VOLUME - II
ISSUE - XV
MAY / JUN 2006

The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

In keeping with our assurance of assisting in obtaining accreditations from the concerned authorities vis-a-vis all related sub-specialties of a pathology laboratory, we are continuing with our efforts that we commenced earlier. As these efforts cannot be anything else except whole hearted, a thorough dose with all mandatory details down to the hilt are to be necessarily transmitted in black and white. In order to maintain completeness and due to space constraints a few of the articles get carried over and overflow on to the next editions. Trust these collectors' communications shall be retained by you in continuation.

The INTERPRETATION of lipid and non-lipid markers of CVD is being continued in this issue. Cardiovascular risk markers, such as hs-CRP(CRP-UV), homocysteine, BNP, and lipid subclass, are available to aid in the detection, evaluation, and management of cardiac disease or risk of cardiac disease. Patients learn about these tests in the media or through searches on the Internet, and may request them. These markers can be advantageous in promoting treatment and long-term management. Patients can see improvement in their levels and remain compliant to the treatment, ultimately decreasing cardiovascular risk and improving outcomes. Given in this issue are the Adult Treatment Panel III guidelines.

Also, the Quality Assurance regimen provided in the 14th issue is making a comeback in order to complete its message in the TROUBLE SHOOTING segment. The recommendations provided are those of the WHO. Additionally, in this issue you will find how to assess performance standards of stains and the QC procedures for commonly used bacteriological tests.

Fighting aliens/foreigners is fine but what about civil wars that are fought against one's own people. The counterpart of this very act in a human body is an autoimmune disorder. The most important among these is Systemic Lupus Erythematosus also known as SLE or Lupus. The DISEASE DIAGNOSIS section here brings to you all clinico-diagnostic aspects of SLE. The criteria used to diagnose SLE are given in length, as are the various laboratory investigations. The interpretation of ANA and positivities of other ENAs in various other autoimmune diseases are also mentioned.

Amongst all this, BOUQUET of jokes, quotations and multiple choice pathology brain teasers has not been overlooked.

DISEASE DIAGNOSIS

SYSTEMIC LUPUS ERYTHEMATOSUS

Introduction

Lupus is a condition of chronic inflammation caused by an autoimmune disease. Autoimmune diseases are illnesses which occur when the body's tissues are attacked by its own immune system. The immune system is a complex system within the body that is designed to fight infectious agents, for example, bacteria, and other foreign invaders. One of the mechanisms that the immune system uses to fight infections is the production of antibodies. Patients with lupus produce abnormal antibodies in their blood that target tissues within their own body rather than foreign infectious agents. Lupus can cause disease of the skin, heart, lungs, kidneys, joints, and nervous system. When only the skin is involved, the condition is called discoid lupus. When internal organs are involved, the condition is called systemic lupus erythematosus (SLE).

Both discoid and systemic lupus are more common in women than men (about eight times more common). The disease can affect all ages but most commonly begins from age 20 to 45 years.

Etiology

The precise reason for the abnormal autoimmunity that causes lupus is not known. Inherited genes, viruses, ultraviolet light, and drugs may all play some role. Genetic factors increase the tendency of developing autoimmune diseases, and autoimmune diseases such as lupus, rheumatoid arthritis, and immune thyroid disorders are more common among relatives of patients with lupus than the general population. Some scientists believe that the immune system in lupus is more easily stimulated by external factors like viruses or ultraviolet light. Sometimes, symptoms of lupus can be precipitated or aggravated by only a brief period of sun exposure.

Dozens of medications have been reported to trigger SLE; however, more than 90% of this "drug-induced lupus" occurs as a side effect of one of the following six drugs: hydralazine (used for high blood pressure), quinidine and procainamide (used for abnormal heart rhythm), phenytoin (used for epilepsy), isoniazide (used for tuberculosis), d- penicillamine (used for rheumatoid arthritis). These drugs are known to stimulate the immune system and cause SLE. Fortunately, drug-induced SLE is infrequent (accounting for less than 5% of SLE among all patients with SLE) and usually resolves when the medications are discontinued.

It also is known that some women with SLE can experience worsening of their symptoms prior to their menstrual periods. This phenomenon, together with the female predominance of SLE, suggest that female hormones play an important role in the expression of SLE. This hormonal relationship is an active area of ongoing study by scientists.

Recent research provides direct evidence that a key enzyme's failure to dispose of dying cells contributes to SLE. The enzyme, DNase1, normally eliminates what is called "garbage DNA" and other cellular debris by chopping them into tiny fragments for easier disposal. The researchers turned off the DNase1 gene in mice. The mice appeared healthy at birth but after 6-8 months, the majority of mice without DNase1 showed signs of SLE. Thus, a genetic mutation that disrupts the body's cellular waste disposal may be involved in the beginning of SLE.

Symptoms, Signs and Tests

Because many symptoms of systemic lupus erythematosus (SLE) mimic those of other illnesses, lupus can be a difficult disease to diagnose.

