). It can only spread between humans by direct contact with infected body fluids and can enter the body through a break in the skin or mucous membranes. The bodies of the deceased people are also infectious. Laboratory tests used in diagnosis are shown in the following table. Diagnostic testing for Ebola virus infection

Timeline of infection	Diagnostic tests available
Within a few days after symptoms begin	Antigen capture enzyme linked immunosorbent assay(ELISA) IgM ELISA Polymerase chain reaction(PCR) Virus isolation
Later in disease course or after recovery	IgM and IgG antibodies
Retrospectively in deceased patients	Immunohistochemistry testing PCR Virus isolation

Further Reading

1. Centers for Disease Control and Prevention. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. 2014.
2. Centers for Disease control and Prevention. Viral Hepatitis B Testing and Public Health Management.
3. Centers for Disease Control and Prevention. Testing for HCV infection. An update of guidance for clinicians and laboratorians. MMWR 2013:62(18).
4. Centers for Disease Control and Prevention. www.cdc.gov/vhf/ebola/diagnosis/index.2014

"We ran your symptoms through the computer and it caused a virus that shut down the internet!"

MOSQUITO- BORNE VIRAL INFECTIONS OF CURRENT PUBLIC HEALTH IMPORTANCE

Dr Deepthi Nair
Microbiology Unit, Department of Laboratory
Ibn Sina Hospital

Mosquito borne viral infections that have gained recent public attention are dengue, chikungunya (CKV), Japanese B encephalitis (JBE) and Zika virus. Recent years have witnessed a global rise in these infections which are transmitted by mosquitoes, Aedes mosquito causing dengue, chikungunya and zika, while Culex causes Japanese B encephalitis. Awareness about these infections is of paramount importance because there are regular seasonal epidemics, especially in tropical countries, in post rainy season, affecting thousands of persons, with high rate of mortalities and deformities. In some cases, these infections give rise to no symptoms while in others the patient suffers from extreme fatigue, pain and ill health that can extend over days and months. In yet other cases, the patient may develop complications that may lead onto death.

Aedes aegypti mosquito

Culex mosquito

General features of different mosquito-borne viral infections

	DENGUE	**CKV**	**JBE**	**ZIKA**
GEOGRAPHICAL DISTRIBUTION	World wide –Asia, Africa, America. Recent epidemic in Vietnam in August 2017.	World wide – Asia, Africa, America Massive outbreak in India in 2016	South east Asia, Western Pacific	Africa, America, South east Asia Epidemic in Brazil in 2015
SYMPTOMS	Fever, rash, muscle pain, joint pain	Fever, rash, muscle pain, joint pain	Mostly without symptoms. Vomiting, neck pain, difficulty in looking towards light if brain affected	Fever, rash, headache, vomiting, joint pain

SPECIAL FEATURES	There may be bleeding from nostrils, gums, gastrointestinal and urinary tracts	Stooping posture	Involvement of the brain (encephalitis)	If pregnant mothers are infected, there may be abortion or the baby may be born with a small head circumference (microcephaly)

Laboratory Diagnosis of Dengue, Chikungunya, JBE and Zika virus infections

1. Detection of virus specific nucleic acid in serum, plasma, blood, cerebrospinal fluid (CSF) or tissue by real time reverse transcriptase PCR (polymerase chain reaction).
2. Detection of virus specific IgM antibody (antibodies are produced in response to an infection) in a single sample of CSF or serum by MAC –ELISA (IgM Capture ELISA)
3. Detection of virus antigen (viral component, presence of which indicates presence of the virus) in tissue by immunohistochemistry
4. Isolation of virus from serum, plasma, blood or tissue in cell culture
5. A four- fold or greater rise in virus specific antibody as measured by haemagglutination inhibition (HI) or plaque reduction neutralisation assay (PRNT) in acute and convalescent phase serum samples. The samples should be collected 14 days apart.
6. In cases where the brain is affected as in JE encephalitis, a lumbar puncture (the procedure of drawing cerebrospinal fluid from the spinal cord by inserting a needle) is done. The fluid is then sent to the laboratory for haematological, biochemical and microbiological analysis. In cases of encephalitis or brain involvement, the cerebrospinal fluid (CSF) pressure may be high, CSF protein may be high and CSF glucose often normal.

Laboratory tests used in regular practice

TEST	**DENGUE**	**CKV**	**JBE**	**ZIKA**
RT-PCR (good results if used in the 1st 5-7 days of illness onset)	✓	✓	Likelihood of a positive result is less since quantity of virus in blood is usually low	✓
MAC-ELISA (IgM antibody detection) –test of choice if onset of symptoms >/=7 days	✓ PRNT to confirm	✓ PRNT to confirm	MAC-ELISA is the standard diagnostic test PRNT to confirm	✓ PRNT to confirm

Demonstration of 4-fold rise in IgG antibody titer between samples taken 14 days apart ((HI or PRNT	Helps to differentiate between a primary and a secondary dengue infection	✓	✓	✓
NS1 antigen test by ELISA	✓	Not applicable	Not applicable	Not applicable

Interpretation of PCR test result

- PCR positive - evidence of current infection
- PCR negative – does not always suggest absence of infection. Because a test with lower sensitivity may miss a low number of copies of viral nucleic acid. If PCR is negative in the 1ST 5-7 days of illness, a second serum sample has to be submitted for MAC-ELISA.

Interpretation of serology results

IgM	IgG	Possible interpretation
Positive	Negative	Current Infection
Positive	Positive	Current Infection
Low or negative or not tested	fold increase in sample taken 2-4 weeks apart	Recent infection
Low or negative	positive	Past infection
negative	negative	Too soon after initial exposure for antibodies to develop or symptoms due to another cause

Mosquito borne infections cause a lot of suffering to those affected. Treatment in most cases is only supportive eg, rest, paracetamol for fever and intravenous fluids for dehydration. Some cases require blood and platelet transfusion. Complications leading to infection of brain (encephalitis) or multi organ failure may lead to death or survival of patient with serious sequelae like intellectual disability.

Prevention of such mosquito borne infections is of paramount importance. This can be achieved by using mosquito nets, insecticides like DEET, applying mosquito repellent body creams, wearing full sleeve tops and full length pants and avoiding travel to areas infested with these mosquitoes. In unavoidable travel to these areas, it is advised to be immunised by vaccines for infections against which the vaccines are available. In Kuwait, however, most of these infections are found to be imported.

Suggested reading

1.www.who.int/mediacentre/factsheet/zika/en/

2.www.who.int/rpc/guidelines/9789241547871/

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PRENATAL SCREENING FOR INFECTIOUS DISEASES: WHY, WHEN, WHAT AND HOW?

Dr Seema Shahedkhan Pathan
Microbiology Unit, Department of Laboratory
Maternity Hospital

Why are these tests performed ?

Mother can pass the Infections to the baby during pregnancy, at the time of delivery, or after delivery, which may lead to fetal death, or a newborn with infections and severe malformations.
These tests enable early detection and treatment of infections during pregnancy so as to improve mother's health and significantly reduce the risk of mother- to-child transmission.Thus, the screening tests help in decisions regarding the specific therapy during pregnancy/labor, and after labor, breast feeding, method of delivery (Caesarian or normal), or abortion (if the baby is to be born with severe malformations).

When are the Screening tests done ?

Prenatal screening tests are usually offered during the first prenatal visit.

Which are the tests performed ?

1. The tests includes the TORCH panel which consists of screening for Toxoplasmosis, Other infections such as Parvovirus (b19), screening for Rubella,Cytomegalovirus (CMV) and Herpes Simplex virus 2 (HSV).
2. Screening for Hepatitis B, Syphilis, HIV, Varicella (chicken pox) is also performed.
3. It also includes screening for group B *Streptococcus* infection from the rectovaginal swab, detection of Chlamydia trachomatis and Nesseriae gonorrhoeae in cervical samples and diagnosis of asymptomatic bacteriuria from urine sample.

TORCH Screening: Blood sample is taken to detect IgG and IgM antibodies. Avidity tests are performed to find out the timing of maternal infections in certain diseases like toxoplasmosis and Rubella. For toxoplasmosis confirmation of recent infection in pregnant women cannot be easily performed and a combination of serologic tests is required, that are done in reference laboratories.

Hepatitis B: Screening in pregnancy is done by serological detection of HBs Ag which will reveal whether you are a Hepatitis B carrier. Detection of HBe Ag, which is associated with high infectivity, can also be done.

Syphilis: It is a sexually transmitted disease. The tests performed are known as RPR or VDRL. A positive test should be confirmed by other specific tests.

HIV testing: It is the virus which causes AIDS. Screening is performed by taking blood for detection of antibodies. Test for direct detection of HIV –RNA can be done in some situations. When urgent results are needed, certain rapid tests can be done.

Varicella: Chicken pox is caused by varicella Zoster virus and it can cause congenital varicella syndrome in babies. If you never had chicken pox, if you have never been vaccinated and you think you are exposed during pregnancy than you should tell your doctor and get tested for the same.

Group B Streptococcus screening: It is performed by taking a swab from the vaginal and rectum at 35-37 weeks of gestation

C. trachomatis and N. gonorrhoeae: Pregnant women are tested forthese organisms by performing a polymerase chain reaction (PCR) to detect the nucleic acid, samples taken from inside the neck of the uterus (endocervical samples).

Asymptomatic bacteriuria: Urinary tract infection comprises the most common infection during pregnancy. The test done is urine culture on a midstream clean catch urine sample. Isolation of single organism in a count of 10 CFU/ml of urine sample is considered as infection.

Recommendations for screening of infectious disease during pregnancy

Condition	Screening when	Test done	Interpretation
Toxoplasmosis	First prenatal visit	Enzyme immunoassays for IgG and IgM. Avidity test and a panel of tests for detecting recent infection for those with IgM positive in the first time	IgG +ve in previously -ve: **+ve test** *IgG and IgM +ve: **recent infection** IgM +ve & low IgG-avidity: **primary infection**
Parvovirus B 19	First prenatal visit	Enzyme immunoassays for IgG and IgM	IgG +ve in previously –ve, or +ve for both IgG and IgM: **recent infection** IgG +ve in a previously +ve case: **the woman is immune to Parvovirus**
Rubella	First prenatal visit	Enzyme immunoassays for IgG and IgM Avidity test for detecting recent infection for those with IgM positive in the first time	IgG +ve: **she is immune and no need for vaccination** IgG & IgM +ve: **recent infection** IgM Positive and low IgG-avidity: **primary infection**
Cytomegalovirus	Routine screening not recommended. Selective screening for women with high risk factors.	Enzyme immunoassays for IgG and IgM CMV IgG Avidity CMV PCR in doubtful cases	IgG +ve in previously -ve: **CMV-infection** IgG & IgM +ve: **recent infection** IgM +ve & low IgG-avidity: **primary infection**

Herpes Simlex virus	First prenatal visit	Enzyme immunoassays for IgG and IgM	IgG +ve in previously -ve: **infection with HSV** IgG & IgM +ve: **recent infection**
Hepatitis B	First prenatal visit Selective screening for women with high risk factors before delivery.	HBsAg serology HBeAg serology	HBsAg +ve: **chronic infection or a carrier** HBeAg +ve: **high infectivity**
Syphillis	First prenatal visit and before delivery	RPR or VDRL TPHA or FTA-ABS for confirmation	RPR or VDRL positive confirmed by performing TPHA. VDRL is repeated after 3-4 weeks to see the effect of treatment. **Once +ve, TPHA remains +ve for life**
HIV	First prenatal visit and before delivery	Conventional or rapid Enzyme immunoassays Confirmation by Western Blot	Antibody test +ve: **HIV+ve** Confirmed by further tests
Varicella	Only if exposed during pregnancy with no past history of chicken pox	Enzyme immunoassays for IgG and IgM	IgG +ve: **she is immune and no need for vaccination** IgG & IgM +ve: **recent infection**
GroupB Streptococcus screening(GBS)	At 35-37 weeks of gestation	Rectovaginal swab for culture of GBS	GBS isolated indicates GBS colonization, which will require prophylaxis during delivery
Chlamydia trachomatis Nesseriae gonorrhoeae	Selective screening for women with high risk factors at first prenatal visit.	Nucleic acid amplification test (NAATs) in urine and vaginal secretions.	Any positive case must receive antibiotic treatment
Asymptomatic bacteriuria	At 12-16 weeks of gestation or at first prenatal visit.	Urine culture of midstream clean catch urine sample	Single organism in a count of 10 CFU/ ml of urine sample is considered as infection

*IgM Toxoplasma antibodies may persist for years after the acute infection. Hence additional tests are recommended. These tests are performed in reference and research laboratories.

What does the test result mean?

The interpretation of the results is very important. Once you have the results you should talk to your doctor about what the results mean and what you should do.

1. Results are usually reported as positive (or reactive), or negative (or non-reactive), indicating the presence or absence of antibodies for each of the infections tested, respectively. If IgG and IgM are negative the test result is considered normal.
2. A positive test result means antibodies were found in the screening .The presence of IgG antibodies and IgM negative usually indicates a past infection. Typically, a second blood test is done four weeks later so that the antibody levels can be compared. If levels increase, it means the infection was recent.
3. IgM positive and IgG negative means it is an acute and recent infection. False positive results can occur. Hence the test is repeated with a second sample of blood.
4. Any positive antibody results should be confirmed with additional specific tests.
5. The timing of maternal infection can be found by avidity testing for the particular infection. A high IgG-avidity during the first trimester would mean an infection more than three months old, and indicates a low risk of transmission to the baby during pregnancy. A low avidity would mean an infection within 3 months.

Further Reading

Mandell, Douglas, and Bennett's principals and practice of infectious diseases, eighth edition volume 2

CLINICAL MICROBIOLOGIST'S PERSPECTIVE OF MOLECULAR DIAGNOSTICS AS APPLIED TO PATIENT CARE: A CULTURE SHOCK !

Dr Rita Dhar
Microbiology Unit, Department of Laboratory
Al Farwania Hospital

Introduction

The conventional microbiological tests like culture and serology may be labor-intensive and time-consuming resulting in delayed reporting of the results. The high burden of infections like tuberculosis (TB), malaria, Human Immunodeficiency Virus (HIV) etc. on the third world countries could partly be explained by the absence of or inaccurate laboratory diagnostic testing systems. With the advent of molecular detection methods it has not only become possible to detect and characterize viral infections but also diagnose diseases due to fastidious bacteria.

The Basic Methodology

Nucleic acid amplification testing (NAAT) has brought a revolution in conventional clinical microbiology laboratory methods. It involves the extraction of the genetic material (Deoxy ribonucleic acid, DNA or Ribonucleic acid, RNA) from the cells of infective microorganisms. These carry specific genes with specific characters like the organism's identity, its resistance to drugs etc. With certain chemicals, the desired genes are multiplied in the laboratory, thus making their detection easy, fast and accurate. The molecular methods involve Polymerase Chain Reaction (PCR) technology, gene sequencing, pyrosequencing, reverse hybridization, mass spectrophotometry and microarray analysis, which aid in specific diagnosis and deciding on the specific treatment options and management of infectious diseases.

Detection of Causative Organism(s)

Bacteriology: Many slow growing &/or fastidious (difficult to grow) bacteria such as *Mycobacterium tuberculosis* (causing TB), *Chlamydia trachomatis, Neisseria gonorrheae* (causing sexually transmitted infection) and *Bordetella pertussis* (causing pertussis) have public health implications, hence early recognition & treatment are of great importance. Mycobacteriology calls for special mention as molecular detection of *M.tuberculosis* allows confirmation of diagnosis with up to 98% sensitivity within a day, compared to two weeks or more by culture. For detection of B. pertussis (causative organism of pertussis) early in the disease PCR has replaced direct fluorescent –antibody and culture as the "gold standard method". Other fastidious respiratory pathogens that can be rapidly diagnosed by molecular methods include *Legionella spp.*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. *Tropheryma whipplei* can only be detected by molecular means as culture is not possible in a routine microbiology laboratory. Other examples where molecular diagnosis can prove helpful include cat scratch disease due to *Bartonella henselae*, Q fever due to *Coxella burnetii*, and male urethritis due to *Mycoplasma genitalium*. Some serious infections such as meningitis or septic shock require early diagnosis for prompt appropriate antibiotic therapy as well as early provision of chemoprophylaxis for

close contacts. Rapid detection of common causes of meningitis or sepsis is now possible by using multiplex PCR method.

Virology: The diagnosis of viral infections suffers from cumbersome culture methods, and detection of antibodies (serology) being inaccurate for many infections, and in early stages. However, PCR technology is now being used to improve the detection of viruses especially Herpes simplex, Hepatitis C, Cytomegalovirus (CMV), HIV, rubella and Varicella-zoster. PCR detection has been helpful as it can rapidly screen for many respiratory viruses including swine influenza (H1N1), avian influenza (H5N1) and SARS-CoV, and prevent major outbreaks. Such an early detection may prove to be cost-effective due to avoidance of unnecessary hospitalization, testing, procedures & unjustified antibiotic usage. PCR has also been used to diagnose viral diarrheal diseases caused by rotavirus, norovirus, astrovirus and enteric adenovisuses.

Mycology & Parasitology: Many fungi and parasites may be missed by conventional methods. Molecular methods have been a great help in differentiation of different fungal & parasitic species like *Candida spp., Aspergillus spp., Pneumocystis jiroveci* and *Plasmodium spp.* (malaria parasites) thus affecting the treatment of these infection significantly.

Detection of Antibiotic Resistance: Applying rapid and reliable molecular tools for a definite identification of resistance genes can have helpful infection control and management implications. It is of great potential benefit to detect infection or colonization with methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum-beta lactamase ESBL)-positive Enterobacteriaceae and multi-drug resistant TB in order to take action to monitor and control the spread. It overcomes the problem of variability in conventional tests. Resistance to antiviral agents like oseltamivir (Tamiflu®, against influenza virus), gancyclovir (against CMV) and various antifungals can also be detected.

Treatment Monitoring: Viral DNA or RNA loads, are monitored by branched chain DNA signal amplification or more recently, real-time PCR. This is an integral component of the management of HIV, HCV, HBV and CMV infections. It not only helps to assess patients regarding need for antiviral therapy but also to monitor their effectiveness.

Molecular Epidemiology: Recently, the whole genome sequencing has been started to discover new pathogens, and also to look for the source of infection outbreaks. It is by far the most discriminatory and rapid method.

Limitations of molecular methods

Apart from the high costs, false positive results due to environment or inter-sample contamination, or presence of dead organisms' DNA or RNA are a possibility. False negative results may be due to difficult DNA extraction in certain organisms.

Further reading

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ROLE OF HEMATOLOGY LABORATORY : AN INTRODUCTION

Dr B Dash
Hematology Unit, Department of Laboratory
Maternity Hospital

Blood is a red colored fluid present in our body. It is composed of cells and plasma. The cells constitute 45% and plasma 55% of the whole blood. On an average blood is about 7% of body weight and average adult has 5 liters of blood. The blood cells consist of red blood cells [RBC], white blood cells [WBC] and platelets. The blood cells are produced after birth in the bone marrow from cells known as stem cells.

The red blood cell is responsible for delivery of oxygen to tissue and carrying of carbon dioxide to the lungs. The white blood cells consist of neutrophils, eosinophils, basophils also called polymorphonuclear leukocytes and mononuclear leukocytes comprising of monocytes and lymphocytes. The white blood cells are critical in detection and elimination of foreign substances in the body including infection causing microorganism. The platelets are the smallest of the blood cells and play critical role in prevention of bleeding. The cells can increase or decrease in variety of disease including infections, inflammation and malignancies as we will see in next chapters.

Examination of blood

Blood is the most frequent body fluid used for analysis. The blood sample is usually drawn from a vein, in special circumstances sample may be drawn by skin puncture or from an artery. The blood drawn is collected in a tube containing anticoagulant. Blood drawn from skin puncture may be used directly for analysis. Some of the common tests done are briefly discussed below

The most common blood test done is called complete blood count (CBC). It is used to evaluate overall health and detect disorders like anemia, infection and leukemia. Automated blood cell analyzers have replaced manual blood testing.

Interpretation of complete blood counts results is done keeping in mind the normal range and correlated with the clinical condition.

Results below and above the normal range may indicate a problem:

Hemoglobin, hematocrit and red blood cell count: A lower than normal result indicates anemia. A higher than normal range indicates erythrocytosis and requires further testing.

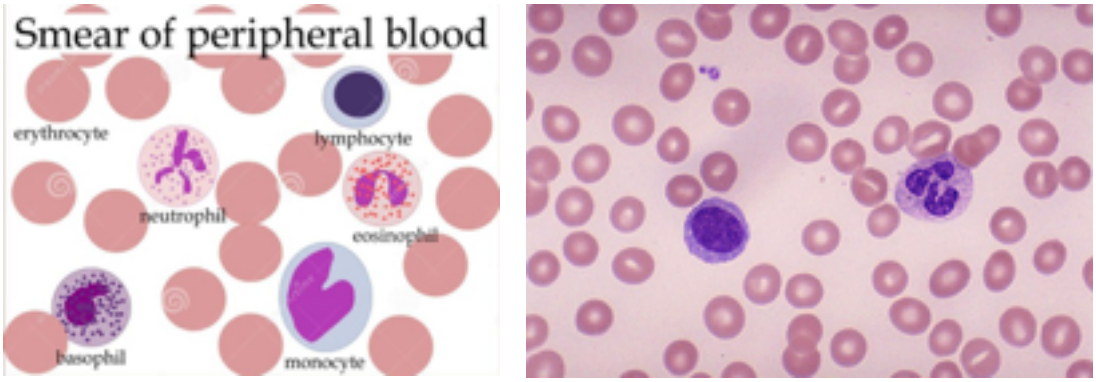
White blood cells count: A low count (leukopenia) may be due to infection, bone marrow disease or medication. A higher than normal count leukocytosis may be secondary to infection, inflammation or bone marrow disease.

