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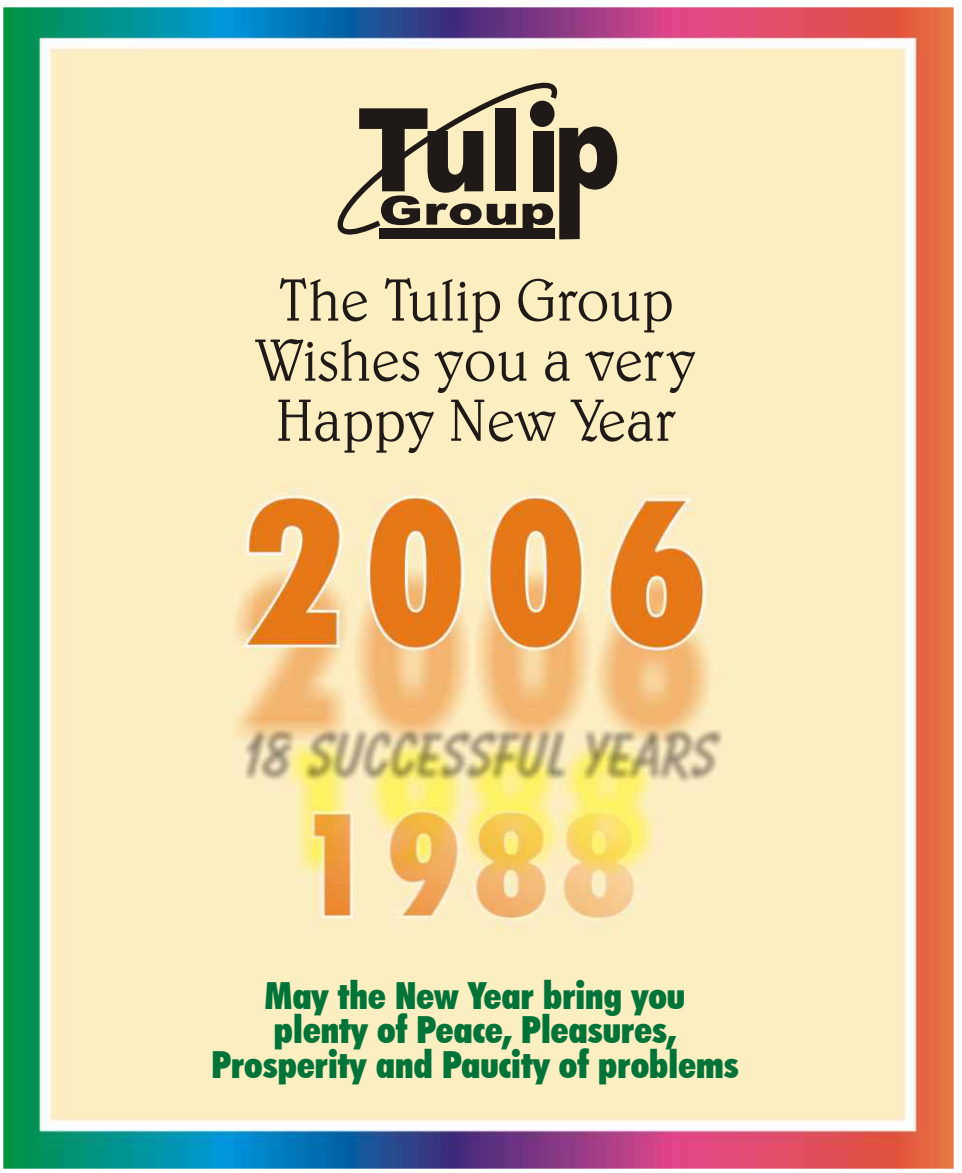

DISEASE DIAGNOSIS

TUBERCULOSIS

Preview

Tuberculosis (TB) continues to be a devastating disease worldwide and is believed to be present in about one third of the world's population. It is estimated that about 8 million new cases of TB and 2.6 to 2.9 million deaths from this disease occur annually around the world. Primary care physicians provide the critical first steps in reducing the incidence of active cases and detecting the emergence of drug resistance in TB. This article discusses characteristics of the disease and diagnostic procedures for rapid identification and drug susceptibility testing.