Diagnosis is usually made by a careful review of three factors:

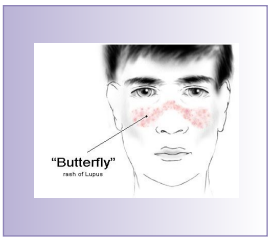
- The individual's entire medical history
- An analysis of the results obtained in routine laboratory tests and
- Some specialized tests related to immune status.

To make a diagnosis of SLE, an individual must show clinical evidence of a multi-system disease (i.e. shows abnormalities in several different organ systems)

Typical symptoms or signs that might lead to suspicion of SLE are:

Skin: Butterfly rash across the cheeks; ulcers in the mouth; hair loss.
Joints: Pain; redness, swelling.
Kidney: Abnormal urinalysis suggesting kidney disease.
Lining membranes: Pleurisy (inflammation of the lining of the lung); pericarditis (inflammation of the heart lining); and/or peritonitis (inflammation around the abdomen). Taken together, these types of inflammation are known as polyserositis.
Blood: Hemolytic anemia (the red cells are destroyed by autoantibodies); leukopenia (low white blood cell count); thrombocytopenia (low number of platelets). There may be pancytopenia.
Lungs: Infiltrates (shadowy areas seen on a chest x-ray) that come and go.
Nervous system: Convulsions (seizures); psychosis; nerve abnormalities that cause strange sensations or alter muscular control or strength.

Butterfly rash



If an individual has several of these symptoms, the physician will then usually order a series of tests to examine how well the individual's immune system is functioning. In general, physicians look for evidence of autoantibodies. Although there is no one test that can definitely say whether or not a person has lupus, there are many laboratory tests which aid the physician in making a lupus diagnosis.

Routine clinical tests which suggest that the person has an active systemic disease include:

- Sedimentation rate (ESR) and CRP (C-reactive protein) binding, both of which are frequently elevated in inflammation from any cause
- Serum protein electrophoresis which may reveal increased gamma globulin and decreased albumin
- Routine blood counts which may reveal anemia and low platelet and white cell counts

Routine chemistry panels which may reveal:

- Kidney involvement by increases in serum blood urea nitrogen and creatinine
- Abnormalities of liver function tests
- Increased muscle enzymes (such as CPK) if muscle involvement is present.

These kinds of abnormalities alert the doctor to the presence of a systemic disease with multiple organ involvement.

Commonly used blood tests in the diagnosis of SLE

- Anti-nuclear antibody test (ANA) to determine if autoantibodies to cell nuclei are present in the blood
- Anti-DNA antibody test to determine if there are antibodies to the genetic material in the cell
- Anti-Sm antibody test to determine if there are antibodies to Sm, which is a ribonucleoprotein found in the cell nucleus
- Serum (blood) complement test to examine the total level of a group of proteins which can be consumed in immune reactions
- Complement proteins C3 and C4 test to examine specific levels

The Antinuclear Antibody (ANA or FANA) Test

Positive ANA

The immunofluorescent antinuclear antibody (ANA or FANA) test is positive in almost all individuals with systemic lupus (97 percent), and is the most sensitive diagnostic test currently available for confirming the diagnosis of systemic lupus when accompanied by typical clinical findings. When three or more typical clinical features are present, such as skin, joint, kidney, pleural, pericardial, hematological, or central nervous system findings as described above, a positive ANA test confirms the diagnosis of systemic lupus.

However, a positive ANA test, by itself, is not proof of lupus since the test may also be positive in:

1. Other connective tissue diseases, such as:
   - Scleroderma
   - Sjogren's syndrome
   - Rheumatoid arthritis
   - Thyroid disease
   - Liver disease
   - Juvenile arthritis
2. Individuals being treated with certain drugs, including:
   - Hydralazine
   - Isoniazid
   - Chlorpromazine
3. Viral illnesses, such as:
   - Infectious mononucleosis
4. Other chronic infectious diseases, such as:
   - Hepatitis
   - Lepromatous leprosy
   - Subacute bacterial endocarditis
   - Malaria
5. Other autoimmune diseases, including:
   - Thyroiditis
   - Multiple sclerosis
6. As many as 30-40 percent of asymptomatic first-degree relatives of people with lupus (siblings, parents, and children).

Weakly positive ANA

The test can even be weakly positive in about 20 percent of healthy individuals. While a few of these healthy people may eventually develop lupus symptoms, the majority will never develop any signs of lupus or related conditions. The chances of a person having a positive ANA test increases as he or she ages.

Negative ANA

A negative ANA test is strong evidence against lupus as the cause of a person's illness, although there are very infrequent instances where SLE is present without detectable antinuclear antibodies. ANA-negative lupus can be found in people who have anti-Ro (SSA) or antiphospholipid antibodies.

ANA Titers and Patterns

ANA laboratory reports include a titer and a pattern

The titer indicates how many times the lab technician had to dilute plasma from the blood to get a sample free of the antinuclear antibodies.