Platelet count: A decreased platelet count also called as thrombocytopenia or higher than normal count termed as thrombocytosis is due to an underlying medical problem and need further testing

The normal ranges are as under:

Full Blood Count			
Haemoglobin	Adult male Adult female	130 - 180 115 - 165	g/L g/L
Total White Cell Count	Adult	3.6 - 11.0	$x10^9$/L
Differential count: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	 Adult Adult Adult Adult Adult	 1.8 - 7.5 1.0 - 4.0 0.2 - 0.8 0.1 - 0.4 0.02 - 0.1	 $x10^9$/L $x10^9$/L $x10^9$/L $x10^9$/L $x10^9$/L
Platelet Count	Adult	140 - 400	$x10^9$/L
Red Cell Count	Adult male Adult female	4.50 - 6.50 3.80 - 5.80	$x10^{12}$/L $x10^{12}$/L
Haematocrit	Adult male Adult female	0.40 - 0.54 0.37 - 0.47	L/L L/L
Mean Cell Volume (MCV)	Adult	80 - 100	fL
Mean Cell Haemoglobin (MCH)	Adult	27 - 32	pg
Reticulocyte Count	Adult / Children Full term infant	0.2 - 2.0 2.0 - 6.0	% %

Blood film examination is required in case of an abnormal CBC for diagnosing type of anemia, detecting abnormal cells or parasites like malaria.



Peripheral blood smear (as seen under microsope)

Special tests like bone marrow aspiration/ biopsy along with checking nature of cells by immunological testing (Flow Cytometry) and genetics testing like chromosomal analysis (karyotyping) and molecular testing are done to diagnose malignant blood disorders.

Erythrocyte sedimentation rate (ESR): This test detects rate of settling down of red blood cells. A faster rate indicates an underlying inflammatory process and is usually done during evaluation of unexplained fever, arthritis or muscle disease. No special preparation including fasting is required for the test.

Coagulation tests: The human body maintains a delicate balance between prevention of bleeding and excessive clotting. Coagulation tests measure ability to clot and time taken to clot. These tests are done to assess risk of excessive bleeding or developing of clots and include condition like hemophilia, where there is inability to clot or thrombophilia where there is tendency to excessive clotting and liver diseases. The tests included are prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), clotting factor assays, platelet count and function tests and thrombophilia testing.

Tests for hemoglobinopathy and thalassemia are done in newborn or patients suspected to have these genetically transmitted disease. The tests include complete blood counts, peripheral smear examination, high performance liquid chromatography (HPLC), hemoglobin electrophoresis.

Blood grouping and compatibility testing are done in blood bank laboratories for safe blood and blood product transfusion.

In conclusion, hematology laboratory plays a critical role in diagnosing and monitoring of blood and systemic disease.

BLOOD: WHEN IT IS TOO LITTLE?
PART I: INHERITED ANEMIAS

Dr Arun Gupta
Hematology unit, Department of Laboratory
Mubarak Al Kabeer Hospital

Anemia is reduced hemoglobin below normal limits for age and sex viz <13 g/dl in adult males and <12 g/dl for adult females. The three main causes of anemia are excessive blood loss, increased red cell destruction and decreased red cell production.

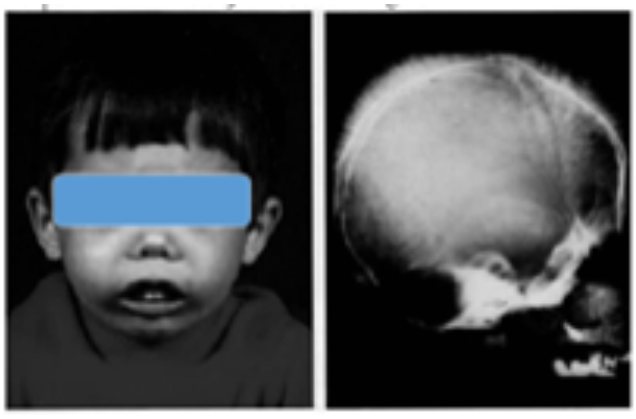
Normal red cells have an average life span of 120 days. Anemia produced by increased red cell destruction and reduced life span is called hemolytic anemia. It may be due to a defect in the red cell (intrinsic defect) or outside the red cell (extrinsic defect). The cause may be hereditary or acquired.

In inherited hemolytic anemia, there is intrinsic defect in red cells. It may be in the red cell membrane like in hereditary spherocytosis or in the metabolism as in enzyme G6PD deficiency or in the hemoglobin as in thalassemia or sickle cell disease.

What is Thalassemia?

Hemoglobin in the red cells consists of haem which carries oxygen and two proteins, alpha and beta globin chain. In thalassemia there is deficient production of alpha or beta globin chain which results in defective red cell production, reduced red cell lifespan and reduced oxygen carrying capacity. Depending on the deficient globin chain, the condition is called as alpha or beta thalassemia. Alpha thalassemia is caused by decreased or absent production of alpha chain. There are normally four genes for alpha chain and the number of genes absent decide the severity of the disease. Absence of all four genes is incompatible with life and results in stillbirth. Absence of three genes produces moderately severe anemia and absence of one or two genes results in red cell changes without anemia (thalassemia trait).

Beta thalassemia is caused by defects in the beta globin chain gene. If both parents carry thalassemia trait gene, one out of four offspring can have severe transfusion dependent disease called as thalassemia major. In some cases the clinical manifestation are less severe and is called as thalassemia intermedia. If only one defective gene is inherited, the person may have no manifestation or may be mildly anemic and this condition is called thalassemia trait/minor.



Thalassemia Major - Bone changes

What are the symptoms and signs of thalassemia?

Thalassemia major presents with

- Severe anemia at 3 to 6 months after birth.
- Liver and spleen enlargement.
- Expansion of bones.
- Osteoporosis/ fractures.
- Recurrent infection.
- Thalassemia minor/trait is however, asymptomatic.

How is thalassemia diagnosed?

Thalassemia is diagnosed by blood tests.

1. Complete blood count and blood smear examination. This test shows low hemoglobin, decreased MCV (small sized RBC) and smear under microscope shows small and abnormal shaped red cells and immature red cells.
2. Special tests like high performance liquid chromatography (HPLC) or hemoglobin electrophoresis show absent normal adult hemoglobin and presence of fetal hemoglobin in thalassemia major. In thalassemia trait/minor hemoglobin A2 is increased.

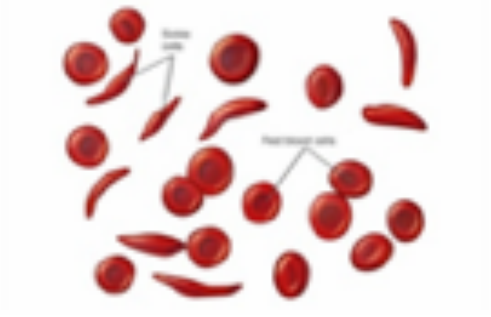
What is sickle cell disease?

- Sickle cell disease is produced by inheritance of sickle globin gene. If the gene is inherited from both parents it produces severe disease (homozygous disease). However inheritance of two different genes like for sickle globin gene and beta thalassemia gene or hemoglobin C gene also causes sickling disease.
- Sickle cell disease is characterized by severe anemia and recurrent blocking of small blood vessels causing severe pain in bones, paralysis (stroke) etc or swelling of hand and feet. Episodes of severe hemolysis/bone marrow failure and irreversible red cell changes (sickling) and clogging of blood vessels called crisis. If only one gene is inherited it produces milder disease called as sickle cell trait. It is a benign condition, however precautions are necessary when these patients are pregnant, receive anesthesia or travel to hot places.

How is sickle cell disease diagnosed?

Sickle cell disease is diagnosed by blood tests:

1. Complete blood counts and blood film examination reveal anemia (hemoglobin 6-9 g/dl), sickle cells and target cells.
2. Screening tests to detect sickling.
3. High performance liquid chromatography (HPLC) or hemoglobin electrophoresis to detect presence of abnormal hemoglobin SS, absence of normal hemoglobin A and presence of hemoglobin F.



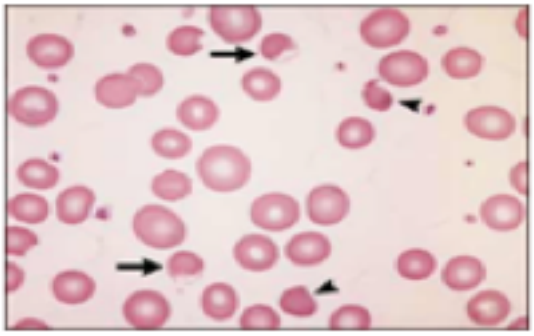
Blood film: Sickle and normal red blood cells

Is it possible to predict or diagnose inherited hemoglobin disease in fetus?

It is important to give genetic counselling to couples at risk of having a baby with major hemoglobin defects. If one partner is found to have hemoglobin abnormality the other partner should be tested and if both have a defect there is a risk of serious defect in the offspring particularly beta thalassemia major. It is important to have antenatal diagnosis by taking a sample from the fetus or chorionic villus and subjecting them to genetic testing to detect the disease.

What is G6PD deficiency? Why is it important?

Glucose 6 phosphate dehydrogenase (G6PD) is an important enzyme to avoid damage to red cell and ensure normal red cell lifespan. G6PD deficiency is sex linked, affecting males and is transmitted by females. G6PD deficiency is asymptomatic however acute hemolytic anemia occurs on exposure to certain drugs, infections or fava bean. It causes neonatal jaundice and rarely chronic anemia.



Blood Film: Damaged cells in G6PD deficiency

The diagnosis of G6PD deficiency is done by screening tests and confirmed by red cell G6PD enzyme assay. The patients have normal counts between hemolytic episodes. During hemolytic crisis, there is increased bilirubin, decreased hemoglobin and blood film may show abnormal red cells and fragments and increased reticulocytes.

Patients with G6PD deficiency should avoid drugs like antimalarials, sulphas, certain antibacterial agents and fava beans.

What is hereditary spherocytosis?

Hereditary spherocytosis is an inherited disorder in which red cell membrane protein are defective resulting in reduced red cell survival. The patients have variable anemia, jaundice, spleen enlargement and may have gallstones. Complete blood counts, blood smear examination and special tests like osmotic fragility or fluorescent flow analysis are required for diagnosis.

In conclusion, thalassemia, sickle cell disease and G6PD deficiency are the most important inherited anemias. Anemias due to bone marrow failure secondary to genetic mutation can also cause inherited anemia especially in children; these are rare disorder.

BLOOD : WHEN IT IS TOO LITTLE ?
PART II : ACQUIRED ANEMIAS

Dr K. Anitha
Department of Hematology
Kuwait Cancer Control Center

Anemia is the most common of all blood disorders. It can be inherited or acquired. "Acquired" means you aren't born with the condition, but you develop it. "Inherited" means your parents passed the gene for the condition on to you.

What is anemia?

Anemia is simply defined as insufficient hemoglobin to carry out oxygen requirement by tissues.
WHO Cut off Criteria for hemoglobin in Venous Blood

- Adult Man - 13gm/dl
- Adult Woman - 12gm/dl (non pregnant)
- Adult Woman - 11gm/dl (pregnant)
- Child 6-12 yrs of age - 12gm/dl
- Child<6 yrs of age - 11gm/dl

Definition of anemia in pregnancy:

- WHO (World Health Organization) Hb Conc.< 11gm%
- CDC (Centre for Disease Control) Hb Conc. 11 gm % in 1st and 3rd trimester of pregnancy and Hb Conc.<10.5 gm %in 2nd trimester.
- For developing countries: cut off level of Hb Conc. is 10 gm %

Anemia is mainly caused by

1. Blood Loss
2. Lack of red blood cell production
3. High rates of red blood cell destruction

1.Blood Loss:

Common cause of blood loss include

- Heavy menstrual periods
- Bleeding in digestive tract or urinary tract
- Major surgeries
- Trauma due to accidents

2. Lack of red blood cell production:

Acquired causes include

- Poor diet(deficient in Iron/Vitamin B12/Folate)
- Abnormal hormone levels(lack of erythropoietin)
- Chronic diseases(kidney disease, rheumatoid arthritis, HIV/AIDS,chronic inflammatory bowel disease(Crohn's),liver disease, heart failure, thyroid disease)
- Treatment for cancers(Chemotherapy damage bone marrow resulting in decreased production of red blood cells)
- Pregnancy(due to low levels of Iron/Folic acid, increased demand and dilution of blood)
- Acquired Aplastic anemia- where RBC production in bone marrow is suppressed because of certain medicines, toxins and infectious diseases
- Pernicious anemia- autoimmune condition where our body cannot absorb Vitamin B12 due to lack of a protein called intrinsic factor which is produced in the stomach

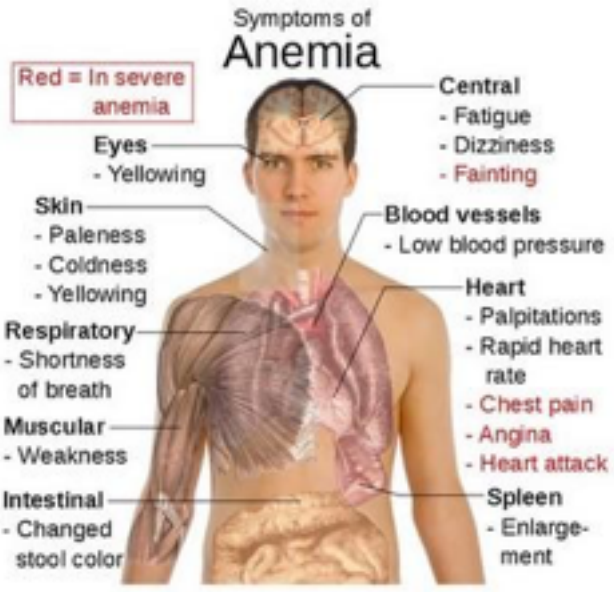
3. Increased red blood cell destruction:

Acquired causes include

- Enlarged spleen
- Acquired Hemolytic anemias due to autoimmune disorders(rheumatoid arthritis) infections, certain medicines or reactions

Symptoms of Anemia

Shortness of breath, extreme fatigue, chest pain, pale skin, dizziness or lightheadedness, palpitation, headache, unusual craving for non-nutritive substances (pica), restless leg Syndrome .In addition to the above symptoms glossitis, tingling in hands and feet, numbness in extremities in vitamin B12/ folate deficiency and discoloration of skin(jaundice) in hemolytic anemia. Dark red colored urine especially in the morning.



When to suspect anemia?

If you experience any of the above symptoms of anemia approach a physician to rule out anemia

Who is at Risk of anemia?

- Women of childbearing age
- Premature infants
- Exclusively breast fed infants
- Infants between 1-2 years
- Older adults

How is anemia diagnosed?

- A detailed medical and family history is very essential to find out a causative factor
- Diagnostic tests and procedures

1. Complete Blood Count(CBC)- includes WBC, hemoglobin(Hb),hematocrit (HCT), MCV, platelets and reticulocyte count

Hb– it is an iron rich protein in RBC that carries oxygen to the body. Low level is a sign of anemia

HCT– (Ref. range in Men - 38.8 -50 %, Women - 34.9-44.5%) it indicates how much space red blood cells (RBC)take up in your blood. A low level of HCT is a sign of anemia. Inaccurate levels may be seen in high altitude, pregnancy, recent blood transfusion and severe dehydration

MCV– (Ref. range 80-96 fl/red cell) gives an average size of the RBC. Low MCV denotes iron deficiency anemia and thalassemia. High MCV seen in Vitamin B12 and Folate deficiency

WBC & Platelets – abnormal results may be a sign of anemia, infection or another blood disorder

Reticulocyte Count – (Ref. range 0.5-2.5 % in adults, 2-6% in infants) measures the number of young blood cells and is an indicator of bone marrow function .Higher than normal is a sign of increas red cell production by bone marrow as a responce to increased red cell loss or destruction.

2. Iron Profile includes:

Serum Iron – (Ref.range 50-100 mcg/dl) Levels are decreased in Infection/inflammation. Low in iron deficiency anemia. Best interpreted in conjunction with total iron binding capacity (TIBC)

Serum Ferritin – (Ref. range >10-20 mcg/l) Indicates the iron stored, increased in infection or inflammation independent of iron status. Low in iron deficiency anemia.

Total Iron Binding Capacity (TIBC)– (Ref. range 250- 410 mcg/dl) High in iron deficiency in anemia.

% Saturation of Transferrin – (Range >20%) Indicates availability of Iron for erythropoiesis. Low in iron deficiency anemia.

3. **Vitamin B12 and Folate Level –** Low levels are an indication of deficiency
4. **Stool for occult blood –** Indicates bleeding in the digestive tract, if found positive other invasive procedures like endoscopy may be required
5. **Stool for parasites/ova/cysts –** rules out parasitic infection like hookworm or roundworm etc. as a cause of anemia
6. **Kidney Function tests –** abnormal results indicate kidney failure which may be a cause of anemia
7. **Serum Erythropoietin –** Low levels are seen in anemia
8. **Hb Electrophoresis -** to look for Thalassemia
9. **Other contributory tests –** include Coomb's test to look for hemolysis and bone marrow examination in few cases

How to prepare for Blood and Stool Tests?

- To test for CBC – No need for fasting
- To assess Kidney or Liver Function tests fasting is required. A 12 hr fasting period is ideal.Avoid coffee, tea or alcohol before any blood tests
- For stool testing, 3 days before the tests avoid fruits and vegetables including broccoli, carrots, cauliflower, turnips , melons , radish ,cucumber, grapefruit, mushrooms and horseradish(peroxidase rich fruits and vegetables). Also avoid red meat, poultry, fish, Vitamin C supplements and pain reliever such as aspirin and ibuprofen

How is severity of anemia graded?

Severity	Hb (gm/dl)	Hct (%)
Moderate	7.0 -10.9	24-37
Severe	4.0 - 6.9	13-23
Very Severe	<4	<13

Treatment of Anemia

Determine and remove the underlying causes

- Dietary Iron – Iron rich food (Spinach, Dried Fruits, Cereals, Egg, Red Meat, Sprouts etc.) should be included. Diet rich in Vit.C increase uptake of Iron.
- Folate rich foods include Green Leafy vegetables, Brussels, Sprouts, Broccoli, Whole Grains
- Treatment of worm infestations
- Oral Iron Therapy
- Parenteral Iron(IM or IV): In situations where correction of Hb is urgent or where Oral absorption is poor or intolerant
- Packed Red Blood Cell (PRBC)Transfusion

Conclusion

Among Acquired Anemia, nutritional anemia is most prevalent in children, adolescents, pregnant women and elderly that is preventable and easily treatable when diagnosed early. Youth need to be educated on diet, sanitation and personal hygiene. Please ensure to approach to a physician as early as possible on noticing any of the above symptoms of anemia.

"Prevention is better than Cure"

Further reading

1. Your Guide to Anemia- National Heart Lung and Blood Institute NIH
2. Acquired Anemias – Evidence Based Hematology- ZumbergWilies on line library
3. Nutritional Anemia in Developing Countries – Intech Open by FT Wieringa
4. Acquired Immune Hemolytic Anemias- Lawrence D.Petz
5. Acquired Hemolytic Anemias- Concise Guide to Hematology
6. The Guide Book Nutritional Anemia – USAID
7. Community medicine with recent advances

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BLOOD – EXCESS OF EVERYTHING IS BAD

Dr Ramesh Pandita
Department of Hematology
Kuwait Cancer Control Center

The blood consists of cells and plasma. The cells consist of white blood cells (WBC), red blood cells (RBC) and platelets. When we do complete blood counts we look for any increase or decrease in the blood cells. In this section we will look into the causes of increase in blood cells and further investigations required.

What are causes of increase in white blood cells?

The white blood cells consist of neutrophils, lymphocytes, eosinophils, monocytes and basophils. Increase in cells can be due to different reasons.

Neutrophils: Neutrophils are body's defense against infection. Increase in neutrophils is one of the most frequently observed blood cell change. Causes of increase in neutrophils also called as neutrophilia are:

- Bacterial infection, inflammation and tissue death e.g. trauma, inflammation of muscles and blood vessels etc.
- Acute blood loss or red cell destruction.
- Drugs.
- Blood malignancies like leukemia.

Monocytes: Monocytes are another defense cell against infection. They ingest the bacteria.

Causes of increase in monocyte (monocytosis) include:

- Chronic infection
- Autoimmune disorders like rheumatoid arthritis etc.
- Parasitic infection.
- Blood malignancies like chronic myelomonocytic leukemia.

Eosinophils: Eosinophils are involved in allergic response and defense against parasitic infection.

Causes of increase of eosinophils (eosinophilia) include:

- Allergic disease like bronchial asthma, urticaria, hay fever etc.
- Parasitic infestation due to amoeba, hookworm, tapeworm, filariaetc infestation.
- Skin diseases.
- Drug sensitivity.
- Blood malignancies.

Basophil increase in uncommon and may increase in chronic blood malignancies.

Lymphocytes: One of the most versatile blood cells, involved in defense against infection and foreign substances in our body. They are important in recognizing foreign substances and assist in their removal.

Causes of increased lymphocytes (lymphocytosis) include:

- Infection: viral infection, chronic bacterial infections
- Leukemias

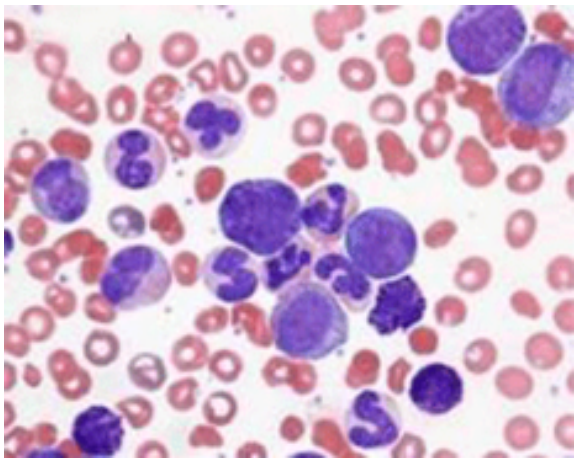
What is leukemia?

Leukemia is a cancer of white blood cells. In this condition too many white blood cells are produced in the bone marrow and leave very little normal cells. The blood cells produced may be immature called as blasts or mature cells. If the cells produced are predominantly immature (blasts), this rapidly progressive disease is called acute leukemia. Depending on the type of cells involved in the cancerous process the acute leukemia may be myelocytic or lymphocytic. In chronic leukemia there is gradual increase in mature and maturing white blood cells either myelocytic or lymphocytic. Due to decrease in normal cells these disease may present with pallor, bleeding or recurrent infections. The cells may also involve lymph nodes, liver and spleen and cause their enlargement.