The *Mycobacterium tuberculosis* complex includes *M. tuberculosis, M. bovis,* and *M. africanum,* all closely related species or subspecies that may cause the disease tuberculosis (TB) in humans. These microbes are currently the leading cause of death from an identified organism worldwide, with about 8 million new infections and nearly 3 million deaths occurring annually. The World Health Organization predicts continued increases in TB and an estimated worldwide incidence of nearly 12 million cases each year by 2006.

An increase in TB rates is attributable to poor social hygiene, high population density, MDR cases, homeless persons, injecting drug users, HIV-infected patients, and prison inmates (congested residential places). TB rates have also increased among residents of nursing homes and mental health institutions and in healthcare workers. TB rates in some Asian and sub-Saharan Africa nations have increased to more than 300 cases per 100,000 population, and in some of these areas, nearly 50% of the HIV-infected population are co-infected with *M. Tuberculosis*.

Diagnosis

The term "tuberculosis infection" refers to a positive TB skin test with no evidence of active disease. "Tuberculosis disease" refers to cases that have positive acid-fast smear or culture for *M. Tuberculosis* or radiographic and clinical presentation of TB.

Traditionally, the diagnosis of TB has been made on the basis of clinical findings and chest radiographs and confirmed by sputum or tissue smears that show TB bacilli. These methods remain the "gold standard" for diagnosis, but development of DNA probes, polymerase chain reaction (PCR) assays, and liquid media now allow more sensitive and rapid diagnosis. Unfortunately, increased sensitivity of rapid techniques is not always associated with increased specificity.

Skin testing should be used in conjunction with other clinical findings and is not a sensitive or specific test for establishing the diagnosis. In extrapulmonary TB, site-specific tissue or fluid samples or both are submitted for smear, culture, and histologic analysis. Typically, histologic features of a tuberculous lesion includes caseating and noncaseating granulomata with giant cells

Pathogenesis and Clinical Features

Primary TB infection typically occurs in the lower lobes (because of preferential aerosol deposition) after inhalation of droplet nuclei. Bacteremia uniformly occurs during primary infection; therefore, tuberculous disease can occur in nearly any organ during the first weeks of infection. In the vast majority of patients, primary infection is asymptomatic or minimally symptomatic, with fever, dry or scant sputum production and, occasionally, development of retrosternal pain or erythema nodosum. Primary infection causes clinical pneumonia in 5% to 10% of adult patients and in an even higher percentage of children and HIV-infected persons.

Secondary TB is often referred to as reactivation disease and is responsible for 90% of TB in patients not infected with HIV. *M. Tuberculosis* microfoci that have remained dormant after primary infection may undergo reactivation, and patients often are asymptomatic in the early stages.

The manifestations of TB are protean, especially if extrapulmonary involvement is present. Therefore, TB should be considered in the differential diagnosis of all patients with fever of unknown origin, night sweats, or unexplained weight loss.

Symptoms (in % of TB patients)	
Cough	31-77
Hemoptysis	6-19
Weight loss	35-50
Fever	22-36
Anorexia	Avg 20
Night sweats	13-33
Chest pain	15-25
Dyspnea	Avg 22
Non respiratory complaints	Avg 33
Chills	Avg 13
No symptoms	5 -10

Patients in whom pulmonary cavitary disease develops generally have a high burden of *M tuberculosis* bacilli and are most likely to transmit the infection. An elevated sedimentation rate, marked leukocytosis (leukemoid reaction), hyponatremia, and hypercalcemia may be seen in active TB.

Radiographic findings

In primary TB, a lobar or segmental infiltrate, characteristically with ipsilateral hilar adenopathy, is seen on chest films. Adenitis may cause hilar adenopathy, leading to compression of the bronchus and displacement of the great vessels and trachea. Resolution of primary *M. tuberculosis* may be associated with development of a parenchymal nodule or Ghon focus, which typically become calcified with time. When a Ghon focus is associated with calcified hilar lymph nodes, it is termed a primary or Ranke's complex.