For example, a titer of 1:640 shows a greater concentration of anti-nuclear antibodies than a titer of 1:320 or 1:160.

The apparent great difference between various titers can be misleading.

Since each dilution involves doubling the amount of test fluid, it is not surprising that titer numbers increase rather rapidly.

In actuality, the difference between a 1:160 titer and a 1:320 titer is only a single dilution. This does not necessarily represent a major difference in disease activity.

ANA titers go up and down during the course of the disease, and a high or low titer does not necessarily mean the disease is more or less active.

Therefore, it is not always possible to determine the activity of the disease from the ANA titer.

A titer above 1:80 is usually considered positive. Some laboratories may interpret different titer levels as positive, so one cannot compare titers from different laboratories.

The pattern of the ANA test can sometimes be helpful in determining which autoimmune disease is present and which treatment program is appropriate.

The homogeneous, or smooth pattern is found in a variety of connective tissue diseases, as well as in people taking particular drugs, such as certain antiarrhythmics, anticonvulsants or antihypertensives.

This homogenous pattern is also the one most commonly seen in healthy individuals who have positive ANA tests.

- The speckled pattern is found in SLE and other connective tissue diseases.
- The peripheral, or rim pattern is found almost exclusively in SLE.
- The nucleolar pattern, with a few large spots, is found primarily in people who have scleroderma.

Because the ANA is positive in so many conditions, the results of the ANA test have to be interpreted in light of the person's medical history, as well as his or her clinical symptoms. Thus, ***a positive ANA alone is never enough to diagnose lupus***. On the other hand, a negative ANA argues against lupus but does not rule out the disease completely.

A Positive ANA Does Not Equate to Having a Disease

The ANA should be looked at as a screening test. If it is positive in a person who is not feeling well and who has other symptoms or signs of lupus, the physician will probably want to conduct further tests for lupus.

If the ANA is positive in a person who is feeling well and in whom there are no other signs of lupus, it can be ignored. If there is any doubt, a consultation with a rheumatologist should clarify the situation. Besides immunofluorescent other techniques available are : ELISA, CLIA and PCR. These techniques provide quantitative measurements of ANA and ENAs.

Other Autoantibodies

In those individuals with a positive ANA, additional tests can be done for certain particular antibodies that may better establish a diagnosis of SLE. The knowledge of which particular antibody is responsible for the positive ANA test can sometimes be helpful in determining which autoimmune disease is present.

Antibodies to **dsDNA** (the protein that makes up the body's genetic code) are found primarily in SLE.

Antibodies to **histones** (DNA packaging proteins) are usually found in people with drug-induced lupus (DIL), but may also be found in those with SLE.

Antibodies to the **Sm** antigen are found almost exclusively in lupus, and often help to confirm the diagnosis of SLE.

Antibodies to **RNP** (ribonucleoprotein) are found in a number of connective tissue diseases. When present in very high levels, RNP antibodies are suggestive of mixed connective tissue diseases (MCTD), a condition with symptoms like those of SLE, polymyositis and scleroderma.

Antibodies to **Ro/SS-A** are found in people with either lupus or Sjogren's syndrome, and are almost always found in babies who are born with neonatal lupus.

Antibodies to **Jo-1** are associated with polymyositis.

Antibodies to **PM-Scl** are associated with certain cases of polymyositis that also have features of scleroderma.

Antibodies to **Scl-70** are found in people with a generalized form of scleroderma.

Antibodies to the **centromere** (a structure involved in cell division) are found in people with a limited form of scleroderma which tends to have a chronic course.

Complement

Laboratory tests which measure complement levels in the blood may also be helpful to the physician in making a diagnosis of SLE.

Complement is a blood protein that destroys bacteria and also influences inflammation.

Complement proteins are identified by the letter "C" and a number.

The most common complement tests are C3, C4, and CH50.

If the total blood complement level is low, or the C3 or C4 complement values are low and the person also has a positive ANA, some weight is added to the diagnosis of lupus. Low C3 and C4 complement levels in individuals with a positive ANA may signify the presence of active disease, especially kidney disease.

Biopsy

Sometimes examination of a tissue sample (biopsy) can be helpful in making a diagnosis. The biopsy is one of the best ways to evaluate an organ or tissue. The procedure involves removal of a small sliver of tissue, which is then examined histopathologically.

The doctor can use the biopsy to identify the amount of inflammation and damage to the tissue.

Further tests can be performed on the specimen to determine whether the problem is due to lupus or is caused by some other factor such as

infection or medication.

Almost any tissue can be biopsied. The most common sites biopsied in lupus are the skin and kidney.

The results of the biopsy, like any other laboratory test, should be examined in combination with the individual's medical history and clinical findings.

Tests to Assess Disease Activity

When a person diagnosed with lupus develops new or recurring symptoms, laboratory testing of blood or urine can help determine if the symptoms are due to an increase in lupus activity.