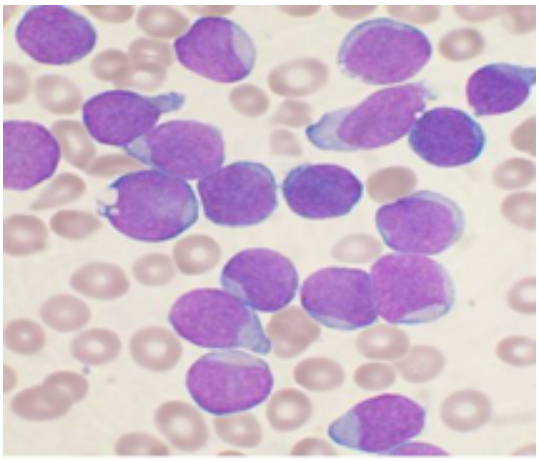
How is leukemia diagnosed?

The diagnosis of leukemia involves a series of tests. The tests are used to confirm whether the leukemia is acute or chronic and their subtypes (myeloid or lymphoid). The tests are:

1. **Blood tests:** Complete blood counts (CBC) show the white blood cells to be increased, normal or decreased in number. The red blood cells and platelets are usually decreased in number. The blood is examined under microscope and the cells are identified and presence of immature cells confirmed.



Chronic Myeloid Leukemia, both mature and immature myeloid cells are present



Acute Lymphoblastic Leukemia, most of cells consist of blasts

2. Bone marrow test:

Bone marrow test involves taking of samples usually from hip bone and occasionally from breast bone or from tibia (in infants). It is usually done under local anesthesia, however in children/ adolescent and apprehensive adults it is done under sedation/ general anesthesia. Bone marrow is aspirated by a needle and a piece of bone marrow is removed by a special biopsy needle. These samples are then processed and examined under a microscope.

Additional samples are taken for special studies like immunophenotyping, cytogenetic and molecular genetic studies.

3. Immunophenotyping test:

This test is done to identify proteins (antigens) in the cells specific to various types of cells and to make an accurate diagnosis of type of leukemia. Flow Cytometry is the commonest way to perform this study.

4. Cytogenetic test:

This test is done to find chromosomal changes in the cells. Specific chromosomal changes are characteristic of certain forms of leukemia. Special genetic tests like fluorescent in situ hybridization (FISH), polymerase chain reaction (PCR) are also now performed for diagnosis and monitoring of leukemia.

Immunophenotyping and genetic studies are essential for confirmation of diagnosis, identifying the best treatment, monitoring response to treatment and outcome (prognosis) in leukemia.

My hemoglobin is 19 g/l, what could be the cause?

Increase in hemoglobin levels above the upper limit of normal for sex and age is called polycythemia. When the hemoglobin increase is because of increase in red blood cells it is called absolute polycythemia and if it is due to decrease in plasma volume it is called relative or pseudopolycythaemia. Absolute polycythemia can be primary, when it is due to the excessive red cell production due to excessive sensitivity of red cell precursor to erythropoietin. Secondary polycythemia is driven by diseases causing decreased oxygen concentration.

Causes of polycythemia:

- Primary – polycythemia vera
- Secondary
  a. Chronic lung disease.
  b. Smoking
  c. Congenital heart disease
  d. High altitude
  e. Abnormal erythropoietin production ?tumou

What are the tests required to find cause of increased hemoglobin/ red blood cells?

Tests required to find causes of polycythemia are done considering clinical findings and are usually done sequentially.

- Complete blood counts, liver& kidney function test.
- JAK II mutational assay mutational assay (A molecular genetic test).
- Serum erythropoietin level
- Arterial oxygen saturation
- Lung function test
- Ultrasound abdomen
- Bone marrow examination (aspiration and biopsy).

Increased hemoglobin with or without increase in white blood cells and platelets, along with JAK II mutation, low serum erythropoietin level and characteristic bone marrow changes are diagnostic of polycythemia vera, a blood neoplasm. This usually requires removal of blood (phlebotomy), low dose Aspirin and in high risk groups chemotherapeutic medication. Secondary polycythemia needs treatment of the underlying cause / disease.

I did my complete blood counts and my platelet count is 600 x 109/l.

What does it mean? Do I need more tests?

A platelet count of >450 x 10^9/l is considered abnormal. Causes of increased platelet counts include:

As a response to an underlying disease (reactive):

- Bleeding, injury, after operation.
- Chronic iron deficiency.
- Chronic infections.
- Chronic inflammations like rheumatoid arthritis.
- Malignancy.

Endogenous: Disease of the bone marrow

- Essential thrombocythemia.
- Polycythemia Vera.
- Chronic Myeloid Leukemia.

Further tests are dictated by clinical findings. Persistent increase in platelets in absence of infections or inflammation requires exclusion of Iron deficiency. Also genetic tests like PCR for chromosomal abnormalities and bone marrow aspiration and biopsy is required to make exact diagnosis.

Increase in blood cells is usually benign, however seeking medical advice is essential to exclude serious disorders, allay anxiety and initiate appropriate treatment.

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BLOOD : BLEEDING TOO MUCH OR CLOTTING TOO FAST

Dr Sunil Bahl
Hematology Unit, Department of Laboratory
Al Jahra Hospital

Blood flows in a fluid state in our arteries and veins under normal physiological conditions. Whenever there is breach in the continuity of the blood vessels it leads to bleeding. To stop the bleeding our body has natural mechanisms which lead to clot formation (coagulation) and arrest of the bleeding. Hence our normal blood flow is a critical balance between excessive bleeding and excessive clot formation. The factors that maintain this balance are blood vessels, blood platelets (small cells in the blood), coagulation factors, coagulation inhibitors and fibrinolytic elements (which breaks down the clot). Let us now review the causes, the sign symptoms, physiology, diagnosis and the treatment of a patient with bleeding or thrombosis (abnormal clot formation).

What are the common causes of bleeding?

Bleeding can occur due to a local cause or a generalized bleeding tendency.

Local causes are: 1) Trauma (injury) and 2) Surgery.

Generalized (Systemic) causes are:

1) Increased dose of medications like aspirin or anticoagulants like warfarin.

2) Reduction or abnormal function of platelets in the blood.

3) Inherited or acquired coagulation factor deficiency or inhibitors. Inherited causes like

- Hemophilia A (Factor VIII deficiency)
- Hemophilia B (Factor IX deficiency)
- Hemophilia C (Factor XI deficiency)
- Any other coagulation factor deficiency. Acquired antibodies against coagulation factors.

What are the signs and symptoms of bleeding?

The signs and symptoms of bleeding are: Bleeding in the skin with small spots called purpura or larger spots called ecchymosis. Mucosal bleeding from nose, oral cavity, from intestines in the stool or from the kidneys in the urine. Major bleeding can occur in the joints or the muscles. Critical bleeding can occur in the brain.

What are the blood tests performed for bleeding patients?

The primary tests performed for bleeding patients are:

1) Platelet count and morphology

2) PT: Prothrombin Time

3) APTT: Activated Partial Thromboplastin Time.

4) TT: Thrombin Time

5) Fibrinogen

Further tests are done depending upon the results of the primary tests and history.

Second line tests may include tests for individual clotting factors, clotting process by products like fibrin degradation products and tests for platelet function.

What is the treatment for a bleeding patient?

The treatment of a bleeding patient includes

1) Local measures in the form of pressure at the local bleeding sight or ice packs at local sight.

2) Surgical suturing of the wound to close the bleeding site.

3) Specific treatment for bleeding is decided according to the lab test results.

    a) Blood components like platelets, fresh frozen plasma (FFP) or packed red cells.

    b) Medications to increase the platelet count if it is low.

    c) Specific coagulation factor replacement like in Hemophilia.

What is Hemophilia?

Hemophilias are inherited bleeding disorders due to congenital deficiency of coagulation factors like Factor VIII or Factor IX. Factor VIII deficiency is known as Hemophilia A, while Factor IX deficiency is called Hemophilia B. Severe deficiency of these factors can lead to lifelong bleeding episodes. Parents can notice bleeding in a child very early in infancy during minor trauma while crawling. The bleeding may occur in a muscle or a joint which becomes swollen and blue. The treatment as mentioned is replacement of deficient factor. To prevent bleeding in such patients it is recommended to give regular infusions of Factor concentrates as home therapy. If no preventive therapy is given then these patients can develop severe joint deformities due to regular bleeding in the joints occurring due to joint movements. International and local Hemophilia societies are established to give financial, moral and vocational support.

What is thrombosis?

Our body is capable of blocking the bleeding sight by clot formation under normal circumstances. Some people can have increased tendency to clot formation (known as thrombosis). The common causes of increased venous thrombosis are: 1) Immobilization (more common in obese people) after operation or illness. This also includes long flights without movement for more than six hours. 2) Medications like

oral contraceptive pills. 3) Long term plasters on fractured limbs. 4) In pregnancy due to the weight of the baby. 5) Congenital tendency to hypercoagulate (known as thrombophilia).

What are the signs and symptoms of abnormal clot formation?

The signs and symptoms are 1) Swelling of the affected limb 2) Redness of the limb 3) Pain at the local sight 4) Restriction of the movement of the limb.

What are the tests done to confirm the diagnosis of thrombosis?

1) Color Doppler ultrasound of the affected limb. 2) D-dimer blood test.

Which patients require further testing?

Special tests are done to exclude acquired or inherited thrombotic tendency in newborn, children and young adults who develop thrombosis, those that have a strong family history or thrombosis at unusual site, and in individuals at ages who have recurrent episodes of thromboembolism. These tests may include looking for lupus anticoagulant (in acquired cases) or protein C , protein S or antithrombin deficiency or more.

What is the treatment of venous thrombosis?

1) The immediate treatment of venous thrombosis is critical to prevent the increase in size and spread of the clot. The aim is to dissolve the clot.

2) Venous thrombosis is treated with anticoagulant medication which are in two forms a) intravenous medication b) oral medication. The immediate treatment is usually with intravenous medication in the form of 'Low Molecular Weight Heparin' (LMWH). It is normally continued for five days and is then substituted with oral medication. It is a fixed dose regime calculated according to the weight of the patient and it requires no laboratory monitoring.

3) The oral treatment is with a) Warfarin. b) Newer oral anticoagulants (NOACs). Warfarin tablet is started on the second or the third day of thrombosis along with the LMWH as the oral Warfarin starts having effect in two to three days. Once the oral Warfarin takes over the LMWH is withdrawn. The dose of oral Warfarin is monitored by the blood test prothrombin time from which INR (International Normalized Ratio is calculated). Ideally the test result value should be between 2 – 3. If the INR blood test result is below 2 then the dose of Warfarin is increased and if it goes above 3 the dose is reduced. The treatment with oral anticoagulation is continued from three month to one year or indefinitely according to the criteria laid down.

What are the side effects of oral Warfarin?

1) Bleeding: If the dose of Warfarin exceeds the requirement the patient can bleed. This would be shown by an increased INR result. Hence a regular monitoring of Warfarin dose is required with INR testing.

2) If the dose of Warfarin is less than required the patient can get rethrombosis or an extension of the previous thrombosis.

What is the treatment of bleeding due to Warfarin overdose?

1) Stop taking Warfarin dose immediately. 2) If only the INR test is high and the patient has no bleeding then holding the drug for two to three days is adequate and the drug can be restarted after two to three days on a lower dose. Monitor with INR again after one week. 3) If the INR test is increased and the patient has only minor bleeding then hold the drug and your doctor will give vitamin K. After the bleeding stops, the drug is restarted on a lower dose. 4) If there is major bleeding then hold the drug and your doctor may have to admit you in the ward. The treatment given would be a) local measures b) Vitamin K injection c) PCC – Prothrombin Complex Concentrate d) Fresh Frozen Plasma (FFP). If the bleeding doesn't stop with any of these measures then as a last resort recombinant factor VII can be used.

What are NOACs ?

NOACs are newer oral anticoagulants which are a substitute for Warfarin tablets. They are Dabigatran, Rivaroxaban, Apixaban. These drugs have many advantages over Warfarin. 1) They are fixed dose regimes and require no laboratory monitoring. Hence the patient doesn't have to go for regular blood tests. 2) They are safer than Warfarin as they have less bleeding side effects. 3) They are also not affected by food intake.

Further reading

1. National Hemophilia Foundation: www.hemophilia.org
2. The Hemophilia Society: haemophilia.org.uk
3. Hemophilia Federation (India): www.hemophilia.in
4. International Society on Thrombosis and Hemostasis, Inc.: www.isth.org

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DONATE BLOOD, SAVE LIVES

Dr Sunny Varghese
Hematology Unit, Department of Laboratory
Al Adan Hospital

What is blood donation ?

It is the voluntary donation of about 450 ml of your blood to the blood bank for use in needy patients. Many patients require to be given blood and blood products for conditions like massive blood loss following accidents or trauma and also in different disease conditions like anaemia where there is decreased production of blood components in the body or there is increased destruction of blood in the body as a result different disease conditions.

Why donate blood ?

By donating blood we are saving another life. It is one such service only a fellow human being can do for you, because blood cannot be produced artificially in factories like any other commodity in the market, so far. Each pack of Blood is unique and it has to come from another human being only.

Who can donate blood ?

Any healthy adult between the ages of 17 and 70 years can donate blood. Pregnant and lactating mothers are excluded along with certain exceptions.

How often can you donate blood ?

One can safely donate blood every three- four months, subject to maximum of three donations per year. Any way our system replaces all the blood in our body every three months

What are the hazards of blood donation to the donor ?

The most common hazard of blood donation is fainting attack, mostly seen in anxious/ apprehensive individuals and in those donating for the first time. It can be avoided by drinking fluids prior to the donation and enforcement of adequate rest immediately after the donation. Once the faint occurs, one needs only to rest in horizontal position with elevation of legs is usually sufficient. For the same reason drivers and machine operators are advised not to return to work the same day after donation. Other minor hazards include hematoma at the site of venepuncture, infection of the venepuncture site are rarely seen.

What happens to the blood after you donate ?

Blood after donation goes through many checks and testing before it can be received by a patient, quite contrary to the myth and what we usually see in movies.

In short the blood is tested for the type/group, tested for transmissible diseases, separated into different blood components and tested for compatibility to the intended recipient blood.

What are the different blood type/ groups ?

Human blood groups are classified/typed by different blood types like ABO system, Rh system, MNS system, Kell, Duffy, Kidd etc. We usually classify blood in the ABO and Rh system because of the increased clinical relevance of the system.

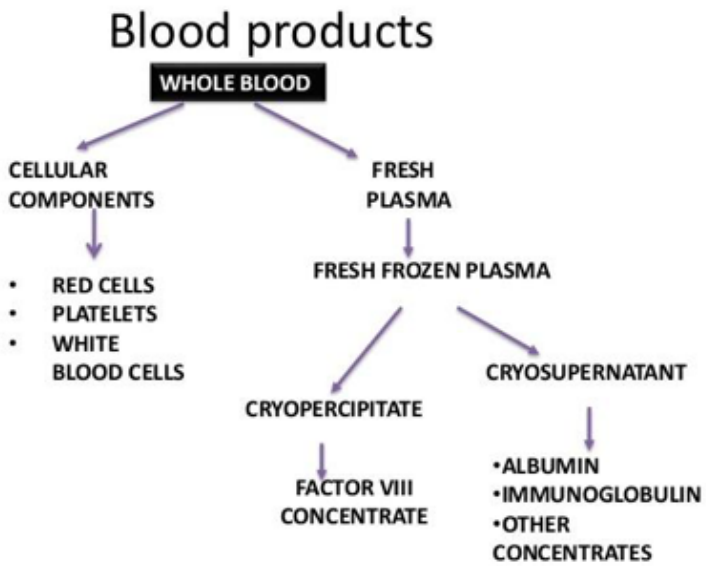
In the ABO system we have four blood groups viz A, B, AB and O and in the Rh system we have Rh Negative (-ve) and Rh Positive (+ve) and accordingly one can usually be belonging to any one of the eight groups like A +ve or A –ve; B +ve or B -ve; AB +ve or AB –ve and O +ve or O-ve

What are the diseases routinely tested for from my blood ?

The blood we donate are routinely screened for transfusion transmissible pathogens like HIV, hepatitis B, hepatitis C and malaria

What are the different blood components ?

They are Packed RBC, granulocyte concentrate, platelet concentrate, fresh frozen plasma and cryoprecipitate



Packed RBC consists mostly of Red Blood Cells and is usually given to patients with anaemia for increasing their haemoglobin level. One unit dose is about 350-450 ml in volume and can be stored at 4-8 ^{0}C up to 42 days after collection with suitable additives. One unit when transfused increases the haemoglobin level by about 1gm/dl.

Granulocyte concentrate consists of the granulated white blood cells (WBC) and are extremely labile and hence required to be transfused within few hours to the patient. It is given for combating severe infections.

Platelet concentrates are concentrated platelet fraction as the name implies, each unit is about 50-55 ml in volume and can be stored up to 5-7 days at room temperature of 20-22 ^{0}C. It is usually given to boost the platelet count of patients with thrombocytopenia or platelet diseases.

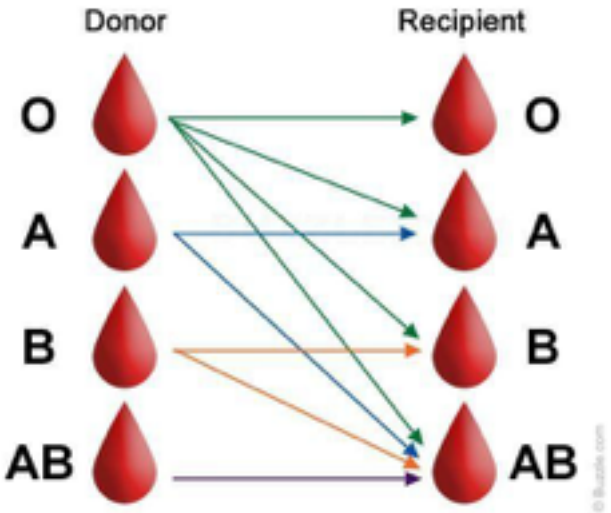
Fresh Frozen Plasma (FFP) is the fluid which remains in the whole blood after removal of cellular elements like RBC, WBC and Platelets mentioned above and are frozen for storage within 24 hours of collection. They are stored at -30 ^{0}C or below up to 24 months. Require to be thawed rapidly to 37 ^{0}C before transfused to the patient. It contains all coagulation factors and is used to correct coagulation factor deficiencies like in haemophilia patients' if the specific factor concentrates are not easily available.

Cryoprecipitate is prepared from FFP and contains factor VIII and Fibrinogen and mostly used as source of fibrinogen.

What is cross matching of blood ?

Cross matching is also referred to as compatibility testing. It is the process by which the donor cells are tested against the potential recipient plasma or serum to detect and identify any antibodies in the recipient that can react with the cells of the donor. It avoids development of transfusion reactions in the recipient as far as possible. It takes at least about 30 minutes to do an uncomplicated cross match in a routine patient and may last up to 2-3 working days in complicated cases.

Who can receive my blood ?

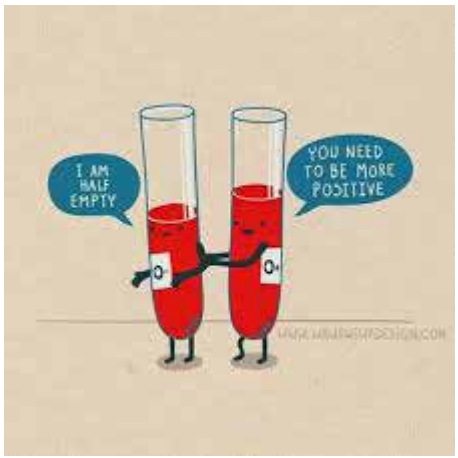


We can see that if

Your blood type is	You can give blood to	You can receive blood from
A+	A+, AB+	A+, A-, O+ & O-
O+	O+, A+, B+, AB+	O+ & O-
B+	B+, AB+	B+, B-, O+ & O-
AB+	AB+	EVERYONE
A-	A+, A-, AB+ & AB-	A-& O-
O-	EVERYONE	O-
B-	B+, B-, AB+ & AB-	B- & O-
AB-	AB+ & AB-	AB-, A-, B- & O-

What is artificial blood ?

Yes that is the future of blood transfusion. Research is seeking for a product which can replace the functions of blood and blood components but at the same time free from the antigens and antibodies which often create problems in the recipient. Clinical Trials are going on worldwide. May be artificial blood will be licensed for use in humans in future.



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(1) Kantarjian H et al. *N Engl J Med.* 2010;362(24):2260-2270.
(2) SPRYCEL® Prescribing information, September 2016.

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RECURRENT INFECTION : WHAT DO WE LOOK FOR

Dr Mohan Ram
Department of Hematology
Kuwait Cancer Control Center

Recurrent infections are infections that are too great in number, too severe, or too long lasting. Recurrent infections are defined as two or more severe infections in one year, three or more respiratory infections (e.g. sinusitis, otitis, bronchitis) in one year, or the need for antibiotics for two months/year.

The recurrent infections are usually due to an anatomic lesion, a primary and secondary cause of Immune Suppression.

The human immune system has a complex defense system, consisting of cells and proteins that act together; to fight infections caused by bacteria, viruses, and parasites.

The Immune System

The major function of the immune system is to recognize foreign substances and react to them. An immune system that is functioning adequately should defend the body from infectious organisms (bacteria, viruses, fungi, and parasites) and protect the body from development of cancers. The major organs of the immune system include the bone marrow, liver, thymus, tonsils, lymph nodes, the spleen blood. These organs manufacture, process and store the major components of the immune system, which include:

- **T- Lymphocytes or T-cells –** White blood cells important in regulating the immune system and helping fighting viral infections.
- **B- Lymphocytes or B-cells –** Cells that make specific proteins called antibodies to protect from bacteria that cause ear infections, sinusitis and pneumonia, etc. The chemical name for antibody proteins is immunoglobulins or gamma globulins. There are four major classes of antibodies or immunoglobulins which are IgG, IgA, IgM and IgE.