Primary *M. tuberculosis* infection may progress in a small percentage of patients, resulting in pleurisy and pleural effusion, progressive caseous pneumonia, extensive bronchopneumonia, or hematogenous spread that leads to disseminated disease. Some studies have indicated that primary *M. tuberculosis* is not recognized initially on chest radiographs from more than half of cases among hospitalized adults subsequently diagnosed with TB

The chest radiograph in patients with secondary TB may show fibronodular changes, most often in the upper lobes. Cavity formation or volume loss may also be apparent. Active disease should be suspected even if calcifications are present. One of the more common reasons for missed diagnoses of active TB is ascribing granulomatous changes to "old granulomatous disease," without ruling out *M. Tuberculosis*.

Other unusual presentations of reactivation disease include lower-lobe infiltrates, adenopathy, pulmonary masses, isolated or associated pleural effusion, pneumothorax, alveolar filling pattern, or presentation resembling adult respiratory distress syndrome.

Imaging techniques

Computed tomography (CT) is more sensitive than chest radiography for detection of cavities, lymphadenopathy, miliary disease, bronchiectasis, bronchial stenosis, bronchopleural fistula, and pleural effusion. The increased sensitivity of CT also is valuable when findings on chest films are absent or inconsistent and for guiding diagnostic evaluations, such as bronchoscopy. High-resolution CT may reveal occult abscesses, cavities, and the extent of pleural disease. CT is also useful in patients with extensive fibrosis, scarring, or postsurgical change. Magnetic resonance imaging is preferred for diagnosis of extrapulmonary disease, such as skeletal and intracranial TB.

Mycobacterial detection and isolation

A number of tests, ranging from acid-fast smears to complex serologic studies, are available or under development for identifying TB infections.

Acid-fast smear

The Ziehl-Neelsen carbolfuchsin or Kinyoun carbolfuchsin stains have been essential in TB diagnosis for nearly 100 years. Although less sensitive than culture, the acid-fast smear is a rapid and inexpensive test that can be performed with a minimum of equipment and is very specific for mycobacteria. Depending on the bacterial load, a single sputum smear has sensitivity between 22% and 80%, but the yield is improved when multiple sputum specimens are examined .

Fluorochrome stain

Most advanced laboratories these days use fluorochrome stains, such as auramine-rhodamine stain. With these techniques, mycobacteria fluoresce with a bright orange color and can be easily seen on low-power microscopy, increasing the sensitivity of the smears.

Nucleic acid amplification

Direct tests of nucleic acid amplification are rapid, widely available, and can be performed in a day.

Gene probes: The Amplified *Mycobacterium Tuberculosis* Direct Test targets mycobacterial ribosomal RNA by transcription-mediated amplification. The test uses DNA probes that are highly specific for *M. tuberculosis* species. It is best used (and only approved for use) in patients in whom acid-fast bacilli smears are positive and cultures are in process. Since specificity is less than 100%, even in patients with positive smears, occasional false-positive results do occur, usually in patients with nontuberculous mycobacterial infections.

PCR testing: This technique amplifies even very small portions of a predetermined target region of *M. tuberculosis*-complex DNA. The test uses an automated system that can rapidly detect as few as one organism from sputum, bronchoalveolar lavage, blood, cerebrospinal fluid, pleural fluid, or other fluid and tissue samples and has shown sensitivity and specificity of nearly 90% in pulmonary disease (Table. 2).

Table 2. Clinical utility of polymerase chain reaction (PCR) in diagnosis of tuberculosis

Smear	PCR	Clinical suspicion	Comment
Positive	Positive	High/low	Diagnosis: active TB
Negative	Negative	High/low	Cannot exclude active TB
Negative	Positive	High	Suspect *M. tuberculosis;* start treatment and review therapy when culture results are final
Negative	Positive	Low	Active TB, old TB, or contaminant; consult specialist
Positive	Negative	High	*M. tuberculosis* or nontuberculous mycobacteria; start therapy and review when culture results are final
Positive	Negative	Low	Most likely nontuberculous mycobacteria but cannot exclude *M. tuberculosis;* consider initiating TB therapy until culture results are final

Firefly luciferase: This ingenious assay uses the fluorescent capabilities of fireflies genetically implanted in *M. tuberculosis*. The procedure offers the possibility of testing mycobacterial drug susceptibility in hours. It is in the development stages but may become widely available in the next few years.