Disease activity correlates with a rise in:

CRP (C-reactive protein) binding
ESR, or sedimentation rate
Anti-DNA
Liver and kidney function tests (AST, ALT, BUN, creatinine)
CPK (muscle enzyme)
Urine protein or cellular casts

Disease activity also correlates with a rise in:

CBC or complete blood count (white blood cell count, hemoglobin, platelets)
Complement components

Systemic lupus erythematosus at a glance

Systemic lupus erythematosus (SLE) is an autoimmune disorder
SLE is characterized by the production of unusual antibodies in the blood.
SLE is eight times more common in women than men.
The cause(s) of SLE is (are) unknown, however, heredity , viruses, ultraviolet light and drugs all play some role.
Up to 10% of patients with SLE isolated to the skin will develop the systemic form of lupus (SLE).
Eleven criteria help doctors to diagnose SLE.
Treatment of SLE is directed towards decreasing inflammation and/or the level of autoimmune activity.
Patients with SLE can prevent 'flares' of disease by avoiding sun exposure and not abruptly discontinuing medications.

BOUQUET

In Lighter Vein

"Doc," says Steve, "I want to be castrated."
"What on earth for?" asks the doctor in amazement.
"It's something I've been thinking about for a long time and I want to have it done" replies Steve.
"But have you thought it through properly?" asks the doctor, "It's a very serious operation and once it's done, there's no going back. It will change your life forever!"
"I' m aware of that and you're not going to change my mind -- either you book me in to be castrated or I'll simply go to another doctor."
"Well, OK.", says the doctor, "But it's against my better judgment!"
So Steve has his operation, and the next day he is up and walking very slowly, legs apart, down the hospital corridor with his drip stand. Heading towards him is another patient, who is walking exactly the same way.
"Hi there," says Steve,"It looks as if you've just had the same operation as me."
"Well," said the patient, "I finally decided after 37 years of life that I would like to be circumcised."
Steve stared at him in horror and screamed, "Shit! THAT'S the word!

"Would you mind telling me, Doctor," Bob asked, "how you detect a mental deficiency in somebody who appears completely normal?"
"Nothing is easier," he replied. "You ask him a simple question which everyone should answer with no trouble. If he hesitates, that puts you on the track."
"What sort of question?"
"Well, you might ask him, 'Captain Cook made three trips around the world and died during one of them. Which one?'
Bob thought for a moment, and then said with a nervous laugh, "You wouldn't happen to have another example would you? I must confess I don't know much about history."

A cardiac specialist died and at his funeral the coffin was placed in front of a huge mock up of a heart made up of flowers. When the pastor finished with the sermon and eulogy, and after everyone said their good-byes, the heart opened, the coffin rolled inside and the heart closed. Just then one of the mourners burst into laughter.
The guy next to him asked: "Why are you laughing?"
"I was thinking about my own funeral" the man replied.
"What's so funny about that?"
"I'm a gynecologist."

Wisdom Whispers

Most folks are about as happy as they make up their minds to be.
The secret of happiness is to make others believe they are the cause of it.
Happiness is nothing more than good health and a bad memory.
The happiness of a man in this life does not consist in the absence but in the mastery of his passions.
Happiness is that state of consciousness which proceeds from the achievement of one's values.
The pursuit of happiness is a most ridiculous phrase; if you pursue happiness you'll never find it.
Cherish all your happy moments: they make a fine cushion for old age.
Slow down and enjoy life. It's not only the scenery you miss by going too fast - you also miss the sense of where you are going and why.
Many persons have a wrong idea of what constitutes true happiness. It is not attained through self-gratification but through fidelity to a worthy purpose.
If we cannot live so as to be happy, let us least live so as to deserve it.
The foolish man seeks happiness in the distance, the wise grows it under his feet.
At the height of laughter, the universe is flung into a kaleidoscope of new possibilities.

Brain Teasers

1. Gleason's pattern/grading system is used for malignancies of which organ?
   A. Pancreas B. Eyes C. Prostate D. Kidney

2. Apolipoproteins A-I and A-II are the principal apoproteins of which of the following?
   A. HDL B. LDL C. VLDL D. IDL

3. In rickets which of the following parameters would definitely be increased?
   A. ALP B. Serum Calcium C. Serum Phosphate D. Calcium excretion

4. D-Xylose test is used to diagnose which of the following disorders?
   A. Malabsorption syndrome B. Diabetes mellitus C. AMI D. CA rectum

5. Schilling test is used to diagnose deficiency of which of the following constituents?
   A. Carotene B. Vitamin B_{12} C. Vitamin C D. Folic acid

ANSWERS : 1. C, 2. A, 3. A, 4. A, 5. B

INTERPRETATION

INTERPRETATION OF LIPID & NON-LIPID MARKERS IN CARDIOVASCULAR DISEASE (CVD) (...contd)

Beyond the Traditional Lipid Panel

A lipid panel can assess the risk of atherosclerosis or cardiovascular disease and help to determine proper therapy. The traditional lipid panel to measure cholesterol includes total cholesterol, triglycerides, HDL, LDL, and cholesterol/HDL ratio. It also monitors the progress of the treatment plan. In many instances, patients with atherosclerosis, cardiovascular disease, or myocardial infarctions have normal levels of LDL and HDL, which should prompt healthcare providers to consider additional lipid testing as a diagnostic tool.