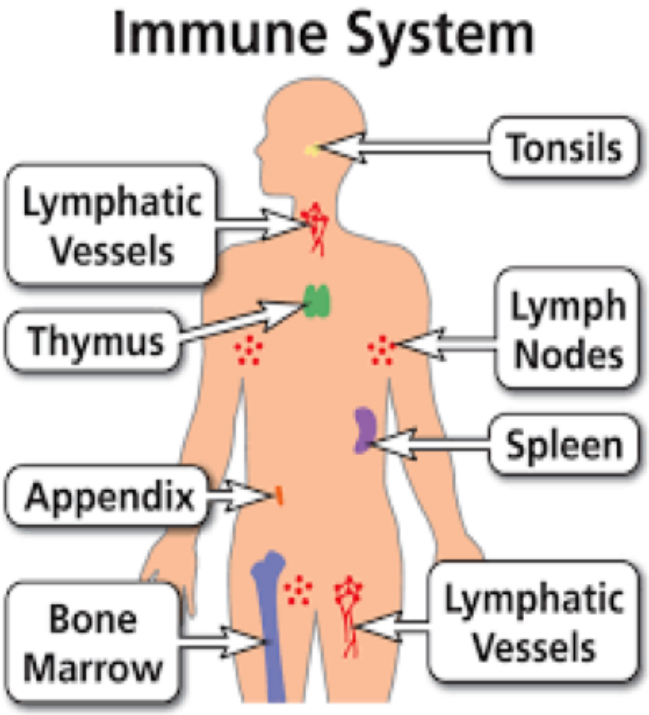
Each immunoglobulin class has special chemical characteristics that provide it with specific advantages. For example, antibodies in the IgG fraction are formed in large quantities, last for over a month and travel from the bloodstream to the tissues easily. The IgG antibodies are the only immunoglobulins that cross the placenta and pass immunity from the mother to the newborn. Because our immune

system can't make the most important antibody, IgG, in sufficient quantity until about six months of age, IgG antibodies passed on

by mother before birth, protects the baby. If the baby's own immune system does not "turn on" at the right time, the child may get recurrent infections. This is called "Transient hypogammaglobulinemia (low immunoglobulin) of infancy", transient because the child will outgrow it by the age of 2 or 3 years, as the immune system matures.

IgA antibodies are produced near mucous membranes and find their way into secretions such as tears, saliva, and mucus, where they protect against infection in the respiratory tract and intestines.

IgM antibodies are the first antibodies to be formed in response to infection. Therefore, they are important in the early days of an infection.



IgE antibodies are responsible for allergic reactions and parasitic infections.

- **Phagocytes –** Cells that ingest foreign bodies and bacteria to kill them.
- **Complement –** Proteins that may kill bacteria that may cause infection.

A Primary Immunodeficiency occurs when the abnormality to the immune system develops from an inborn defect in the cells. The cells that are affected include T-cells, B-cells, phagocytic cells, or the complement system. Most primary immune deficiencies are inherited diseases. **The disease is not always evident** at birth. There are about 95 known primary immunodeficiency diseases. Some examples of Primary Immunodeficiencies include: Selective IgA deficiency, severe combined immunodeficiency (SCID), Common variable immunodeficiency (CVID), X-linked Agammaglobulinemia (XLA).

Secondary Immune deficiencies occur when damage is caused by an environmental factor. Radiation, chemotherapy, burns, and infections contribute to the many causes of secondary immune deficiencies. Acquired Immune Deficiency is a secondary immune deficiency caused by the Human Immunodeficiency Virus (HIV). In Leukemia, Lymphomas, and metastatic cancer, abnormal cancerous cells crowd out normal stem cells of the bone marrow. These abnormal cells reduce the number of B cells and lead to hypogammaglobulinemia or secondary immune deficiency.

Recurrent infection in Adult

Adult patients who present with recurrent infections pose a dilemma to the generalist. In most cases, there is a secondary cause, such as an anatomic abnormality. However, secondary immune defects

due to other medical disorders are sometimes identified, while primary immune defects presenting in adults are rare.

In adults, recurrent infections are usually due to an anatomic lesion, a functional disorder, or to a secondary cause of immunosuppression.

- Anatomic lesions, whether congenital or acquired, and disorders affecting the function of specific organs are important causes of recurrent infections in adults e.g. Recurrent otitis media, sinusitis, recurrent lymphadenitis, bronchopneumonia, cystitis, cellulitis, abscesses and meningitis.
- Secondary immune disorders due to other medical conditions or treatments for these conditions are a much more common cause of recurrent infections than primary immunodeficiency e.g. Immunosuppressive agent, microbial infection, malignancy, metabolic disorders, autoimmune diseases and trauma.
- Most congenital (primary) immunodeficiency do not present in adulthood, but rather are diagnosed in infancy or childhood.

Recurrent infection in Childhood

In Children, recurrent infections are usually due to primary immune deficiency and sometimes secondary immune deficiency.

- Primary Immunodeficiency (Most common)
- Secondary Immune disorder due to malnutrition, HIV, lymphoma, leukemia, sickle cell disease, diabetes mellitus, renal failure, severe liver diseases and asplenia.

Recurrent or persistent infection is the major manifestation of primary immunodeficiency. While most children with recurrent infections have a normal immunity, it is important to recognize the child with an underlying immunodeficiency in order to investigate and treat appropriately. Early diagnosis and treatment of immunodeficiency will improve the quality of life. It may also be life-saving in patients with primary immunodeficiency (PID).

Laboratory Investigations for Recurrent Infection

Initial laboratory evaluation

The initial screening test should include a complete blood count including a differential white blood cell count with platelet determination. The total leukocyte count and the absolute numbers of lymphocytes, neutrophils, and eosinophils should be noted and interpreted according to age-appropriate normal values. Persistent or cyclical neutropenia (if ANC is <1500) can predispose the patient to abscesses. On the other hand, persistent neutrophilia occurs in patients with Leukocyte Adhesion Defect (LAD). A peripheral blood smear can be very informative. The presence of giant cytoplasmic granules in leukocytes in the peripheral smear may suggest Chediak-Higashi syndrome, whereas Howell-Jolly bodies in erythrocytes occur in patients with asplenia. Thrombocytopenia and small platelet size is characteristic of Wiskott Aldrich syndrome.

It is important to include electrolytes, glucose, urinalysis, renal, and liver function tests in the initial assessment of every patient. HIV testing is indicated in every patient with suspected T cell defect. Testing is either done by determining the antibody titers or by PCR.

To exclude anatomical factors, a chest x-ray is indicated if the person has frequent respiratory symptoms or infections. In addition, if sinopulmonary disease is the major presenting feature, cystic fibrosis and immotile cilia must be excluded by Sweat Chloride test and nasal ciliary biopsy, respectively.

Specific Immunological Assessment

A wide variety of tests is available for the evaluation of immune response. For ease of discussion, the laboratory approach to immunodeficiency is subdivided into investigation of defects primarily affecting humoral immunity, cellular immunity, the complement system, and the phagocytic system. Within each system, screening tests are considered first, followed by more sophisticated assays.

Evaluation of Humoral Immunity

To diagnose various humoral immunity deficiency disorders,in vitro evaluation of lymphocytes is important, and involves identification of B lymphocyte with the use of flow cytometry. B cells can be identified by using monoclonal antibodies against the cell surface markers, termed clusters of differentiation (CD nomenclature). CD19 or CD20 are considered the cell surface markers of B cells. B cells are usually normal in patients with transient hypogammaglobulinemia of infancy, distinguishing them from patients with X linked agammaglobulinemia.

Evaluation of Cellular Immunity

Cellular immunodeficiency testing includes the measurement of T cell surface markers by flow cytometry. The CD3 marker serves as identification of T cells in general, CD4 marker serves as identification for T helper cells; and CD8 marker identifies cytotoxic T cells.Cellular immune function can also be assessed by the delayed hypersensitivity skin tests to microbial antigen to which the patient has been exposed such as Candida albicans,and by the lymphocyte proliferation assays to mitogens, antigens, and allogeneic cells.

Evaluation of cellular immune deficiency requires specialized laboratory facilities and expertise not available in most clinical testing laboratories. The advice of an immunologist is extremely important for the interpretation of the results, even during the diagnostic process.

Evaluation of the Complement System

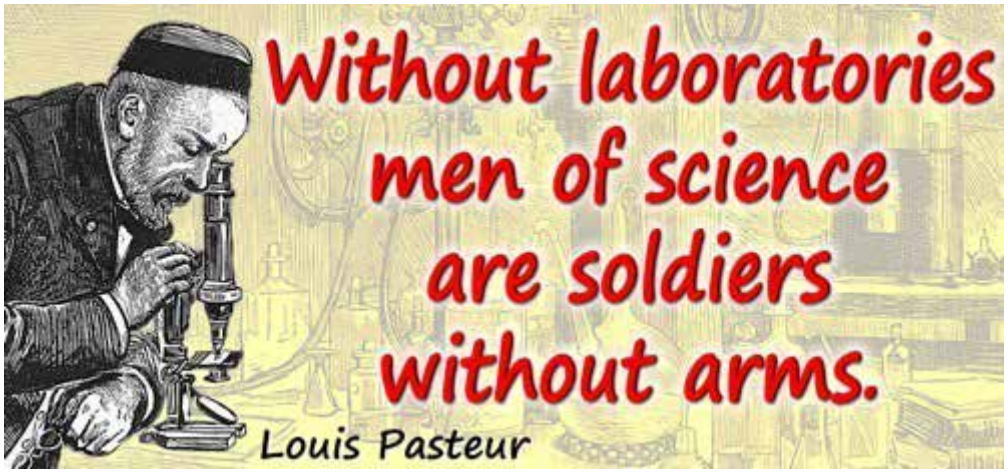
The total hemolytic complement (CH50) assay evaluates the functional integrity of the classic complement pathway.It is a useful screening test for detecting functional deficiencies of the components of the classical and alternate pathways of the complement system. An immeasurable or low value suggests a

complement deficiency and may need to be followed by more specific tests to delineate the abnormal complement component since it is possible to assay each individual complement component in order to identify the abnormal protein.

Evaluation of the Phagocytic System

Evaluation of phagocytic function can be done by microscopy through enumeration and characterization of neutrophils and monocytes. Assessment of patients for CGD is done by nitrobluetetrazolium (NBT) test or flow cytometry to evaluate oxidative burst. Assays for neutrophil phagocytic and chemotactic function is only performed in specialized immunodiagnostic laboratories and requires expert interpretation.

In conclusion, patients with recurrent infections need a thorough clinical and laboratory evaluation. Correct diagnosis is essential for appropriate treatment.



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HISTOPATHOLOGY - ROLE IN THE DIAGNOSIS OF DISEASES

Dr Elizabeth Joseph
Histopathology Unit, Department of Laboratory
Kuwait Cancer Control Center

Histopathology is the microscopic examination of biological tissues to diagnose diseases by observing the appearance of diseased cell and tissues in very fine details. The word histopathology is derived from a combination of 3 Greek words, histos = tissue, pathos = suffering or disease and logia = study of. In medical practice, histopathology refers to the study of surgical specimen or biopsy material by a pathologist. Pathologist is a medical doctor specialized in identifying the cellular changes in disease at the microscopic level.

Uses of Histopathological examination

1. **Diagnosis of various diseases:** Histopathological examination of tissues helps in diagnosing inflammatory conditions, cancers as well as non-cancerous conditions. It is also useful in diagnosing early lesions which may progress to cancer (precancerous lesions).
2. **To assess the completeness of surgical excision of a tumor:** The extension of abnormal cells to the margins of surgical excision indicates incomplete removal of the tumor
3. **Staging and grading of cancer:** The stage of a tumor refers to its size or extent and whether or not it has spread to other organs and tissues. Tumor grade is the description of a tumor based on how abnormal the tumor cells and the tumor tissue look under a microscope.
4. **To study the response of disease to treatment:** Sometimes patients with certain cancers e.g. breast cancer, may receive chemotherapy before radical surgery. In such cases, histopathological examination of the tissue removed after chemotherapy helps to assess how far the cancer has responded to treatment.

Types of specimens for histopathological examination

1. **Surgical specimens:** All the tissues removed during surgery (e.g. breast, intestine, uterus, prostate etc) are usually sent to the histopathology lab for detailed examination.
2. **Biopsy specimens:** The small piece of tissue removed from a diseased area (lesion) for diagnosis is subjected to microscopic examination. When the entire suspicious portion (lesion/lump) is removed it is called excision biopsy. Core biopsy or incisional biopsy refers to removal of only a portion of the abnormal tissue without attempting to remove the entire diseased area.
3. **Tissues obtained from autopsy (Post mortem):** The tissues removed during post mortem examination are subjected to histopathological examination to diagnose or confirm the cause of death.

How specimens are sent for Histopathological examination ?

After surgery, all specimens are submitted with a proper labeling including identification details and clinical details. The specimens are collected in wide mouthed containers filled with 10% buffered formalin, which is the commonly used fixative. Fixative is the chemical used to prevent or arrest the degenerative changes which commence as soon as a tissue is deprived of its blood circulation. It also prevents autolysis which results in tissue digestion by intracellular enzymes and bacterial decomposition which is brought about by the microorganisms. So, proper preservation by fixation of the collected specimen is a very important step. This is because improper fixation can result in permanent tissue damage which will interfere with proper interpretation and diagnosis.

There are different types of fixatives, the most commonly used is 10% buffered formalin. Formalin is used because it does not significantly interfere with subsequent histochemical and immunohistochemical staining. However in certain situations eg: if electron microscopic study is required, 3% glutaraldehyde is used as the fixative. Thus the selection of fixative depends on the type of study planned.

What Pathologists do with the specimen ?

Once the specimen is received in the histopathology laboratory, it is identified by a specified number (pathology number). The pathologist examines the whole specimen and describes the abnormalities seen. Then the specimen is cut into thin slices and the diseased part is identified from which multiple representative pieces are taken for processing. This entire process is described as 'grossing'.

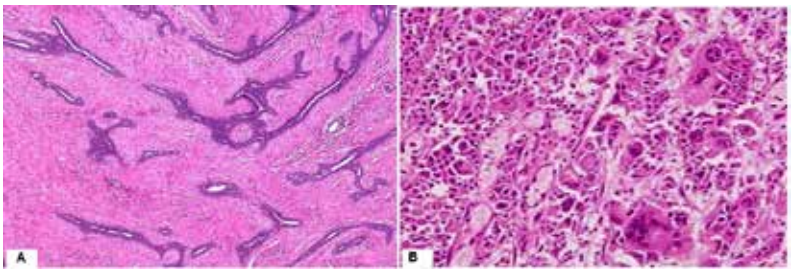
Processing of the specimen

The steps include:

1. Dehydrating the tissue
2. Placing it into a wax block to harden it
3. Slicing it into extremely thin layers of block, less than half mm thickness. This is done using a machine known as microtome.
4. Mounting the thin layer on a glass slide
5. Staining them so that tissue becomes visible under the microscope
6. Covering with a cover slip so that tissue on the slide will be preserved for many years

The processing of tissue can be done manually as well as by automated machines. The whole process may take one to two days.

What are the observations which help to make the diagnosis?



The pathologist examines the slides under microscope and studies the arrangement of cells (cellular architecture) and morphological features of the cell nucleus and cytoplasm. (e.g. size, shape, pattern of chromatin, mitosis etc). Based on these features distinction between normal, inflammatory, cancerous or non-cancerous (benign) tissue is made.

Infections caused by different organisms (e.g. Tuberculosis, fungal or parasitic infections) can also be identified.

What are the ancillary tests used ?

When the features of the lesion cannot be clearly assessed by ordinary staining further ancillary tests are useful. These include:

1. Immunohistochemistry

Several diseases or disease sub-types may look alike or appear to have similar cells under the microscope but have different behavior . The best way to differentiate them is to detect specific molecules on these cells that act as markers. Immunohistochemistry is a technique that uses antibodies that can seek out, identify and attach themselves to these markers on the cells. Since antibodies are highly specific, the antibody will bind only to the protein of interest in the tissue section. The antibody-antigen interaction is then visualized using either chromogenic detection, in which an enzyme conjugated to the antibody cleaves a substrate to produce a colored precipitate at the location of the protein, or fluorescent detection, in which a fluorescent chemical compound is conjugated to the antibody and can be visualized using fluorescencent microscopy.

2. Molecular Pathology and Cytogenetics

Increasing number of genes are being recognized that, if faulty, may be involved in the development of disease including cancers. Molecular pathology is an umbrella term for the analysis of the genetic material (chromosome and DNA) of cells for the diagnosis. One of the subdivisions of molecular pathology is cytogenetics, which is the analysis of DNA in the chromosomes. Different techniques like fluorescence in situ hybridization (FISH) or direct sequencing of DNA are used for this. These tests reveal areas where genes may have been deleted, duplicated or broken. Direct sequencing of cell DNA help to identify individual genes or groups of genes, to detect and characterize which mutation is present in a particular patient's tumor. For example in case of breast cancer, apart from the information regarding the stage and aggressive nature of the cancer obtained from routine histopathology and immunohistochemical studies, cytogenetics identify whether the patient has a faulty gene which predisposed them to the development of breast cancer. If present, this would mean that they have an increased chance of developing cancer in the opposite breast and also of developing other specific

cancer types like ovarian cancer. Hence early steps to prevent or screening of cancer recurrence can be made. This also helps selecting specific treatments since new treatment regimes often target the products of specific gene mutations in a patient.

3. Flow cytometry

This technique is used most commonly as an adjunct in the diagnosis of cancers of the blood cells.

4. Electron microscopy

Certain diseases can be diagnosed only at subcellular level. In these cases, a very powerful type of microscope called the Electron microscope which uses a beam of accelerated electrons as a source of illumination is used. Thus studying the ultra structural details of cells help in the diagnosis.

How long does it take to get the final histopathology report ?

The report on small specimens like core biopsy may be ready within two days. But the big specimens take at least more than a week for the final report. Specimens like bony tissue need more time as they need removal of calcium by chemical reagents before routine processing. If additional ancillary tests are required it may take even more time.

What should be done if external consultation is required ?

The slides and blocks can be taken by hand or sent by courier if external consultation is required or if the patient goes for treatment at a different centre.

How long the materials are preserved in the lab ?

Depending on the institutional policy the specimens are discarded within the stipulated time. The wax blocks and the stained glass slides are usually preserved for at least 5 to 10 years.

Summary

All the surgical specimens including small or big tissue should be submitted for histopathological examination. This is very important because in rare occasions a focus of cancer may be missed if the tissue is not evaluated and later the patient may present with spread of cancer to other sites (metastasis). Histopathology is considered as the gold standard for the diagnosis of cancer. Also in many other clinical situations histopathology provides the 'final diagnosis' when all other investigations have failed.

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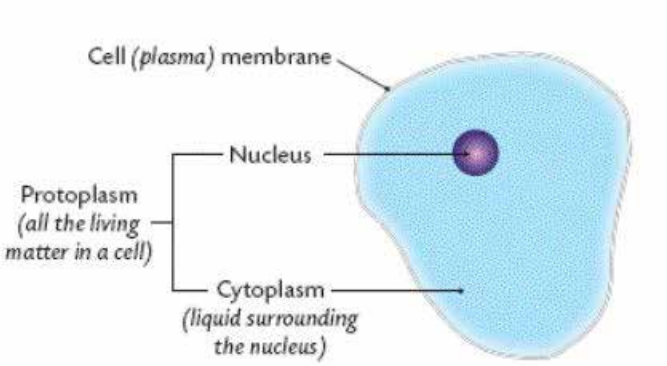
CANCER-NOT A DEAD END

Dr Rajan Arora
Histopathology Unit, Department of Laboratory
Al Farwaniya Hospital

Human body is made up of approximately 37.2 Trillion cells.Cell (Latin-small room) is enclosed by a membrane(cell membrane) and contains jelly like substance (cytoplasm) in which all the biochemistry of life takes place.These biochemical reactions are commanded by the Nucleus which contains hereditary material(DNA) packed into 23 pairs of thread like structures called as Chromosomes.These chromosomes carry approximately 25000 genes.Gene is a small segment of DNA carrying specific instructions for various biochemical reactions in the cytoplasm. About 37.2 Trillion cells with about 25000 genes carried on 23 pairs of chromosomes in each cell-Indeed Human body is a marvelously complex system.



Normal Cell

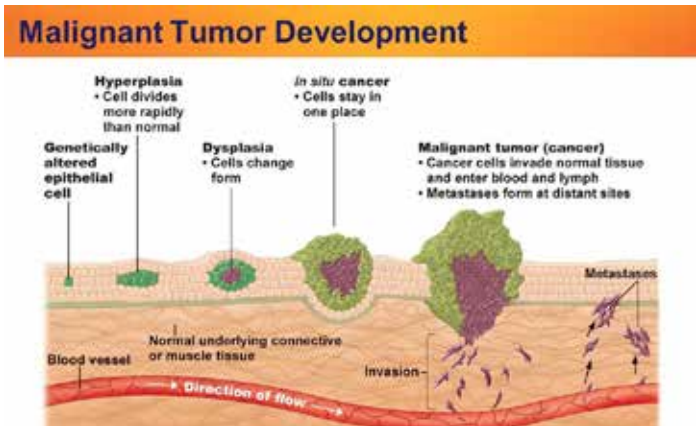
WHAT IS CANCER?

Cells grow,sustain,divide,decay and die.This cycle of cell life is controlled by genes. If genes undergo certain alterations (mutation), the cells grow uncontrollably and form an abnormal growth (tumor).The localized tumors which do not invade the surrounding tissue are called Benign tumors.Those tumors which invade the surrounding tissue and can also spread to distant parts of the body are called as Malignant tumors (Cancer).

Thus Cancer is a genetic disease caused by mutations in genes which control the growth and division of cells.There are three kinds of such genes:

- Facilitators of normal growth and division (Proto oncogenes)
- Suppressors of abnormal growth and division (Tumor suppressor genes)
- Repairer of damaged DNA (DNA Repair genes)

Mutations are either inherited from parents or acquired in the lifetime. A significant number of



Evolution from normal to cancer with intermediary stages

acquired genes is because of exposure to certain substances in the environment. Cancer can occur anywhere in the body, and there are over 100 major types of cancers.

SOME FACTORS THAT ARE KNOWN TO INCREASE THE RISK OF CANCER

- Cigarette smoking, tobacco chewing, Air pollutants
- Infections-Human Papilloma Virus,Hepatitis B and C Virus,Helicobacter pylori
- Radiation-Ultraviolet rays of sun

SOME FACTORS THAT MAY AFFECT THE RISK OF CANCER

- Diet - Diet poor in fruits and vegetables; rich in fats and calories
- Physical inactivity
- Obesity
- Diabetes

PRECANCER

Cancer does not develop suddenly.Theoretically speaking all cancers have a precancerous state. In some cancers these precursor states can be diagnosed by the Histopathologist. These states are called DYSPLASIA/ATYPIA. Many precancers do not progress to cancers. The surveillance system (Immune system) of body is able to detect and overcome these precancerous states. However, with severe and persistent damage to DNA, the precancers may sometimes evolve to cancers.

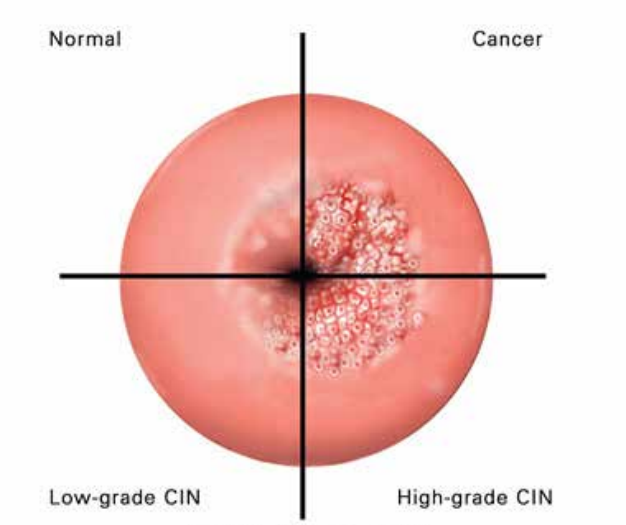


Fig.2 Pre-Cancerous Cells

Normal, Precancer (CIN) and cancer of Cervix

SOME COMMON PRECANCERS

Cervical dysplasia----------	Cancer of Cervix
Oral dysplasia----------	Cancer of Oral cavity
Intestinal Adenoma----------	Cancer of Intestine
Endometrial Atypical Hyperplasia----	Cancer of Endometrium (Uterus)

SIGNS AND SYMPTOMS OF SOME PRECANCERS

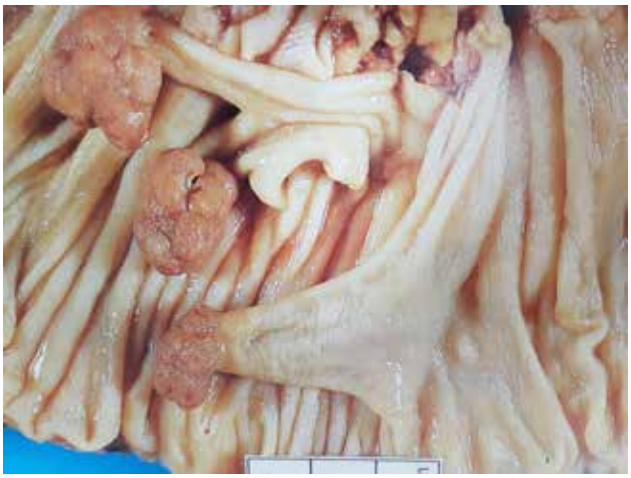
- If one has a Mole/Birthmark and it is increasing in size, changing color or becoming painful
- A white/red patch, nodule, growth in oral cavity or on genitals
- Considerable loss of appetite/weight or fatigability
- A lump in Breast or anywhere in the body

IS CANCER PREVENTABLE?

- HPV vaccination with PAP test screening has considerably reduced the incidence of cancer of Cervix
- Hepatitis B vaccination protects from Liver cancer
- Avoidance of smoking, alcohol intake and exposure to hazardous industrial chemicals reduces the risk of certain cancers
- Moderate exercise and nutritious diet is protective

CAN CANCER BE TREATED?

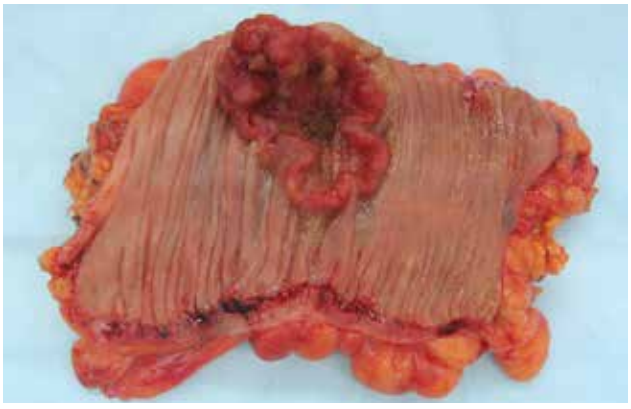
- Cervical dysplasia (precursor of cancer of cervix) is curable by Electrocautery or local surgical resection
- Intestinal Adenoma (precursor of intestinal cancer) is curable by endoscopic Removal
- Treatment of Helicobacter Pylori infection by drugs reduces the risk of stomach cancer
- Many cancers when localized to the organ or with a limited spread are potentially treatable by a wide array of treatment choices(Limited or extensive surgery,chemotherapy, radiotherapy)



Intestinal polyps (precancerous)

ROLE OF HISTOPATHOLOGIST

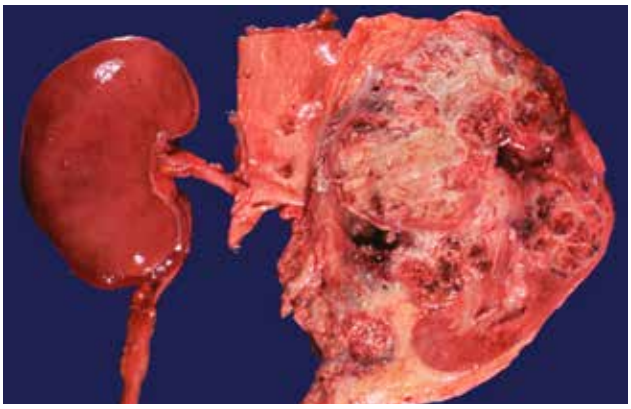
Histopathologist diagnoses precancerous states and the cancers by examining tissue slides under the microscope.He also comments on the tissue of cancer's origin and also about its grade-stage.His opinion on these parameters is final.Based on the histopathology report,an appropriate treatment plan is selected.



Intestinal cancer

SUMMARY

- Cancer is a genetic disease caused by mutations in the genes that control the growth and division of cells.
- Some cancers are preventable to a considerable extent by right life style choices, vaccination, hormones and drugs.
- Precancers, if detected, are mostly curable.
- Some cancers are reasonably treatable by a wide array of treatment choices.
- Treatment of cancer is a meticulously coordinated effort by a Histopathologist, Radiologist, Surgeon, Oncologist and Radiotherapist.
- **Cancer is not a dead end.**



Cancerous Kidney and Normal Kidney

Abbott

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*Huynh D.T.T., Estorninos E., Capeding R.Z., Oliver J.S., Low Y.L. & Rosales F.J. (2015) Longitudinal growth and health outcomes in nutritionally at risk children who received long-term nutritional intervention. J Hum Nutr Diet. doi:10.1111/jhn.12306. ‡48-week clinical study in children at nutritional risk, when given in conjunction with dietary counseling. 1. Fisberg M, Maulen-Radovan IE, Tomo R, et al. Effect of oral nutritional supplementation with or without synbiotics on sickness and catch-up growth in preschool children. Int. Pediatr. 2002;17: 216-222. † Studied in children at risk of malnutrition.

FROZEN SECTION
A USEFUL RAPID DIAGNOSTIC PROCEDURE
DURING SURGICAL OPERATIONS

Dr Surendra Nayak Kapadi
Histopathology Unit, Department of Laboratory
Maternity Hospital

Histopathologists routinely receive tissue biopsy samples for diagnosis from any part of the body-Kidney, Liver, Brain, Bone, Ovary etc. The biopsy sample processing routinely takes a minimum of 24 hours. However sometimes the surgeon needs to know during surgery about the type and extent of the tumors for the right management.

Benign tumors need limited surgery and malignant tumors need extensive surgery. For this rapid intra operative diagnosis FROZEN SECTION technique is applied.This technique needs a special machine called as CRYOSTAT.In this technique tissue sample is frozen and thin sections are cut from a built-in microtome and stained by rapid staining procedure .Total time taken for diagnosis from this technique is around 20 minutes.

Cryostat machine

Indications & Uses of Frozen sections

Rapid diagnosis during surgery especially in case of suspected malignancy.

- To identify the presence or absence of metastatic disease especially in sentinel lymph node.(first lymph node that drains the tumor).
- To assess the surgical margins for the presence or absence of cancer.
- To know the extent of the disease and its pathological staging.
- Rarely also used to demonstrate fats, lipids, certain enzymes, silver demonstration in brain tumors. These contents in the tissue are destroyed in routine paraffin sections where temperature above 56 degree is used during tissue processing.

Limitations

- Tumor is examined only on limited number of sections.
- Sections are not as satisfactory as routine paraffin sections for critical examination.
- Slight distortion of structural details due to lack of embedding media.
- Difficult to get very thin sections and the staining quality is inferior to the routine paraffin sections.

Cryostat

It is a refrigerated cabinet in which microtome is placed in a deep freeze cabinet ,maintained at a temperature of -15 to -30 ^{0}C. All the controls for the microtome are operated from outside the cabinet.

Handling the Tissue Sample

- Tissue sample should reach the frozen section room besides the operation theatre or in the nearby laboratory without any delay.
- It should be sent in a fresh state in a container without any fixative.Tissue should not be sent in a gauze piece because it absorbs water and affects quality of the sections.
- As soon as the tissue is received in the laboratory, accession is done by the technician. The pathologist checks the relevant clinical details on the request form and takes weight, measurements, and records the gross appearance of the surgical specimen.
- Specimens are handled by wearing gown, gloves, goggles and mask to avoid the risk of potential health hazards since the tissue is unfixed.
- Tissue sampling is done by taking tissue from representative, suspicious areas which usually appear different from the adjacent normal areas. Minimum of 3-4 tissue pieces are taken for evaluation when the mass is big. The tissue thickness is usually 3 mm.
- The tissue is kept on the block holder with proper orientation and cryoembedding media is used to cover the tissue section.The Cryostat temperature must be between -15 to -30 ^{0}C. Lymph node, liver, kidney, thyroid, spleen are best cut at the temperature between -12 to -16 ^{0}C whereas breast, muscle, skin are best cut between -18 to -30 ^{0}C.
- Cryostat needs several hours to attain operating temperature and should be kept on all the time and routine maintenance including decontamination should be done.
- Thin sections are cut using disposable blades. The thickness of sections cut are 6-10 µm.Sections are directly attached on to the coated glass slides,air dried,fixed in alcohol and stained by rapid hematoxylin and eosin (H&E) technique.
- The pathologist examines the prepared slides and diagnosis is conveyed verbally to the operating surgeon over the phone and a printed report is sent to the operation theatre immediately.
- Most of the times an experienced Pathologist is able to distinguish the benign from the malignant (cancer). However rarely this distinction could be difficult.In such a situation the surgeon is advised to carry out the limited surgery and wait for the final report on paraffin sections.
- All Frozen section diagnosis are always confirmed and refined with necessary details on paraffin sections prepared by the standard tissue processing technique.

In a hospital carrying out surgeries, Frozen section facility is mandatory.This needs a cryostatmachine, a trained histo-technician and an expert histopathologist.

THE PAP TEST - A SUCCESS STORY OF A PREVENTABLE CANCER

Dr Pushpak Dighe
Cytology Unit, Department of Laboratory
Maternity hospital

PAP smear also called a Pap test is a screening procedure for the cancer of cervix. It detects the presence of precancerous or cancerous cells in the cervix.The test was developed by a Greek doctor Georgious Papanikolaou in 1940.

Cervical cancer is the most common cancer in Indian women and the second most common cancer in women worldwide. However, if detected early, it is preventable. Sexually transmitted Human Papilloma Virus (HPV) infection is the most important risk factor. Other important cofactors implicated in the progression from HPV infection to cancer are - long term use of hormonal contraceptives, multiple sex partners, early initiation of sexual activity, high parity, tobacco smoking and infection with HIV.

HPV is a DNA virus. Over 100 serotypes of HPV have been discovered, of which 15-20 are capable of initiating cancer.The lag period between the HPV infection and the appearance of cervical cancer is 10-15 years. High risk type HPV 16 and 18 contribute to over 70% of all cervical cancers.

What are cervical cancer screening guidelines?

The US Preventive Services Task Force (USPSTF) and the American Cancer Society (ACS) recommendations are :

Age	Pap Smear Screening frequency
< 21 years,not sexually active,no known risk factors	Not needed
21 - 29 years	Every 3 years
30 - 65 years	Every 3 years
> 65 years	Not needed

How to prepare for a Pap smear?

• Let your doctor know if you are menstruating,because it may affect the results.

• Avoid sexual intercourse or use of spermicidal products the day before the test.

The procedure of taking Pap smear is fairly simple and quick where the Gynecologist uses a cytobrush to take cells from the cervix and sends the sample to the laboratory in an appropriate container.

Thin Prep Machine

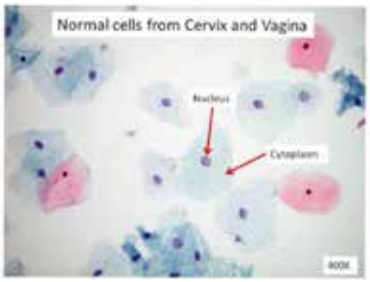
Screening programs for cervical cancer are done by Conventional

Pap smear technique or Liquid based cytology (LBC). Nowadays, LBC is the method of choice in many laboratories across the globe.ThinPrep Pap test and SurePath Pap test are the two liquid based cytology tests approved by the US Food and Drug Administration(FDA).

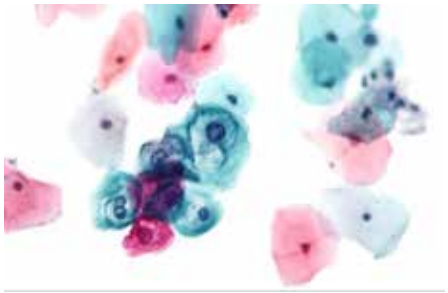
Reporting

The Bethesda System(TBS) for reporting cervical/vaginal cytology was developed in 1988 at the National Institutes of Health in Bethesda, Maryland to provide uniform diagnostic terminology that would facilitate communication between the cytologist and the clinician. Further Bethesda workshops were held in 1991,2001 and 2014 and newer criteria and terminologies were introduced.

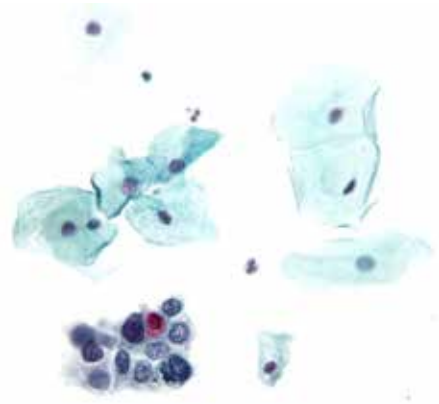
The Bethesda system recommended a 2-tiered reporting system for squamous intraepithelial lesions(SILs): low-grade SIL(LSIL) and high-grade SIL(HSIL).A normal Pap smear is reported as Negative for intraepithelial lesion or malignancy.



Normal smear



LSIL



HSIL

Prevention of cervical cancer

To reduce the risk of cervical cancer

- Get vaccinated against HPV. HPV vaccines are recommended for girls between 11-13 years old
- Get routine Pap test done every 3 years
- Practice safe sex
- Avoid smoking

CONCLUSION

Pap test can effectively diagnose precancerous lesions. LSIL are followed up by PAP test for any regression or progression. HSIL are further confirmed by biopsy procedure and can be treated by limited local surgical procedure.

In developed countries, the widespread use of cervical Pap smear screening program has reduced the incidence of cervical cancer by 50% or more. Current preventive vaccines reduce, but do not eliminate the chance of getting cervical cancer. Therefore, experts recommend that women combine the benefits of both programs by seeking regular Pap smear screening,even after vaccination.

EXAMINATION OF BODY CAVITY FLUID BY CYTOLOGY FOR DETECTION OF CANCER CELLS

Dr Meera Balakrishan
Cytopathology Unit, Department of Laboratory
Mubarak Al Kabeer Hospital

Cytological study of body fluid is a complete diagnostic modality. In 1943, Papanicolaou and Traut popularized the cytological methods for body fluids. The information provided by body fluids like urine, blood or body-cavity fluids analysis serves several functions. First, it assists the clinician in formulating and pointing out the etiology of effusion and list of differential diagnoses. Second, it allows one to follow the results of therapy and prognosis. The appearance of malignant cells in body fluids is a clear indication for the existence of a tumor. Therefore, the examinations of urine or body-cavity fluid by cytologic methods are routine diagnostic tests today.

The accurate identification of cells as either malignant or reactive mesothelial cells (the lining cells of body cavities) is a diagnostic problem in conventional cytological smears. Distinguishing benign from malignant cellular changes may require meticulous screening, careful scrutiny of cellular features and an understanding of the range of reactive changes. Since cellular overlapping, artifacts, suboptimal processing and preparatory cytotechnique causes lower diagnostic yield in conventional smear methods, the residual material can be very useful in increasing diagnostic yield by the use of cell block method. The cell block (CB) technique is one of the oldest and complementary methods for the evaluation of body cavity fluids. Bahrenberg first described the cell-block method and in the year 1928. Zemansky concluded that the cell-block method was superior to the smear technique. Cell block preparation increases the sensitivity of detecting malignancies, and also has the ability to reduce false-positive interpretations. This method is simple and inexpensive which requires no extra material compared to other methods. The main advantages of the CB technique are preservation of tissue architecture and obtaining multiple sections for special stains and immunohistochemistry.

The smears and sections are examined with the low-power objective using a microscope. Suspicious cells and cell clusters are marked by an ink dot, and these are then checked with higher magnification. If no clumps or suspicious cells are seen with low-power magnification, the smear or section is then checked with high power. The criteria for malignancy that we use are those suggested by Papanicolaou. In general, the tumor cells are seen in clusters. Whenever possible, biopsies are obtained to confirm positive diagnoses made by the cytologic methods.

Types of Body fluids

Pleural and Peritoneal Fluid: Cancer cells in pleural or ascitic fluid are almost indicative of metastatic cancer, as tumors arising from mesothelial cells lining these spaces are rare. When present, the tumor cells are usually numerous, and frequently found in clusters. The demonstration of mucin in tumor cells is evidence that they arise from glandular epithelium. The occasional tissue fragments occurring in these smears may be broken or may be thick, so that cells are difficult to examine. However, single cells

or small clusters are much better preserved and more readily identified in smears than in cell blocks. At times, mesothelial cells may be confused with tumor cells, especially if the fluid has been allowed to stand before fixation. The nuclear membrane may become dense and the nucleoli prominent, vacuoles are common, and mitoses may be found. Mesothelial cells, however, will be found evenly dispersed throughout the smears or cell block and their cytoplasm appears continuous.

Sputum: Sputum cytology is a medical test in which a sample of sputum (mucus) is examined under a microscope to determine whether abnormal cells are present. Sputum is different from saliva, and is produced in the lungs and the airways leading to the lungs. It is best to collect the samples first thing in the morning. To obtain the best results you should remove dentures if you wear them, rinse your mouth with water and take about four deep breaths followed by a few short coughs, then inhale deeply and cough forcefully into the container. Indications of sputum examinations are 1) lung cancer 2) noncancerous lung conditions, such as pneumonia or inflammatory diseases, tuberculosis, or the buildup of asbestos fibers in the lungs (asbestosis).

Bronchial Brush, Bronchial Wash and Bronchio-alveloar Lavage: Bronchial brushing and wash is a procedure in which cells are taken from the inside of the airway mucosa or bronchial lesions through catheter-based brushing under direct visualization or fluoroscopic guidance. Flexible brushes are passed through the bronchoscope, and the bronchial surface is gently abraded to obtain the specimen. It is used for the detection and characterization of pulmonary lesions which are either malignant pulmonary lesions and/or identification of some microbiologic pathogens (primarily viral and fungal).

Bronchoalveolar lavage (BAL) is a diagnostic procedure by which cells and other components from bronchial and alveolar spaces are obtained for various studies like diagnosing bacterial pneumonias, tuberculous lesions, fungal infections, and malignancies.

Brush cytology in Oesophageal and Gastrointestinal Tract (GIT) Lesions: Brush cytology often complements and increases the sensitivity and specificity of detection of oesophageal and GIT lesions. Brushing of the mucosa covers a larger surface area, provides rapid interpretation and early diagnosis. It is less invasive and a risk-free technique compared to biopsy. It is more useful when the lesion is large or multiple. It has also the advantage of penetrating to the basement membrane and collecting cells from sub-epithelial layers. It is used in diagnosing fungal infections like Candida and to rule out pre malignant and malignant lesions.