Cholesterol is transported throughout the body as a component of a lipoprotein complex. Each lipoprotein complex contains different density lipoprotein and apolipoprotein particles that provide additional information about the characteristics of the lipoprotein complex. Chylomicrons carry dietary fat from the intestines to the liver, deliver triglycerides to muscle tissue, and deposit excessive triglycerides in fatty tissue. Low-density lipoprotein cholesterol is a heterogenous lipoprotein comprised of intermediate-density lipoprotein (IDL) and very low-density lipoprotein (VLDL), which distributes triglycerides to muscle cells, deposits excess triglycerides in fatty tissue, and can contribute to the build up of cholesterol in the arteries. Very low-density lipoprotein has a density of <1.006 g/mL, IDL has a density of 1.006 to 1.019 g/mL, and LDL has a density of 1.019 to 1.063 g/mL. In contrast, HDL has a density of 1.063 to 1.210 g/mL.

Apolipoproteins (Apo-A) are the major protein components of lipoproteins and have a variety of functions. Apo-A is a structural protein of HDL with LpA-I as the major protein component. The current thought is that the LpA-I protein component of HDL, as opposed to LpA-II, provides it with its antiatherogenic properties. The provider must differentiate LpA-I from Lp(a), which is a different lipoprotein particle.

Lp(a) is the lipoprotein that contributes to increased cholesterol deposition in the arterial wall and is considered highly atherogenic. Whether or not a patient's lipoprotein complex contains Lp(a) is mostly a genetic issue.

Low-density lipoprotein contributes 60% to 70% of the total serum cholesterol. Low-density lipoprotein can be separated into seven different kinds of particles, and based on the size of these particles, two LDL subclasses have been created: LDL subclass A and LDL subclass B. Although everyone possesses both subclass A and subclass B, the proportion of each determines an individual's cardiovascular risk. The particles in LDL subclass B are smaller and denser than those found in LDL subclass A. These small particles infiltrate the arterial wall approximately 40% to 50% faster than the larger LDL subclass A particles. An abundance of small LDL particles (measuring less than 257 angstroms) classifies a patient as LDL subclass B and serves as an independent marker for coronary artery disease. Additionally, in patients with a LDL subclass B pattern, HDL is reduced, further increasing the risk of cardiovascular disease. A patient with the LDL subclass B pattern has a 300% greater risk for developing cardiovascular disease than a patient with LDL subclass A.

One value not reported in a traditional lipid panel is the non-HDL-C component, which is a calculated value. To determine this value, the HDL value is subtracted from the total cholesterol value. The values should be < 130 mg/dL for patients at high risk for cardiovascular disease or patients with diabetes and <160 mg/dL for others. It has been shown to correlate highly with LpB levels.

If LDL values fail to reach optimal levels after treatment, a nuclear magnet resonance (NMR) LipoProfile test may be performed. An NMR spectroscopy measures particle numbers and sizes. Lipoprotein subclasses send out different sound signals; each subclass has a unique sound that is recorded on graph paper as amplitude. The amplitude is measured and provides subclass type and quantification information to the provider. Another blood test is the Verticle Auto Profile (VAP) test. The VAP cholesterol test provides patient values and reference range values for lipid subclass types so risk can be more easily identified and treated.

Once specific particle numbers and sizes have been assessed, treatment can be individualized for the patient. For example, certain subclasses of cholesterol such as Lp(a) may not be responsive to the HMG-Co-A-reductase inhibitors alone. A patient with elevated Lp(a) may benefit from the addition of niacin or a fibric acid derivative such as gemfibrozil or fenofibrate.

Treatment goal is based on the Adult Treatment Panel III (ATP-III) Guidelines. (see Table: "ATP III LDL-C Treatment Goals").

TABLE : ATP III LDL-C Treatment Goals

Risk Category	LDL-C Goal	Initiate Therapeutic Lifestyle Changes	Consider Drug Therapy * *
High-risk CHD* or CHD risk equivalents † (10 -year risk 20%)	100 mg/dL (optional goal; < 70 mg/dL ¶	100 mg/dL #	100 mg/dL †† (<100 mg/dL: consider drug options) * *
Moderately high-risk 2+ risk factors‡ (10-year risk 10% to 20%) §§	130 mg/dL	130 mg/dL	130 mg/dL (100-129 mg/dL: consider drug options) ‡‡
Moderate risk 2+ risk factors (10-year risk 10%) §§	130 mg/dL	130 mg/dL	160 mg/dL
Lower risk 0-1 risk factors §	160 mg/dL	160 mg/dL	190 mg/dL (160-189 mg/dL: LDL lowering drug optional)

* CHD includes history of myocardial infarction, unstable angina, coronary artery procedures (angioplasty or bypass surgery) or evidence of clinically significant myocardial ischemia.
† CHD risk equivalents include clinical manifestations of non coronary forms of atherosclerotic disease (peripheral arterial disease, abdominal aortic aneurysm and carotid artery disease) transient ischemic attacks orstroke of carotid origin or 50% obstruction of a carotid artery) diabetes, and 2+ risk factors with 10-year risk for hard CHD 20%.
‡ Risk factors include cigarette smoking, hypertension (BP 140/90 mm/Hg) or on anti hypertensive medications, low HDL cholesterol (40 mg/dl) , family history of premature CHD (CHD in male first-degree relative 55 years of age; CHD in female first degree relative 65 years of age) and age (men 45 years ; women 55 years)
§§ Electronic 10 year risk calculators are available at http//www.nhlbi.nih.gov/guidelines/cholesterol
§ Almost all people with zero or one risk factor have a 10 year risk 10% and 10-year risk assessment in people with zero or one risk factor is thus not necessary.
¶ Very high risk favors the optional LDL-C goal of 70 mg/dl , and in patients with high triqlycerides , non-HDL-C 100 mg/dl.
+ Optional LDL-C goal 100 mg/dl
# Any person at high risk or moderately high risk who has lifestyle related risk factors (e.g., obesity , physical inactivity, elevated triglyceride, low HDL-C or metabolic syndrome) is a candidate for therapeutic lifestyle changes to modify these risk factors regardless of LDL-C level.
* * When LDL-lowering drug therapy is employed, it is advised that intensity of therapy be sufficient to achieve at least a 30% to 40% reduction in LDL-C levels.
†† If baseline LDL-C is 100 mg/dl, institution of LDL- lowering drug is a therapeutic option on the basis of available clinical trial results. If a high-risk person has high triglycerides or low HDL-C, combining a fibrate or nicotinic acid with an LDL-lowering drug can be considered.
‡‡ For moderately high-risk persons, when LDL-C level is 100 to 129 mg/dl, at baseline or on lifestyle therapy, initiation of an LDL-lowering drug to achieve an LDL-C level 100 mg/dl is a therapeutic option on the basis of available clinical trial results.

TROUBLE SHOOTING

QUALITY ASSURANCE IN BACTERIOLOGY

Quality Control of Media and Stains (...contd)

Quality Control of Media

Selective media

Since selective media are designed not only to support the growth of organisms but to inhibit the growth of others, it is necessary to inoculate the medium with representatives of both groups of organisms.

To demonstrate the inhibitory effect, one can challenge the medium with a heavy inoculum, since, if the medium will prevent the growth of a large inoculum, it will inhibit the small number of organisms that may be present in the primary specimen. The medium must also support the growth of the selected organisms.

As a matter of general principle, each batch of culture medium should be checked before use with control strains to ensure that it supports the growth of bacteria and, in the case of selective media, inhibits the growth of undesirable organisms. However, if economics does not permit this approach, those media which are known from experience to be trouble free and reliable need not be subjected to such a regular quality control regimen. The laboratory has to identify such reliable media and accordingly establish quality control schedules. This concept must be periodically reviewed. However, whenever a new batch of medium, new supplier or a new product is to be used it is prudent to subject it to rigorous quality control measures until confidence in the quality of the product is established. A "batch" of the medium refers to all the tubes, plates or containers of medium prepared at the same time in the laboratory, or all the plates, tubes or containers having the same lot number that are received in a single shipment from an outside supplier.

Spectrum of Quality Control

The frequency of performing quality control procedures needs to be determined from the experience of the laboratory. To meet certification requirements, laboratories need to perform quality control procedures according to a prescribed pattern. Careful records of quality control procedures should be made and maintained which should be reviewed periodically to determine the stability of media so that corrective measures can be taken in time.

Quality control of culture media should not be a blind procedure, but should be approached in a rational and disciplined manner.

Performance of Plated Media

Samples of plates from each batch are selected for performance testing and are inoculated with the appropriate stock cultures. For each type of medium, at least two or three microorganisms having growth characteristics with 'positive' and 'negative' results for the medium should be used. The size of inoculum and method of inoculating the test plates must be standardized as closely as possible.

In general, control organisms should be selected from an actively growing broth culture and a standard loopful of culture seeded directly onto the test medium, which is then streaked so as to obtain isolated colonies. After appropriate incubation, the results of the performance test are recorded.

The medium is released for use in the clinical laboratory only if the results indicate satisfactory performance. In initiating a quality control programme, one must establish some priorities, such as beginning by testing those media that are most likely to demonstrate deficiencies.