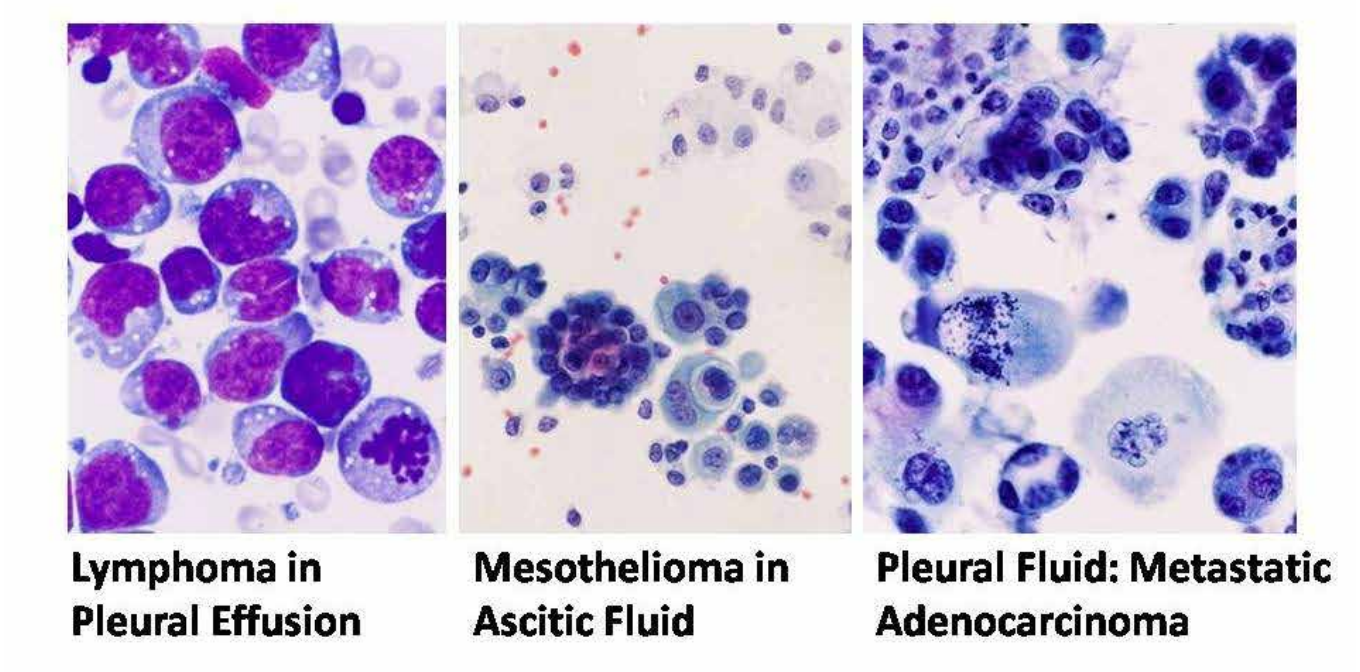
Urine: Either catheterized or voided specimens of urine may be used for cytological examination. This test is best performed on three consecutive days, each day a fresh specimen is collected. In case of voided urine collection does not collect the first urine of the day, a mid stream urine sample must be collected for cytological examination. Catheterized specimens usually contain more and better preserved cells. The diagnosis of cancer should be based on clusters of malignant cell. Cancer cells from various parts of the urinary tract are similar, so that their site of origin is not evident by this method.

Spinal Fluid: The amount of sediment obtained from spinal fluid is inadequate for the preparation of cell blocks, but direct smears may be prepared. Analysis of CSF may exclude infectious, inflammatory and neoplastic diseases affecting the central nervous system. The most common purpose is in suspected meningitis. Malignant cells, when present, may be scant but exhibit the same morphologic changes as neoplastic cells in other sites. The presence of carcinoma cells in cerebrospinal fluid is indicative of meningeal involvement. CSF cytology is also diagnostic in Leukemia, lymphoma and primary CNS malignancies.

Aspirated Purulent Exudates: Cytologic examinations of aspirated material from lesions thought to be infectious and are sent for routine and Mycobacterial culture and sensitivity.

Summary

The examination of body fluids for the presence of malignant cells is a proved and accepted routine diagnostic procedure. In the examination of such fluids care must be taken to obtain good fixation of fresh material. Any routine stain may be employed, but Papanicolaou stain offers excellent nuclear and cytoplasmic details. Either the smear technique or the cell-block method may be employed. False-negative diagnoses may be expected in many instances. If any doubt exists, more material or a biopsy should be requested. Positive diagnosis should be confirmed by biopsy whenever possible. Lastly, The presence of organisms in smears or cell blocks should not be ignored by the pathologist.



Effusion Cytology

Suggested reading

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2. Jain D, Mathur SR, Iyer VK. Cell blocks in cytopathology: a review of preparative methods, utility in diagnosis and role in ancillary studies. Cytopathology. 2014; 25:356-71.

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FINE NEEDLE ASPIRATION CYTOLOGY: IT'S ROLE IN THE DIAGNOSIS AND MANAGEMENT OF DISEASES

Dr Shahed Khan
Cytology Unit, Department of Laboratory
Mubarak Al Kabeer Hospital

What is Fine-needle aspiration (FNA)?

FNA is a diagnostic procedure used for investigating lumps or masses. In this technique, a thin (22 to 25 gauge), disposable needle attached to a disposable plastic syringe is inserted into the mass for sampling of cells. The syringe is fitted with a Franzén handle to facilitate the process of FNA. The smears prepared from the FNA material after being stained, are examined under a microscope. As with other types of biopsies, the sample collected during fine needle aspiration can be of help in making a diagnosis of specific and non-specific inflammation/ infections and to or rule in/ rule out conditions such as cancer. Fine needle aspiration is generally considered a safe procedure. Complications are infrequent.

Historical perspective

First reports of FNAC as a technique for obtaining the diagnostic material was performed at St. Bartholomews hospital, London in 19th century; it was undertaken on a large mass in the liver by surgeons Stanley and Earle. First large scale study was carried out at the Memorial hospital, New York, around 1921, by pioneering team of Martin, Ellis and Stewart. Despite the pioneering work by American pathologists, the technique initially did not have a following among American pathologists. It was in Europe, and particularly Scandinavia, that FNAC, as a technique, began to flourish in 1950's and 1960's, by Lopez-Cardozo in Netherlands and Soderstrom in Sweden. Josef Zajicek was among the first of pathologists to embrace FNA in collaboration with Sixten Franzén at Karolinska hospital, Sweden and applied the requisite scientific rigor to define precise criteria and to determine diagnostic accuracy in variety of conditions. Disciples of these pioneers have spread the gospel to Europe, America, Australia and Asian countries like India, China and Japan. The technique is now part of the service in all sophisticated laboratories of pathology. Fine-needle aspiration cytology is a safe, minor surgical procedure. Often, a major surgical (excisional or open) biopsis can be avoided by performing fine needle aspiration cytology instead. Today, this procedure is widely used in the diagnosis of cancer and inflammatory conditions. When used sensibly, aspiration cytology offers a relatively cheap, quick, and accurate tool for the diagnosis and follow-up of cancer.

FNAC as a tool in clinical investigation and role of imaging guidance

FNA was initially used as means of confirming a clinical suspicion of local recurrence or metastasis of known cancer without subjecting the patient for further surgery. Following the success in this area, the FNAC is also performed for preoperative diagnosis of all kinds of lumps or a tissue masses (tumor) in

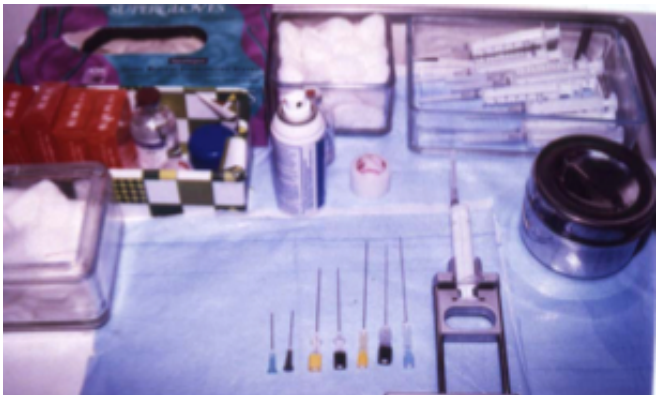
any organ/ tissue of the body, when its nature is in question. When the lump can be felt, the biopsy is usually performed by a cytopathologist or a surgeon. In this case, the procedure is usually short and simple. This method is applicable to lesions that are easily palpable, such as superficial growths of skin and soft tissues, and other organs such as thyroid, breast, salivary glands, and testes and superficial lymph nodes of neck, axilla (arm pit) and groin. Otherwise, it may be performed by an interventional radiologist under ultrasound or CT guidance in non-palpable lesions or in lesions of deep seated organs in chest and thorax such as lung, pleura, mediastinum and abdominal organs like liver, kidney, ovary, adrenal glands and retroperitoneal lymph-nodes. Using endoscopy, doctors can also reach areas deeper in the body. An endoscopy uses a flexible tube with a light and camera attached. During an endoscopy, a doctor can do a fine needle aspiration on certain abnormal spots in the chest or abdomen. In certain organs such as mediastinal and hilar lymph nodes as well as Pancreatic mass, FNAC is usually performed under guidance of endoscopic ultra-sound (EUS). Intraoperative cytology during surgery is another application of FNAC.

Preparation

FNA does not require any elaborate preparation unlike the surgical procedures. However, patients taking aspirin or other blood thinners, such as Plavix (clopidogrel) or Warfarin are advised to stop it 5 days prior to the procedure. The patient should do blood tests (bleeding profile) in case he/she is on blood thinners.

Technique of FNA

For the procedure, a written consent is taken before performing the FNAC. For people undergoing fine needle aspiration through the skin (percutaneous FNA), the skin over the area of the procedure is cleaned with antiseptic solution. The area may be sprayed with a local numbing medication (anesthetic spray) over the skin, at the site of aspiration. Ultrasound may be used during the procedure, in cases of non-palpable lesions. This helps to locate the right area for fine needle aspiration. A thin needle attached to a syringe is inserted through the skin into the abnormal area or mass/lesion. A vacuum inside the syringe causes body fluid or tissue to be suctioned (aspirated) into the needle and syringe. The needle is detached, and smears are prepared from the drops at the tip of the needle. The procedure can be repeated with new needles. The cytological specimens are stained with May-Grünwald-Giemsa (MGG) and modified Wright stain (Diff-Quik) and Papanicolaou stains before being evaluated by a cytopathologist. People undergoing fine needle aspiration especially from mediastinal lymph nodes/mass or from pancreatic or gastro-intestinal mass, during endoscopy undergo an additional preparation, such as fasting and sedation. Endoscopic procedures usually take longer time than fine needle aspirations through the skin. The biopsy sample is examined under a microscope right away. This helps to 1) Verify that a adequate sample was obtained and 2) help in making a rapid diagnosis. The cytopathologist should indicate the degree of certainty when offering a diagnosis, making clear when a clinical or radiological correlation is required and when histological confirmation is necessary.



FNA Equipment

FNA Clinic

Advantages of fine needle aspiration cytology (FNAC)

FNAC is safer and less traumatic and gives fast results and is cost-effective than an open surgical biopsy. The procedure is less painful, so even the children can tolerate it. It is sensitive and specific for the diagnosis of cancer (malignancy) and is an out-patient procedure. The rapid diagnosis possible with FNA can shorten or avoid hospital admissions, and speed up a patient's referral to an appropriate specialist.

In symptomatic breast disease, FNAC used alongside clinical and radiological assessment allows rapid, inexpensive, and accurate diagnosis. However, review of published series has shown that core biopsy with histology is more sensitive and specific than fine needle aspiration in diagnosing most impalpable radiological lesions. Histology facilitates assessment as to whether carcinoma is invasive or in situ, and gives some indication of grade and subtype of carcinomas. Thyroid nodules are common; most are part of the spectrum of nodular goiter. Fine needle aspiration is most sensitive at detecting anaplastic (almost 100%) and papillary (around 90%) carcinomas. Although FNAC has greater accuracy in identifying tumors than alternative imaging or biochemical methods, it misses 5-10% of cancers. . Even so, its incorporation into the diagnosis of thyroid nodules reduces the requirement for excision by at least 25% and doubles the yield of cancer in those that are excised. Cytology is ideal for confirming metastasis from a clinically or radiologically suspected primary site and distinguishing between limited alternatives such as small cell or non-small cell lung cancer. Testicular fine needle aspiration (FNA) is an established technique for the evaluation of testicular and intrascrotal tumors. Recently however, testicular FNA has gained popularity as both a diagnostic and therapeutic tool for the management of clinical male infertility.

Final results of testing after a fine needle aspiration can be ready in one day or longer. In difficult cases it may be longer when it requires special staining or immunocytochemistry. In these cases cell block can be prepared, and immunocytochemistry can be done on cell block sections. In certain cases of infections, the needle washings or the aspirated fluids can be sent for routine culture or mycobacterial culture and sensitivity to rule out of some specific infections, and antibiotic sensitivity can be performed on the organisms grown, so that specific treatment given with the sensitive antibiotics.

Used appropriately, FNAC remains a powerful tool in the diagnosis and management of patients with malignancy. Diagnosing metastatic or recurrent malignancy by FNAC generally has a high specificity and sensitivity.

Limitations/complications of the procedure

Common complications include bruising and soreness and other complications like minor bleeding under the skin at the biopsy site can occur. This can result in a tender, swollen area called a hematoma. Some serious complications like blood infections (septicemia) and pneumothorax (air in the thorax) in case of lung FNAC and abdominal infections (peritonitis) in case of abdominal FNAC are extremely rare. Infection at the biopsy site is also rare, because sterile techniques and equipment are used for all fine needle aspirations. The risk of complications from fine needle aspiration during endoscopy is slightly higher. But it is still quite low for most people. Used sensibly, aspiration cytology offers a relatively cheap, quick, and accurate tool for the diagnosis and follow-up of cancer.

Suggested Reading

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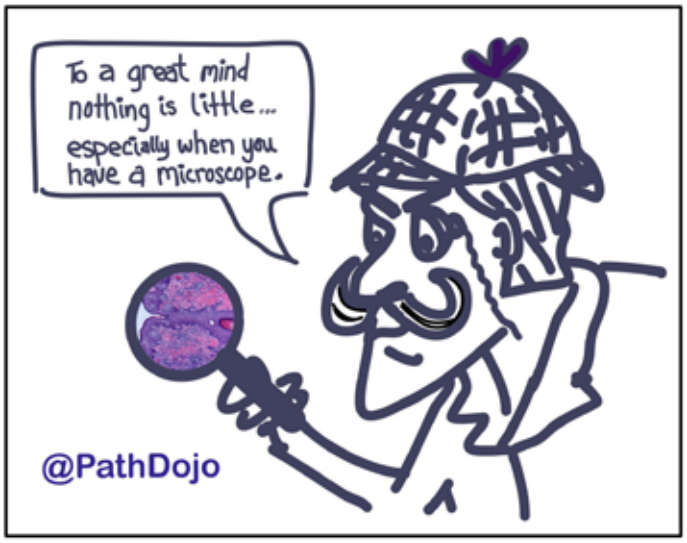
2) Fine Needle Aspirate; a Vital Technique in the Characterization of Masses and Lesions. Okoye JO, Okoye FO Ann Clin Cytol Pathol (2015)1(2): 1008



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THYROID CANCER: IT'S DETECTION BY FINE NEEDLE ASPIRATION CYTOLOGY

Dr Dilip K. Das
Department of Pathology, Faculty of Medicine
Kuwait University
Cytology Unit, Department of Laboratory
Mubarak Al Kabeer Hospital

The thyroid gland situated in the front and lower part of neck, below and anterior to the larynx, consists of two lateral lobes connected by a thin isthmus. Thyroid is formed by follicles lined by follicular cells which form colloid from the trapped iodine and store it in the central lumen of follicles; thyroid hormones are synthesized from colloid by follicular cells and release into the circulation through interfollicular capillaries. The thyroid hormones stimulate the carbohydrate and lipid catabolism, and protein synthesis in various cells, with net result of increase in basal metabolic rate. Thyroid hormones also play an important role in brain development in foetus and neonates. When iodine is deficient in food, the thyroid undergoes hyperplasia and thereby enlarges to perform extra work in order to meet the demand of excess colloid. 1998 World Health Report reveals that the prevalence of goitre (thyroid enlargement) due to iodine deficiency is nearly 844 millions. Thyroid also enlarges when the regulation of body's won defence system becomes faulty and as a result it tries to destroy its own thyroid follicular cells giving rise to an autoimmune disease called Hashimoto thyroiditis. Besides radiation, Hashimoto thyroiditis is a risk factor for papillary thyroid cancer, the commonest cancer of thyroid. Both Hashimoto thyroiditis and thyroid cancer are common in Kuwait, the latter being second to third most common cancer among females.

To diagnose various conditions giving rise to thyroid enlargement clinical examination, thyroid hormone estimation, ultrasonography, iodine 123 or technetium Tc99m scan, and biopsy are various diagnostic modalities available in our hospitals. It is not possible to know by clinical examination as to which thyroid swelling harbours a cancer. Scintigraphy (Iodine 123 or technetium Tc99m scans) and ultrasonography help in the diagnosis of thyroid lesions which may present as diffuse or nodular enlargement. Scintigraphy is a useful method in differential diagnosis of cold thyroid nodules. Ultrasonography (US) can visualize the thyroid nodules with remarkable clarity and provide structural information about location, number, size and consistency of the nodule. But these modalities cannot diagnose neoplastic lesions including cancer with certainty. Thyroid biopsy with pathological examination can only give the final answer for thyroid malignancy. However, thyroid being a highly vascular organ, surgical biopsy or even a thick needle biopsy is not an ideal modality for getting a tissue diagnosis of thyroid diseases. Fine needle aspiration (FNA) cytology of the thyroid, on the other hand, is a simple, safe, inexpensive and minimally invasive procedure without appreciable complications and side effects for the diagnosis of thyroid lesions and more so for nodular thyroid disease. Therefore, in most Centers, FNA cytology has supplanted imaging studies as the routine initial procedure to separate benign from malignant thyroid nodules. A review of 10 studies shows that the average frequency of benign, suspicious/ indeterminate, malignant and inadequate cases are 65.7%, 17%, 5.5% and 11.8%, respectively. The

sensitivity, specificity and diagnostic accuracy of FNA cytology for thyroid nodules is 85.2%, 95.6% and 94.4%, respectively, indicating that it is a very useful diagnostic feature. Whereas in international scenario, papiilary thyroid cancer comprises 67% to 87% of all thyroid cancers, at the Cytology Unit of Mubarak Al Kabeer Hospital, inflammatory goiter, most of which are Hashimoto thyroiditis, accounts of 20% of all thyroid FNAs and papillary thyroid cancer is nearly 65% of all thyroid neoplasms, benign or malignant.

Most of the patients with thyroid enlargement are females.When there is mild enlargement of thyroid, it gives rise to fullness of neck resembling that of a swan and adds to beauty but when the enlargement goes beyond that and becomes irregular due to development of nodularity, it is a cosmetically challenging situation. The patient may also be emotionally disturbed from a hyper- or hypothyroid state. The fear of cancer may further add to this problem. In such a situation,FNA cytology rather than a more radical procedure like core biopsy would be the ideal diagnostic tool of choice. Infact, because of the simplicity of the procedure and lack of complications, it is readily accepted by patients including children, and can be repeated when necessary, during the initial diagnosis and follow-up of the cases. Of the FNA cytology, clinical data and laboratory findings, FNA cytology alone has the highest diagnostic values. FNA cytology is a cost effective diagnostic tool in thyroid nodules which can save substantially on health care resources. FNA cytology of cold thyroid nodules gives more diagnostic information than nodules size, dynamic radio-isotope scan or ultrasonography studies. Since morphological diagnosis is readily available, time consuming and expensive investigations are bypassed.

FNA Cytology in the Diagnosis of Developmental Anomalies of Thyroid and the Associated Pathological Lesions

Thyroid in fetal life migrates from base of tongue to its position from the lower part of neck and following this the tubular travel path disappears. On rare occasion the thyroid fails to migrate and remains as a swelling at the base of tongue (lingual thyroid). If accidentally removed by surgery without proper identification, the patient has to take thyroid hormone supplement throughout life. Sometimes the tubular travel or migratory path (duct) along with residual thyroid tissue persists and appears as a swelling in adult life (thyroglossal duct cyst). The descent of thyroid can also be excess, in which case thyroid may go further down to be located inside the chest or can be displaced laterally to be confused as a lymph node mass. As a simple tool, FNA cytology helps in the identification of lingual thyroid and thereby prevents its accidental removal by surgery; FNA cytology has also been utilized for the diagnosis of thyroglossal duct cyst and malignant lesions associated with the cyst, ectopic thyroid and carcinoma arising in it, and substernal thyroid in the chest.

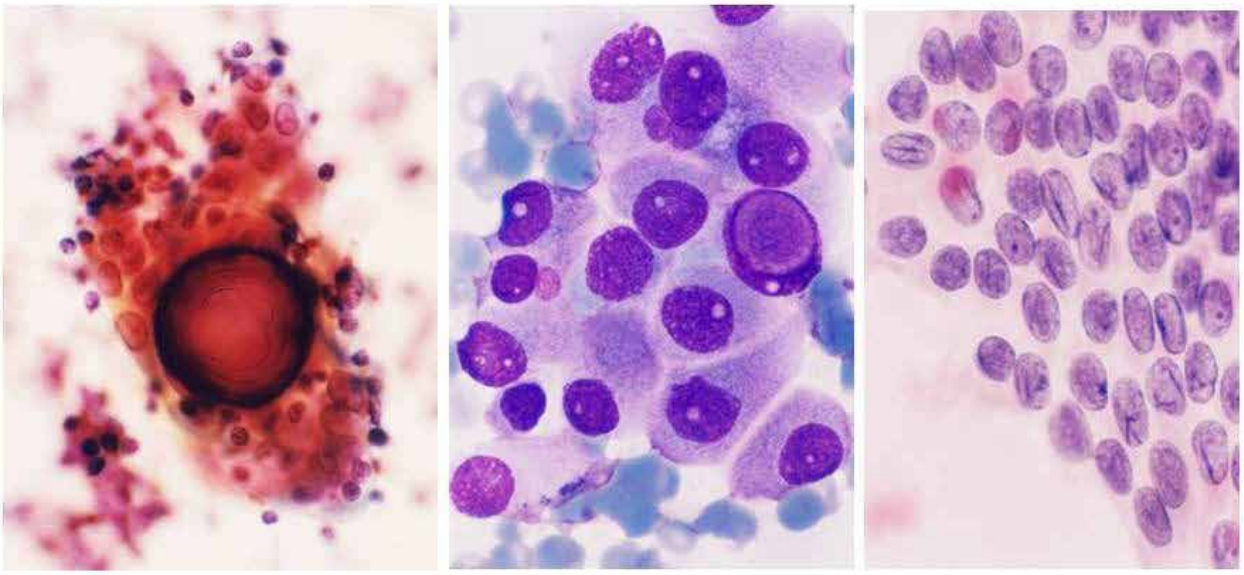
FNA in the Diagnosis of Acquired Lesions of the Thyroid

The cytologically diagnosed benign lesions comprise non-toxic hyperplasia (colloid goiter), diffuse toxic hyperplasia (thyrotoxic goiter or Graves' disease) and thyroiditis (acute, subacute and chronic). The malignancy cases are papillary carcinoma, medullary carcinoma, anaplastic carcinoma, and malignant lymphoma. The most common swelling of the thyroid is because of iodine deficiency goiter in which accumulated colloid and associated hemorrhage (bleeding) may give one or more nodules raising suspicion regarding malignancy. FNA not only offers a benign diagnosis (with great relief from the fear of malignancy), it also evacuates the contents, which acts as a therapeutic (treatment) measure.

The sudden disappearance of the swelling following evacuation of its content may give rise to an expression of surprise and disbelief in the patient's face. Subacute or viral thyroiditis follows an episode of viral infection of the upper respiratory tract (throat) and presents as a painful and hard swelling of the thyroid raising suspicion of cancer. The patient shows the features of hypothyroid state in the following few weeks. By FNA cytology not only the diagnosis of a subacute (granulomatous) thyroiditis is given, the patient is assured that this swelling and hypothyroid state is temporary, and he/she is going to recover in a few weeks. In case of chronic lymphocytic or autoimmune thyroiditis, on the other hand, such assurance is not possible since the patient requires follow up throughout life with repeat FNA to monitor the progress of the disease and to exclude emergence of associated malignancy like papillary thyroid carcinoma and lymphoma. But the diagnosis of Hashimoto thyroiditis itself helps in the management of hypothyroid and much less frequently a hyperthyroid state of the patient (Hashitoxicosis). The patients suffering from Hashimoto thyroiditis sometimes present in a state of depression with history of treatment by a psychiatrist for variable length of time and the FNA diagnosis changes the approach to management completely.

By FNA cytology, papillary thyroid carcinoma and medullary carcinoma, which have well-defined cytomorphological features, can be easily diagnosed. Among the malignant cases, papillary thyroid carcinoma, which has an excellent prognostic outcome (with 20-year survival up to 90% and 30-year-survival up to 80%), accounts for 70% to 85% of all malignancies. However, despite all its merits, FNA unfortunately cannot distinguish between a benign follicular adenoma and follicular carcinoma and therefore, the term follicular neoplasm is used with an advice to rule out malignancy by histopathology.

FNA cytology is the preferable initial investigation as compared to 99 mTc scan and ultrasonography (US), and usually gives results adequate for a decision on surgical or medical management. However, ultrasonography has the advantage in locating the most appropriate nodule for FNA and guiding the needle in small and non-palpable nodules. Further, FNA is known since long as a useful tool in assessing the need for surgery in high risk patients and in selecting patients for thyroid suppression therapy. With adequate clinical follow up, FNA cytology can accurately and approximately guide the non-operative management of nodular thyroid disease, and that the technique does spare patients the cost and risk of thyroidectomies both initially and during subsequent follow up evaluation. A review of studies shows that as a result of FNA cytology, the rate of reduction of surgery ranged of from 21.5% to 35% with an average of 26.3%. Furthermore, as a result of FNA cytology and selected surgery, the yield of cancer also increased by 2% to 33% with an average of 16.3%. During the recent years, the results of FNA cytology has been supplemented by immunocytochemistry for galectin3, CD44, HBME1 and CK19 as well as molecular parameters like RET-PTC rearrangements, and RAS and BRAF mutations with a hope for improved management of malignancies in future.



FNA Cytology features of papillary thyroid carcinoma

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3. Baloch ZW, LiVolsi VA, Asa SL, et al. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: A synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. Diagn Cytopathol 2008; 36:425-437.

> A natural law regulates the advance of science. Where only observation can be made, the growth of knowledge creeps; where laboratory experiments can be carried on, knowledge leaps forward.
>
> — Michael Faraday


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BREAST CANCER MANAGEMENT: ROLE OF FINE NEEDLE ASPIRATION

Dr Meera Balakrishan
Cytology Unit, Department of Laboratory
Mubarak Al Kabeer Hospital

Palpable breast mass is a common problem in female patients. Breast cancer is the most common cancer in women both in the developed and the developing countries. Incidence rates vary from 19.3 per 100,000 women in Eastern Africa to 89.7 per 100,000 women in Western Europe. In 2008, breast cancer caused 458,503 deaths, and as per World Health Organization estimation, over 508,000 women died in 2011 due to breast cancer worldwide. Many early breast cancers are asymptomatic when they were identified during a cancer screening program. Larger breast cancers may present as a painless breast mass. Early diagnosis and treatment of breast cancers are very important because of high incidence and mortality rates. Therefore, it is essential to evaluate tissue diagnosis in clinically suspicious breast masses. Most countries have now adopted a triple assessment approach, i.e. clinical, imaging and pathology for breast diagnosis, with FNAC as the first-line pathological investigation in both screening and symptomatic populations. Cytology is a well established method of investigating breast lesions in symptomatic patients, but there is little recorded information about its use as part of a well woman screening program.

Fine-needle aspiration cytology (FNAC) of the breast is a minimally invasive diagnostic method. It is cheaper to perform and its results can be available within a short time. Moreover, owing to finer needle size, it is easier/safer in certain lesions, such as very small lesions, lesions just under the skin or very close to the chest wall compared with core biopsy. In addition, FNAC maintains tactile sensitivity, allows multidirectional passes allowing a broader sampling of the lesion and immediate reporting where necessary. For the procedure, a written consent is taken before performing the FNAC .The skin overlying the mass is prepared with betadine. An ordinary 21 to 23 gauge needle is introduced into the tumor, and suction is applied with a 10 ml plastic syringe fitted to a Franzen handle. The needle is detached, and smears are prepared from the drops at the tip of the needle. The procedure is repeated twice with new needles. The cytological specimens are stained with May-Grünwald-Giemsa or modified Wright (Diff-Quik) stain and Papanicolaou stain before being evaluated by a cytopathologist.

Indications

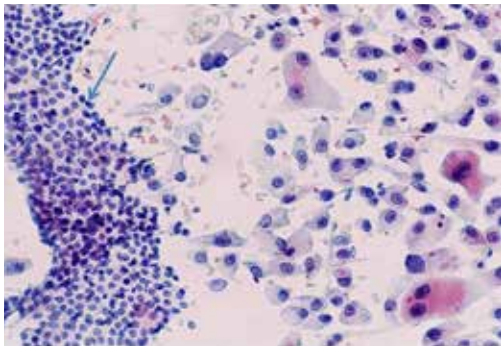
1. Palpable breast lesion
2. Clinical staging: suspicious lymph node
3. Appearance of new lesion in a patient treated for breast cancer
4. Evaluation of biomarkers like estrogen receptor, progesterone receptor and HER2 for management of patients with breast cancer

5. Metastasis, and
6. When core needle biopsy (CNB) technique is not available.

Accuracy of diagnosis by FNAC

It is well known that FNAC has sensitivity of 92.7% and a specificity of 94.8%. False positivity of the procedure is generally low. FNAC helps in diagnosis of inflammatory breast lesions like breast abscess caused either due to bacterial infections or tuberculosis and can be combined with further workup including microbiologic culture correlation. It helps in management and treatment of the patient and avoiding unnecessary surgery. However, in certain cases it is less reliable. For example, differentiating invasive cancer from ductal carcinoma in-situ (DCIS) is not possible and may be limited in some cases in the assessment of tumor grade and type. An initial indeterminate FNAC result would lead to further assessment; either further sampling of the lesion by core biopsies, vacuum-guided biopsies or surgery or, occasionally, with less suspicious lesions, an early recall of patients for further mammograms and assessment in 6–12 months time as a cancer is likely to be picked up eventually.

FNAC is also an operator-dependent procedure and reporting of breast cytological results is more demanding than histological analysis, and requires more experience on the part of a pathologist/cytopathologist. As cellular samples leading to overcrowding of cells in the smears limit identification of the tumor grade, pathologists specialized in cytopathology are best qualified to collect and interpret FNAC samples, but this is not always possible or practical. Radiologists involved in breast imaging should ensure that they have the necessary skills to carry out FNAC under image guidance. Best results are achieved on the lump by the image-guided FNAC in the presence of the cytopathologist.



A compact cluster of Benign breast cells and numerous dissociated breast cancer cells in a fine needle aspiration smear from a case of breast cancer (Apocrine carcinoma of breast) Cytology

To sum up, FNAC has its own advantages with its

1. Rapidity of diagnosis
2. High acceptance
3. Cost-effectiveness
4. High sensitivity and specificity
5. Ability to sample multiple areas at a single go
6. Preoperative planning
7. Sampling of metastatic as well as the primary site
8. Performance of ancillary techniques, and
9. A rapid psychological relief to the patient following a negative diagnosis

Despite the fact that core needle biopsy has progressively replaced FNAC in the investigation of non-palpable lesions or micro-calcifications without a clinical or radiological mass lesion, FNAC has yet a role in palpable lesions and is a valuable diagnostic tool. Adhering to the principle of "Triple-test,"

and acquisition of technical, observational, and interpretative skills will further enhance the diagnostic accuracy of proliferative conditions with atypia or suspicious lesions of breast.

Nipple discharge is the next most common complaint of patients seeking medical attention for breast disease, accounting for about 5% of all breast symptoms. Nipple discharge can be either pathologic or physiologic. Pathologic nipple discharge typically is unilateral; involves a single duct, and is spontaneous; it may be serous (clear and watery) or bloodstained in nature. Physiologic nipple discharge is usually bilateral, involves multiple ducts, and is white or green. Alcohol fixed and air-dried smears are prepared from the nipple discharge and examined under the microscope to arrive at diagnosis such as benign, atypia and malignancy.

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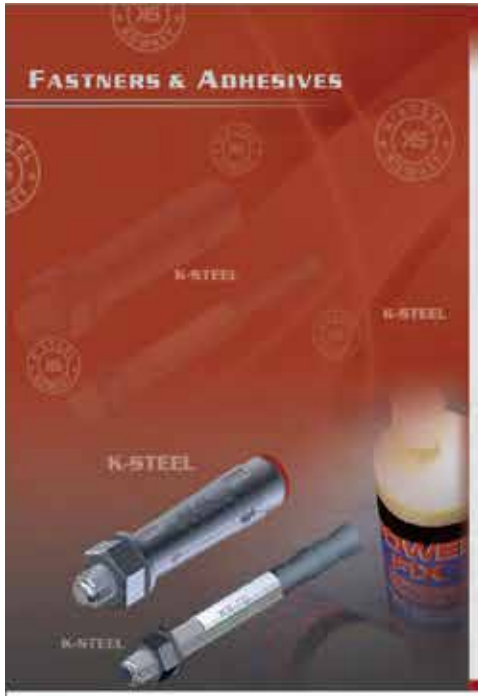

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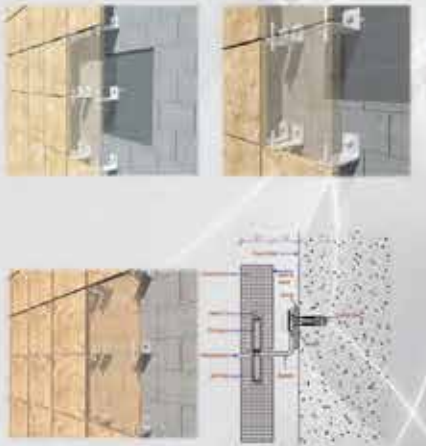

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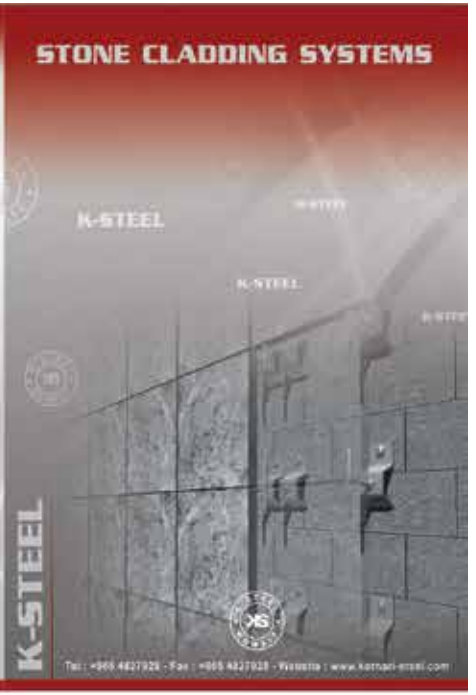

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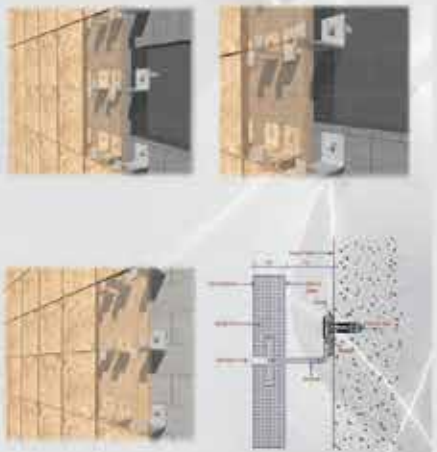

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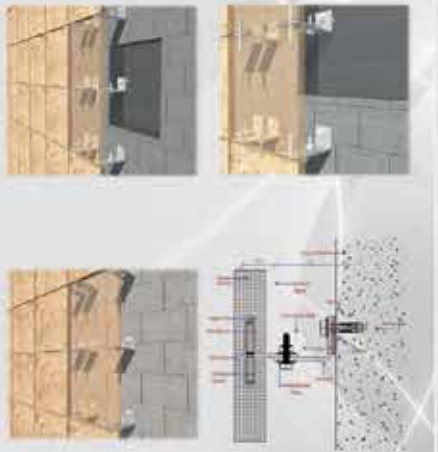

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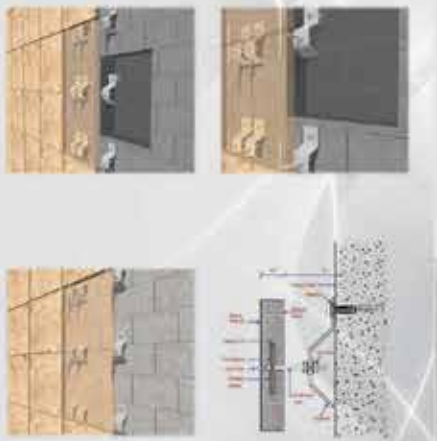


Large Panel System

LYMPH NODE ENLARGEMENT IN NEOPLASTIC AND NON-NEOPLASTIC DISEASES: CONTRIBUTION OF FINE-NEEDLE ASPIRATION CYTOLOGY IN DIAGNOSIS AND MANAGEMENT

Dr Thasneem Amir
Cytology Unit, Department of Laboratory
Kuwait Cancer Control Center

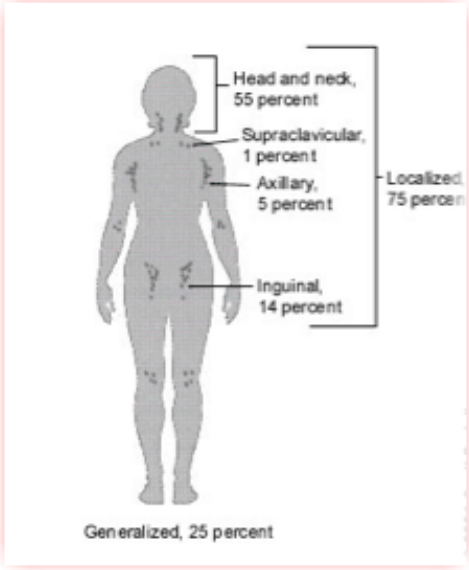
Enlarged lymph nodes are the first organs to be biopsied by fine needle aspiration (FNA); today they are one of the frequently sampled tissues.

What are Lymph nodes?

The lymph nodes are small bean shaped glands present throughout the body. The body has approximately 600 lymph nodes.They are found in single or groups. They play an important role in the defense system against disease.

What is lymphadenopathy?

- Lymphadenopathy refers to nodes that are abnormal in size, consistency or number.
- In adults lymphadenopathy is considered when the short axis of one or more lymph nodes is greater than 10mm, regional variation exists.
- Lymphadenopathy can be localized if limited to a single group of lymph nodes or generalized.
- Lymph nodes enlarge or swell in response to problems in or near the lymph nodes: e.g. infections (non-specific or specific), and malignancy: primary (lymphoma) or secondary (metastasis) from cancer of organs in the catchment areas.



Distribution of Lymphadenopathy

What is Fine needle aspiration?

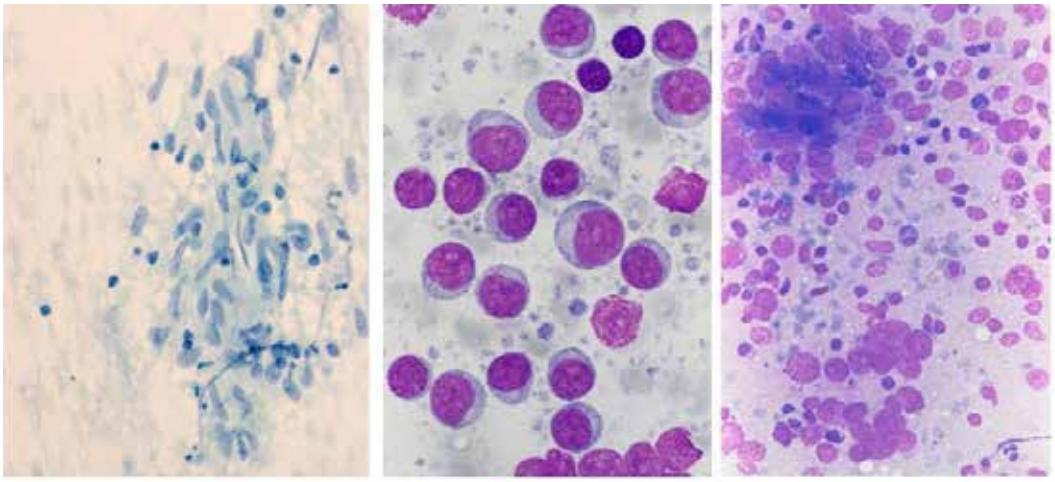
A fine needle aspiration (FNA) is a procedure that helps provide information about the cause of a lump or mass. A pathologist uses a thin needle with syringe to remove cells from the lump. Smears are made on slides for cytopathological examination under a microscope.A lymph node FNA is a relatively minor procedure that can help your doctor determine the cause of your swollen lymph nodes.

What are the indications for FNAC of lymph nodes?

Lymph node enlargement are readily felt under the skin or detected by imaging tests such as CT scan or ultrasound. Clinical management of patients with enlarged lymph nodes varies with factors such age and clinical features like suspicion for presence of infection and malignancy. Children can present with massive local lymphadenopathy even after mild infection. In contrast adult or elderly patients react to infection with only slight to modest lymph node enlargement. Therefore, massive lymphadenopathy in elderly generally arouses suspicion of malignancy.

How is FNA procedure of lymph node done?

The doctor (pathologist, radiologist or the surgeon), performs the procedure. FNA removes a small sample of cells from lymph node. This is an outpatient procedure and takes about 10 minutes. While the patient lies on an examination table, the nurse cleans the biopsy site and applies anesthetic medication to numb the area. A fine needle is attached to a disposable plastic syringe that is in turn fitted with a handle. The doctor inserts the needle into the lymph node and aspirates a sample of cells. The aspirated material is expelled on to glass slides and smears are made. The procedure may be repeated until adequate sample is obtained. A bandage is applied at site of procedure which can be removed after two hours.



Tuberculosis (Cervical lymph node)

Lymphoma (cervical lymph node)

Metastasis from breast carcinoma (Axillary LN)

FNA Lymph node

What are the conditions generally diagnosed by lymph node FNAC?

BENIGN	MALIGNANT
Reactive hyperplasia	Hodgkin lymphoma
Infections-Tuberculosis, fungal, viral infections	Non Hodgkin lymphoma
Kikuchi lymphadenitis	Metastatic tumor
Dermatopathic lymphadenitis	Leukemic infiltration

What are the reasons for the failure to obtain a representative FNAB sample?

- Needle has missed the target
- Needle is in central cystic/hemorrhagic area with no diagnostic cells. Whereas necrotic material from a tumor may not show tumor cells, such material from a tuberculosis lesion may reveal causative organism (Mycobacteria).
- Needle in dominant benign part and missed a small adjacent malignant part
- Fibrotic tissue giving a scant cell yield

What are the ancillary /special studies done with FNA samples?

Ancillary techniques can be done with aspirated material such as microbial culture, immunocytochemistry, flow cytometry, cytogenetics and polymerase chain reaction (PCR)

What are the advantages of FNAC?

- Simple and rapid procedure not requiring hospital admission
- Low cost
- Quick diagnoses
- Sampling of multiple sites at the same sitting
- High diagnostic accuracy
- Ancillary techniques can be done with aspirated material

What are the Complication/side effects?

Usually lymph node FNAB is free of any major complication. Mild pain lasting for one to two days is relieved with the use of analgesics. Bruising and hematoma at the biopsy site is rare.

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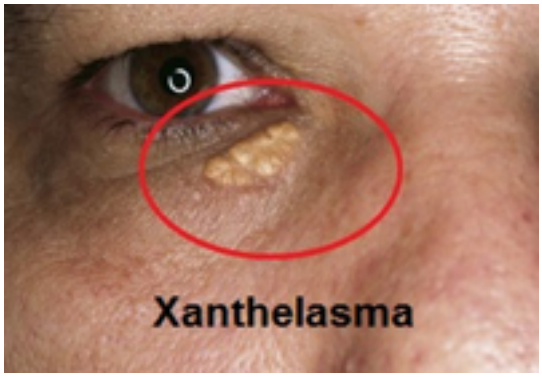
EYE DISEASES AND CLINICAL LABORATORIES

Dr Sebastian Mathew
Vitreoretinal Unit
Al Bahar Eye Center

Eyes very often provide a window to general health of an individual. A large number of diseases affecting the body can show signs and symptoms in the eye. At times, it is the sign of a particular systemic disease in the eye which leads to the diagnosis of the disease itself. For instance a patient may be diagnosed with diabetes during a routine eye check-up. Therefore, laboratory investigations in ophthalmology are important not just in the diagnosis of eye diseases but also in the diagnosis of systemic diseases. Some of the common lab tests done in Ophthalmology are:

CBC (Complete Blood Count)

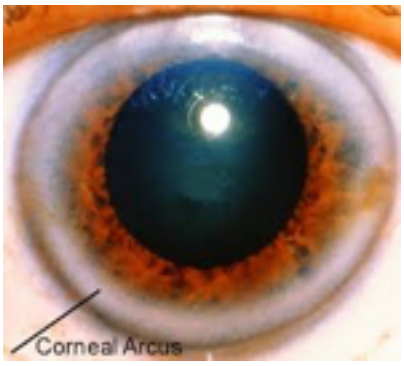
One of the most commonly ordered and extremely useful medical laboratory tests, a complete blood count with differential, provides specific information about red blood cells, white blood cells and platelets. A CBC (which includes differential white cell count) may be ordered for several conditions that exhibit eye manifestations. It is most helpful for patients with persistent infections, recurrent inflammation, or in those who exhibit signs of anaemia or leukaemia. Typically, CBC is part of a battery of tests performed prior to surgery. It also can be used to monitor patients for negative side effects associated with certain medications.