Top priority should be given to blood agar, chocolate agar and Thayer Martin agar media. Secondary priority should be accorded to selective enteric media such as MacConkey agar, EMB, XLD and bile salt agars.

A quantitative approach may be more useful for testing of performance of selective or inhibitory media such as Thayer Martin agar. *N gonorrhoeae* and *N.meningitidis* usually grow on Thayer Martin agar when the inoculum is heavy, but when a fairly light inoculum is used, the pathogens might be inhibited.

Consequently, a somewhat quantitative performance test could detect deficiencies that would be overlooked if one simply inoculated test plates with undiluted stock cultures.

Quality Control of Stains

Test all stains at appropriate intervals for their ability to distinguish positive and negative organisms and document the results. The performance standards for some of the commonly used stains in the bacteriology laboratory alongwith their desired frequencies of testing so as to have continuous reliable results have been shown in Table given below.

Stain	Control organism/ material	ATCC No	Expected result
Ziehl-Neelsen	*Mycobacterium sp.* *Esch. Coli*	25177 25922	Pink red bacilli Blue bacilli
Acridine orange	*Esch. Coli* *Staph.aureus*	25922 25923	Fluorescent bacilli/cocci
Giemsa	Thin film blood smear		Distinct staining of WBCs and RBCs
Gram	*Esch. coli* *Staph.aureus*	25922 25923	Gram -ve bacilli Gram +ve cocci
Iodine solution	Formalin treated stool specimen with cysts		Visible cyst nuclei
Spores	*Bacillus species*		Spores stain one colour and bacillus stains with counterstain

Quality control of stains need to be performed on weekly basis and also as and when a new lot of reagents for staining are procured.

Quality Control of Bacteriological Techniques

Various Biochemical tests are performed in the laboratory on the isolates obtained from the clinical specimen. These tests help in identification of the organism. Quality control procedures are essential for these tests to avoid generation of wrong results which may lead to erroneous diagnoses. Organisms known to give positive or negative reactions with various biochemical tests have been identified. These must be used frequently in the laboratory to assess the authenticity of results of biochemical reactions.

It is also essential to undertake quality control procedures at regular intervals.

These should be performed:

- With each new batch of reagents
- With each new vial of reagent
- Daily for catalse, oxidase, and coagulase
- Weekly for bacitracin, optochin and ONPG

A test procedure not giving anticipated results with the control organisms should not be used till such time that remedial steps have been taken to correct the problem.

Quality Control of commonly used media: suggested control organisms and expected reactions

Medium	Control organism	Expected reaction
Blood agar	Group A *Streptococci* *S.pneumoniae*	Good growth, - haemolytic Good growth, - haemolytic
Bile-esculin agar	*Enterococcus species* - haemolytic *Streptococcus*, not Group D	Good growth, black No growth
Chocolate agar	*H. influenzae* *N. gonorrhoeae*	Good growth Good growth
Christensen urea agar	*Proteus mirabilis* *Klebsiella pneumoniae* *Escherchia coli*	Pink throughout(positive) Pink slant (partial positive) Yellow (negative)
Simmon's citrate agar	*K. pneumoniae* *E coli*	Growth or blue colour(positive) No growth, remains green(negative)
Deoxyribonuclease	*Serratia marcescens* *E. cloacae*	Zone of clearing (add 1N HCl) No zone of clearing
Motility (semisolid agar)	*P. mirabilis* *K. pneumoniae*	Media cloudy (positive) No feather edge on streak line (negative)
MacConkey agar	*E. coli* *P. mirabilis*	Pink colonies (lactose positive) Colourless colonies, no spreading
Sucrose	*E. coli* *N.gonorrhoeae*	Yellow (positive) No colour change (negative)
Maltose	*Salmonella species* *N.gonorrhoeae*	Yellow (positive) No colour change (negative)
Lactose	*N.lactamicus* *N.gonorrhoeae*	Yellow (positive) No colour change (negative)
Lysine	*K. pneumoniae* *Enterobacter sakazakii*	Bluish (positive) Yellow (negative)
Arginine	*E.cloacae* *P. mirabilis*	Bluish (positive) Yellow (negative)
Ornithine	*P. mirabilis* *K. pneumoniae*	Bluish (positive) Yellow (negative)
o-Nitrophenol-p-Dgalactopyranoside (ONPG)	*Serratia marcescens* *S.typhimurium*	Yellow (positive) Colourless (negative)
Phenylalanine deaminase	*P. mirabilis* *E. coli*	Green (add 10% $FeCl_3$) No colour change (negative)
Salmonella -Shigella (SS) agar	*S.typhimurium* *E. coli*	Colourless colonies, black centre No growth
Voges Prauskauer	*K. pneumoniae* *E. coli*	Red (add reagents) No development (negative)
Xylose-Lysine-Dextrose	*Salmonella* species *E. coli* *Shigella* species	Red colonies (positive lysine) Yellow colonies(positive sugars) Transparent colonies (negative)