Blood Glucose and Lipid Profile

These are also commonly ordered tests. At times, examination of the eye can reveal signs which may indicate a previously undiagnosed diabetes or elevated lipids and these tests can confirm the same. These tests are also used in general workup preoperatively.

Certain conditions like xanthelasma (which is a yellowish deposition in relation to the eyelids), corneal arcus in young patients (whitish ring around the cornea), retinal vascular occlusions etc. can indicate atherosclerosis or increased levels of lipids (LDL-Cholesterol). The detection of diabetic retinopathy has sometimes lead to the diagnosis of diabetes in a previously undiagnosed patient.



Inflammatory markers

A number of inflammatory autoimmune diseases have manifestations in the eye. ESR and C-Reactive Protein (CRP) levels are sensitive but

non-specific markers of inflammation and as they are relatively inexpensive, can be used for general screening for inflammatory diseases. Inflammatory diseases in the eye like Uveitis can be localised to the eye itself or maybe part of a systemic inflammatory disorder. It is important therefore to investigate for systemic causes of inflammation if a patient has recurrent episodes of uveitis as treatment will need to be modulated accordingly.

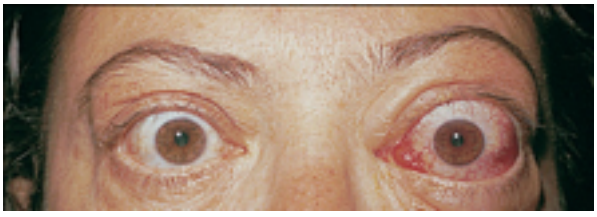
Infectious diseases

Infections can cause manifestations in the eye either due to localised infections confined to the eye or due to systemic infections. Bacterial and fungal infections of the eye can be confirmed by taking samples of discharge from the eye and subjecting them to microscopic examination after staining with appropriate dyes. They are also plated onto special media for culturing them. The cultured growth is further subjected to tests which indicate the medication to which the pathogenic organism is susceptible (sensitive) to. These Culture & Sensitivity tests are useful in the diagnosis and treatment of resistant conjunctivitis, corneal ulcers, endophthalmitis (infections involving the internal structures of the eye) etc.

Systemic infections like tuberculosis, syphilis or parasitic infestations like toxoplasmosis can also cause eye disease. Blood tests and other tests specific to the suspected condition are ordered by the treating doctor along with ancillary radiological testing. In suspected ocular tuberculosis, tests like the TB Skin test (Mantoux test/PPD test), Sputum examination for AFB (acid fast bacilli), TB Gold test or the T-spot test are important to confirm the diagnosis.

Thyroid related eye disease

Eye manifestations are common with thyroid diseases. As mentioned before often the eye signs lead to the diagnosis of thyroid disease. Levels of TSH (Thyroid stimulating hormone), FT4 (Free thyroxine) and FT3 (Free Triiodothyronine) along with radiological evaluation of the orbit are useful in the diagnosis and treatment of thyroid related eye disease.



Exophthalmos

Bulging or protruding of one or both eyes is called proptosis or exophthalmos which occurs in Graves disease, a disorder causing over activity of the thyroid gland (hyperthyroidism). In this condition, TSH is low and FT4 is high.

Congenital and Inherited disorders

A number of congenital and inherited disorders have been described in the eye. Most of these disorders are fortunately rare.

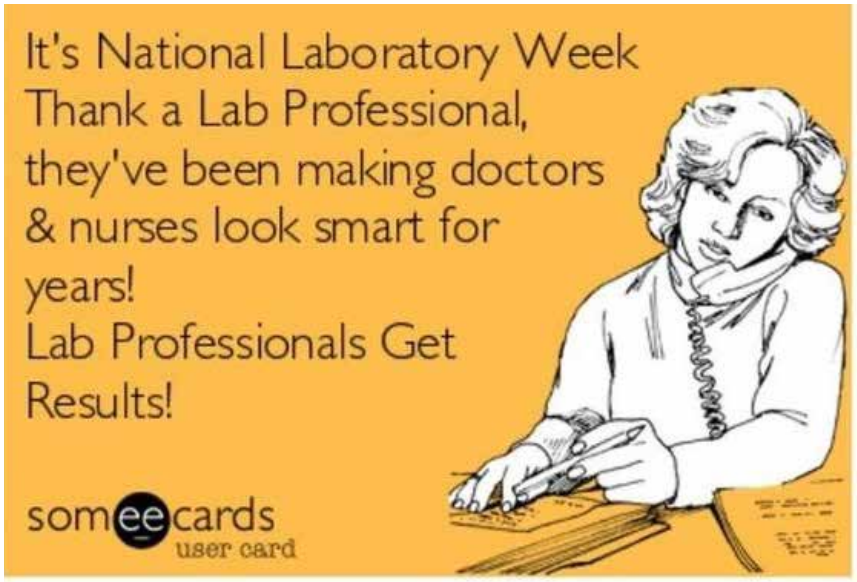
Congenital eye defects include congenital cataracts, dislocated or subluxated lens, congenital glaucoma, various dystrophies affecting the cornea, retinal dystrophies, optic nerve defects etc. Laboratory tests

of these conditions depend on the suspected underlying disorder. As for example, Homocystinuria is an autosomal recessive inherited disorder of methionine metabolism, in which there is an abnormal accumulation of homocysteine and its metabolites (homocystine) in blood and urine. Elevated levels of methionine is also there in blood and urine. On examination of eye, there is dislocation or subluxation of the lens.

Some conditions may require genetic and chromosomal testing to confirm the diagnosis, e.g. Retinitis pigmentosa.

Inherited disorders like sickle cell disease can cause significant eye problems. Sickle cell disease can be detected by blood testing for Hemoglobin S or sometimes by examination of a peripheral blood smear.

In conclusion, lab tests in eye disease are tailored to the underlying condition. Interpretation of lab tests should not be done in isolation. Lab tests form an important part of diagnosis but the final diagnosis rests heavily on good clinical examination and judicious interpretation of all results.

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LABORATORY INVESTIGATIONS FOR SKIN DISEASES

Dr Arun Joshi
Department of Dermatology
Al Farwaniya Hospital

Skin is the largest and the most easily accessible organ of the body. Skin diseases are among the most common conditions for which patients seek consultation from doctors. By virtue of being visible to the patient, skin conditions are detected early. Most of the time they are easily diagnosed by careful history taking and thorough examination of the affected skin, nails, hair, eye, oral and genital mucosa, and other systems of the body. Information related to demography (age, sex, occupation, hobbies etc.), and the skin condition [duration, mode of onset (sudden/gradual), distribution (localized/generalized), progression/evolution of lesions], associated symptoms (pain/itching/redness/swelling/discharge/ulceration etc.), family, personal, social and treatment history give important clues to the diagnosis. Number, size, shape, color, pattern, type, and distribution of skin eruptions guide the doctor in making a quick diagnosis which is straightforward in majority of cases. However in certain situations the skin diseases require laboratory investigations in many patients.

Indications for Laboratory investigations for skin diseases

1. To confirm the diagnosis
2. To know the status of other systems of the body before starting certain treatments
3. For detection and monitoring of the expected side effects of treatments
4. To follow the response to treatment

Investigations performed in skin diseases

A. Bedside tests

A number of skin diseases can be diagnosed rapidly by tests done on the patient in the clinic/outpatient/office/ward during the first consultation. These are called bedside tests as the samples are not sent to the laboratory for further testing. These tests involve:

Scraping the surface of the skin, or

Slitting it with a scalpel and then smearing the collected material on a glass slide, and

Staining it with specific chemicals to highlight certain constituents of the skin cells, and the infecting organisms.

Seeing (examining) the stained slides under the microscope to give a quick diagnosis. Such tests are useful in diagnosing many skin infections (fungal, viral, bacterial), blistering diseases, and parasitic infestations.

i. KOH examination: This is a simple non-invasive test done by scraping scaly material from the surface of the skin, hair, nails and mucosal covering of the mouth and genitalia in cases of superficial fungal infections.

ii. Tzanck Smear: is a rapid, simple and reliable bedside procedure for confirming diagnosis of viral infections, blistering autoimmune skin conditions and certain tumors.
iii. Tissue touch smear: A small piece of skin is removed by blade or biopsy punch. The material is smeared/touched/crushed between two slides, dried, fixed and stained with specific stains and examined under the microscope to look for the presence of causative organisms for diagnosis of skin tuberculosis, leishmaniasis, subcutaneous and deep fungal infections and some STDs.
iv. Saline or direct wet mount preparation for candida fungal infections or parasitic infestations
v. Wood's lamp Examination: Certain conditions of the skin produce pigments (molecules) that fluoresce with characteristic colors/hues when seen under a special lamp (Wood's Lamp emitting UV light) enabling diagnosis of conditions such as fungal infections (tinea versicolor, tinea capitis), bacterial infections (eryhthrasma), pigmentary disorders (vitiligo, Pityriasis alba) and genetic disorders of metabolism e.g. porphyrias.

B. Laboratory Investigations

1. Blood Tests

a. CBC: A very basic investigation, important to look for anemia (for hair loss and nail disorders), check white cell count (WBC), platelet count and ESR. CBC is useful for diagnosis of infectious, malignant or autoimmune skin conditions, to monitor acute myelotoxicity of immunosuppressant therapy (e.g. methotrexate, azathioprine, cyclosporine, cyclophosphamide, dapsone etc.).
b. Blood Glucose: To know the presence/absence of diabetes as oral/injectable steroids are routinely used for treating skin conditions. Prolonged use of steroids can give rise to diabetes/worsen the pre-existing diabetes requiring anti-diabetic drug dose adjustment.
c. Liver function tests: are requested before starting and during follow up treatment at regular intervals for certain drugs routinely used in dermatology that are hepatotoxic (can damage liver) e.g. Methotrexate, acitretin, isotretinoin, oral antifungals, dapsone etc.)
d. Lipid profile: is mandatory before starting very commonly used anti-acne medicine isotretinoin as it can increase serum lipids.
e. Renal functions: are essential before starting many oral medicines as some of these drugs are nephrotoxic.
f. Thyroid function tests (TSH, FT4): for many skin conditions such as hair loss, nail and skin dryness, temperature sensitivity etc.
g. Other Hormone assays
   - LH, FSH, Prolactin: For polycystic ovary disease
   - Testosterone, DHEAS, Androstenedione: For hirsutism
h. G6PD levels: Before starting drugs that can cause hemolysis such as dapsone and chloroquine.
i. Ferritin level: for iron stores in body, important investigation for hair loss.
j. Serology for detecting autoantibodies: For conditions such as auto-immune or connective tissue disorders, many of which have important cutaneous features.

2. Special stains for specimens collected from skin lesions

a. Acid orcein-Giemsa stain: For elastic fibers (dark brown), collagen (pink), melanin (black), hemosiderin

(green/yellow), amyloid (light blue), mast cell granules (purple).

b. AFB Stain for leprosy *(Mycobacterium leprae)* and skin TB *(M. tuberculosis)* They appear as pink rods after AFB stain.

c. Alcian Blue stain: For acid mucopolysaccharides (blue)

d. Congo red: Amyloid (red)

e. Gram's Stain: For bacterial and fungal infections

f. Gomori's: Reticulin (black)

g. Grocot's: Fungus wall (black)

h. Masson's trichrome: Collagen (green), muscle & fibrin (red)

i. Masson's Fontana: for melanin (black) in melanoma

j. Perl's Prusssian blue: Iron (blue)

k. PAS (Per iodic Acid Schiff): Glycogen (magenta red), mucopolysaccharides (red)

l. S 100 Stain: For nerve tissue (neurofibromas, other neural tumors)

m. Toluidine blue: For acid mucopolysaccharides (metachromatic purple, mast cells)

n. VVG (Verhoeff-Van Gieson): collagen (red), muscle, nerve (yellow)

o. Von Kossa stain: For calcium in various skin conditions

p. Immunohistochemistry: For typing of cellular infiltrate in premalignant and malignant conditions of the skin.

3. Skin Biopsy: is an invasive procedure usually carried out in a minor operation theatre. A small part or the whole lesion is removed under local anesthesia and sent to the histopathology laboratory where it is subjected to further staining and examination under the microscope. Tissue diagnosis by biopsy is considered the ultimate gold standard diagnostic method in skin diseases. Skin biopsy is taken by various techniques such as :

- Incision: A small piece of tissue is incised from the edge or center of the skin lesion.
- Excision: The lesions is removed completely in toto.
- Shave: A superficial part of the skin lesion is shaved off.
- Punch: The most commonly employed method. A cylindrical piece of skin is removed by special instrument called biopsy punch.

C. Special Techniques (Procedures)

1. Dermoscopy/dermatoscopy (also known as epiluminescence microscopy): is one of the most recent and innovative technique developed to diagnose skin diseases. It is completely non-invasive, painless and bloodless. In this technique skin lesions are seen directly with an instrument called dermatoscope. The main use of dermoscopy is in distinguishing pigmented skin conditions such as melanoma. It is also useful in distinguishing haemangiomas, angiokeratomas, pigmented basal cell carcinomas and seborrhoeic keratoses from melanocytic lesions, identification of scabies burrows and mites, as well as diagnosis of other parasitic infections.
2. Amniocentesis: is the method of collecting fluid from inside the uterus during pregnancy and to process and analyze it for diagnosing certain rare genetic conditions.
3. Fetal Skin Biopsy: similar to amniocentesis but in this case, a piece of fetal skin is taken with special instrument.

4. Electron microscopy: is useful for diagnosing rare skin diseases such as epidermolysis bullosa characterized by extreme fragility of skin and mucosae that are very disfiguring and sometimes fatal.

Tests for allergic skin conditions

A. Blood Tests for Total and specific IgE: For allergic skin conditions such as urticaria, contact dermatitis and atopic dermatitis, to know specific allergens, blood tests are performed by immunofluorescent technique called 'ImmunoCap' or enzyme immunoassay (EIA) (both are available and used in Kuwait).

B. Patch Test: is the gold standard for detecting causative agent (allergen) of allergic contact dermatitis. A series of chemicals impregnated on a special square shaped absorbent material attached to a special tape is placed on the uninvolved skin on the back of the patient for 2 to 4 days. The tape is removed after 48 hours and the skin is observed for any reaction in the form of redness with induration, papule formation or vesiculation. The reaction is graded (0 to 3+). Positive reaction to the chemicals/allergens help in diagnosing and pin pointing the cause of contact dermatitis.

C. Prick test: In this test a very small quantity of suspected allergen in introduced into the skin with the help of needle and local reaction (redness with edema/induration, papules and vesicles with itching) is looked for.

Lab Investigations for sexually transmitted diseases (STDs)

A. Blood tests (also called serology): Detects specific antibodies against causative microbes of STDs
   i. VDRL/TPHA: For diagnosis and follow up of treatment response.
   ii. HIV testing
   iii. Serology for chlamydia and gonorrhea
   iv. Hepatitis B and C virus screening: Both HBV and HCV are transmitted through blood/sexually or from mother to child. HBV and HCV are associated with certain specific skin conditions such as cryoglobulinemia, vasculitis, Gianoti Crosti syndrome, lichen planus etc. Also done before starting any hepatotoxic (liver damaging) drug.

B. Dark Ground Microscopy: A special technique to demonstrate causative organism of syphilis *(Treponema Pallidum)* from lesions of primary and secondary syphilis.

GENOME SEQUENCING

Dr A. Rao
Biochemistry Unit, Department of Laboratory
Al Sabah Hospital

Human body structure is coded in the 23x2 chromosomes present in every cell in the body. In the chromosome, there is double strand of DNA coiled like rope and tightly packed. DNA is made of units called nucleotides linked to each other by chemical bond. There are 4 nucleotides in DNA, A, G, C and T. Nucleotide triplets are specific codes for each of the amino acids in the proteins of the body. The order of these triplets in the DNA decides the order of the amino acids in the proteins. The proteins are finally responsible for different structures and functions in the body.

The unit of DNA coding for a protein is its 'gene' and for years tests have been done on individual genes with known defects in nucleotide sequence to establish the presence of a gene carried disease. Pharmacogenetics and personalized medicine is another area where drug action and metabolism is affected by variation in the proteins related to the drug's action. Of the 331 drugs listed by www.pharmgb.org as affected by variation in genes, more than 50 drugs are said to benefit from gene testing prior to drug therapy. Familial cancers are predictable with the help of identification of the offending gene. Actress Angelina Jolie decided to have double mastectomy as the BRCA1 Gene mutation that she had increased her breast cancer risk to 87% and ovarian cancer risk to 50%. Similarly, APC gene mutation causing familial adenomatous polyposis raises the risk of colorectal cancer to almost 100%. However, a number of other mutations of other genes predispose to similar cancer too, which makes way for a case for whole genome sequencing which can identify multiple genes by one test. Similarly, ancestry testing (using testing of Y-chromosome and mitochondrial gene sequencing and SNP testing) and parentage testing would be easier with whole genome sequencing.

Diagnostic testing for suspected inherited metabolic disease and other genetic diseases

Many diseases are inherited by a gene altered by change in one nucleotide (single nucleotide polymorphism, SNP) or combination of nucleotides. Many times, testing for the substances accumulating in the body, or for the culprit protein is possible and it may be enough for diagnosis and management. In many cases genetic testing is the only diagnostic tool possible. Tests for culprit genes have a problem that for any such disease there may be more than one SNP responsible in different individuals. Testing the genome broadly has the advantage that it can uncover any alternate mutation or when more than one gene is responsible, it can identify such changes in one go.

Because of the great advantage of determining the sequence of the whole genome (body of genes), in 1993 a project to sequence the genome was started targeting to complete by 2003. The technology was fast improving and the project was completed in 2000. Nowadays, the time required for sequencing the entire genome has dropped to 50 hours and is expected to be less than a day shortly. Cost has also come down to anything from 100 to 3,000 USD, depending upon the purpose of testing, compared to over USD 3 billion for the Genome Project. This has spawned commercial activity testing the genome for various purposes.

Genome testing in the sick neonate in the NICU is seen as the most beneficial diagnostic use. About 10% of the newborn are admitted to neonatal ICU with illness. A sizeable portion of these have been shown to have diseases of genetic origin. These infants are subjected to a battery of tests, mostly without reaching any conclusion and when an initial diagnosis is made, later they are proven wrong in about 40% of the cases. A diagnosis reached early could help these infants enormously, not just by directing proper management, but also by avoiding un-necessary investigations and procedures. Same disease can have different mutations of the same gene or different genes may have mutations causing similar syndromes- whole genome sequencing has the ability to identify them all, unlike testing for individual genes. With a 50 hour turn-around of results currently possible with 'New Generation Sequencing' (NGS) at a price of USD 1,000, whole genome screening is seen as a harbinger of new era of management of sick newborn infants.

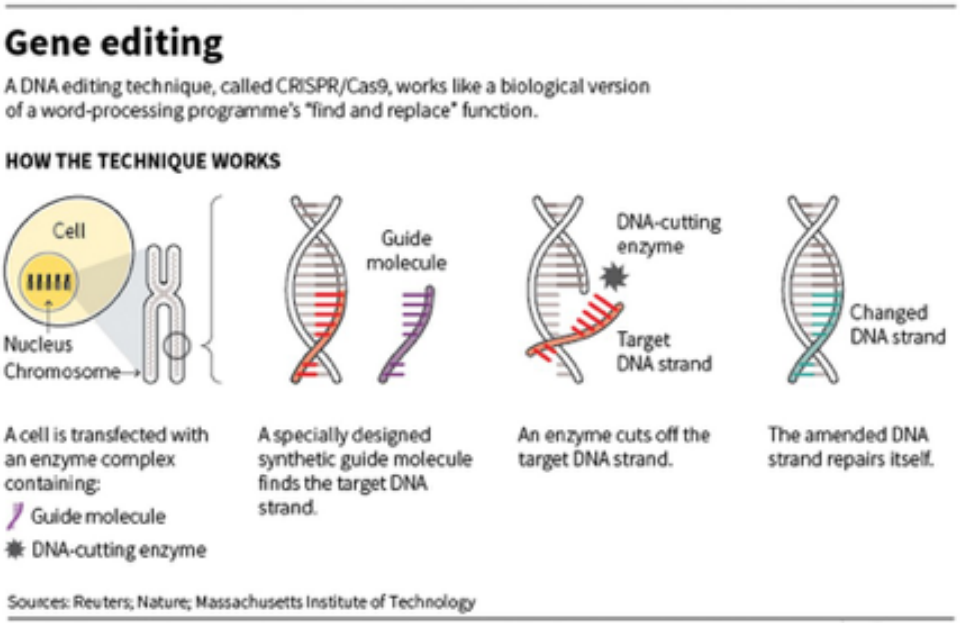

Diagram of CRISPR/Cas9 gene editing method

Hemophilia A is a disease with genetic defect in Factor VIII which helps in the clotting process; this disease has been recently successfully treated using infecting with a virus carrying the correctly functioning Factor VIII gene. Gene editing has been carried out recently to correct defective gene causing hypertrophic cardiomyopathy, a genetic heart disease and is bound to become more and more common. With these developments in treatment of genetic diseases, whole genome screening is coming into the realm of everyday laboratory service very soon. As the cost of sequencing comes down with increased use of the test and with increasing sophistication of the software interpreting the multitude of variations existing in the genome, only some of which are disease related, it may soon replace the present format of newborn screening techniques.

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