Quality Control procedures for commonly used tests

Procedure/ Test	Control organism	Expected result	Expected reaction
Catalase	*Staph. aureus* *Streptococcus species*	+ –	Bubbling reaction No bubbling
Coagulase	*Staph. aureus* *Staph. epidermidis*	+ –	Clot formation in 4 hours No clot
Indole	*Esch. coli* *Enterobacter aerogenes*	+ –	Red ring at surface Yellow ring at surface
Methyl red	*Esch. coli* *Ent. aerogenes*	+ –	Instant red colour No colour change
Oxidase	*P. aeruginosa* *Esch. coli*	+ –	Purple colour in 20 seconds No colour in 20 seconds
Voges Proskauer	*Enterobacter aerogenes* *Esch. coli*	+ –	Red colour No colour change
Bacitracin disc	*Streptococcus group A* *Enterobacter faecalis*	+ –	Zone of inhibition No zone of inhibition
Optochin disc	*Strept. pneumoniae* *Strept. viridans*	+ –	Zone of inhibition No zone of inhibition
ONPG disc	*Esch. coli* *Proteus vulgaris*	+ –	Yellow colour No change in colour
Oxidase disc	*P. aeruginosa* *Esch. coli*	+ –	Purple colour in 30 seconds No change in colour

TULIP NEWS

NOW

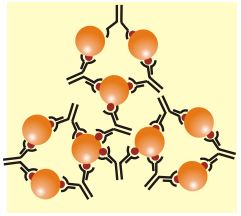
Enhanced agglutination in Rhelax RF/CRP/ASO! Interpret endpoints accurately!

Known for its innovativeness Tulip brings advancement in its latex agglutination slide tests for RF, CRP and ASO.
Visual interpretation of latex agglutination slide tests are subject to variation in endpoint readouts. This variation can be attributed to **MICROSPHERES** utilized for development of agglutination pattern. To improve the readability and minimize the variation in endpoint readouts our R & D has developed significant improvements in the agglutinating features of the latex reagents.

READ ENDPOINTS ACCURATELY

Functionally enhanced MICROSPHERES in RHELAX RF/ASO/CRP

Tulip now utilizes functionally enhanced **MICROSPHERES** that are aesthetically **brighter** and agglutinate in a **easy to visualize pattern.** This enhanced agglutination makes **interpretation of endpoints much easier.**



NEW IMPROVED RHELAX RF, RHELAX ASO & RHELAX CRP

The successful assay design and calibration of Tulip's Rheumatology reagents has been practically demonstrated at various laboratories on several occasions.
The CE certification of Tulip's Rheumatology reagents endorses acceptance of its quality internationally.

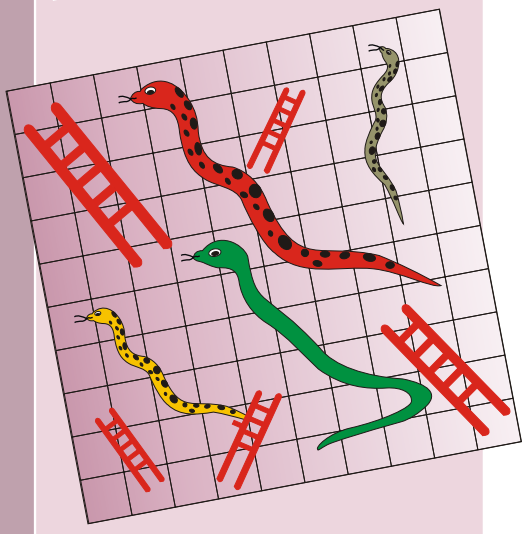
Salient Features

- Functionally enhanced Microspheres for correct determination of Endpoints
- Accurately calibrated reagents for true classification of positives and correct classification of titres that aid differential diagnosis and effective monitoring of various conditions
- Reagents Standardized against IRPs (International reference preparation) to ensure that the users report stays aligned to International standards
- Lot to Lot consistency ensures effective monitoring
- CE mark signifies conformity to international quality standards

RHELAX®

RF · CRP · ASO

SUCCESSFUL ASSAY DESIGN & CALIBRATION



Product	Sensitivity	Prozone limit	Standardization
Rhelax RF	10 IU/ml	2300 IU/ml	International reference preparation of Rheumatoid arthritis serum
Rhelax ASO	200 IU/ml	4000 IU/ml	International Standard for Antistreptolysin 'O' (97/662)
Rhelax CRP	0.6 mg/dl	100 mg/dl	International reference standard 85/506) for Human C-reactive protein

Interpret with ease!

Printed and published by D.G. Tripathi, Edited by Dr. R.J. Sood M.D. (path.) for and on behalf of Tulip Diagnostics Private Ltd, Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh Alto Santacruz, Bambolim Complex Post Office, Goa - 403202, INDIA. Fax: (0832) 2458544, E-mail: sales@tulipgroup.com. Website: www.tulipgroup.com