Culture techniques

The ability to culture mycobacteria is the "gold standard" in the diagnosis of TB. Although more sensitive than smears, cultures are less sensitive than nucleic acid amplification. Lowenstein-Jensen culture medium is the most commonly used solid medium, but mycobacterial growth may take up to 6 weeks. Therefore many laboratories simultaneously culture specimens in a broth-based medium that takes only 2 to 3 weeks for mycobacterial growth. Mycobacteria grown in liquid media are usually speciated and subcultured in the presence of different antimycobacterial agents to assess drug sensitivity. One of the newest and fastest techniques for growing *M. tuberculosis* is the Mycobacterial Growth Indicator Tube. The advantage of this system is the rapidity with which it detects isolates .

The World Health Organization recommends initial susceptibility testing for all *M. tuberculosis* isolates because of the emergence of drug resistance worldwide.

Serologic assays

Development of a sensitive and specific serologic assay for *M. tuberculosis* has been attempted for several decades but remains elusive. However, newer procedures using enzyme immunoassay may make serologic testing possible in the future. Such a procedure would be particularly helpful in detection of *M. tuberculosis* in children or in patients who have extrapulmonary infection and when obtaining specimens is a problem. The latest immunochromatographic platforms can be utilized as important screening devices.

Tuberculin skin testing

The Mantoux test is the preferred and standard skin test for detecting TB. It involves injection of 5 TU -10 TU of purified protein derivative (PPD, tuberculin), usually 0.1 mL, intradermally. Induration is then assessed at 48 to 72 hours. The extent of induration (not erythema) should be measured across two diameters at right angles and the two measurements then averaged. Interobserver variability can be decreased through the use of a ballpoint pen carefully brought from outside the zone of induration toward the center. As the area of induration is reached, resistance increases, and marking should stop at the outer edge of induration.

About 20% of patients with active TB may have negative skin tests, and some populations have an even higher incidence of false-negative results. For example, false-negative rates up to 50% have been reported in patients with advanced HIV infection. Alternately, false-positive results may occur in patients infected by other non-tuberculous mycobacteria (eg, *Mycobacterium avium* complex). Therefore, a negative skin test never rules out TB, and a positive skin test alone does not establish the diagnosis.

A person with a positive skin test who has had a negative test in the previous 2 years is considered a skin test converter. If a person currently has a positive skin test and a last negative skin test more than 2 years previously (or has had no previous skin test), he or she is considered a skin test reactor. In a patient with a positive reaction to allergy panel skin tests and a negative tuberculin test, tuberculous infection or disease cannot be excluded.

BCG vaccine and skin testing

The skin test reactivity associated with BCG (bacille Calmette-Guerin, a highly modified strain of *M. bovis*) vaccination in childhood usually diminishes in 5 years. A positive skin test in a person vaccinated with BCG more than 5 years before skin testing should be considered as caused by *M. Tuberculosis* infection and not attributed to BCG vaccination. Many of these patients are from countries in which TB is prevalent, and the likelihood of infection is high.

Booster effect

Although repeated exposure to tuberculin itself will not sensitize an uninfected person, it may sensitize patients who were infected previously and have experienced waning immunity. This is termed the booster effect and can occur with a second skin test even a year after the first test. This makes it difficult to assess whether the reaction represents new conversion or boosting of an old infection. One recommendation is to repeat the skin test 1 week after an initial negative test. If the second test is positive, boosting of former infection is likely. An increase in induration of more than 10 mm on the second test in persons less than 35 years old or of 15 mm in those more than 35 years old is considered positive for patients undergoing periodic testing. Recommendations for skin test interpretation are outlined in Table 3. Converters and reactors are not contagious to others unless active TB develops.

Table 3. Size of induration in relation to other risk factors in patients with positive tuberculin skin test

Induration ≥ 5 mm	• Contacts of person known to be infected • Patients with abnormal chest film • HIV-positive patients • Organ-transplant recipients • Other immunosuppressed patients (receiving equivalent of >15 mg/dl of prednisone for >1 month)
Induration ≥ 10 mm	• In countries with high incidence of TB • Residents of prisons, nursing homes, institutions • Injecting drug users • Healthcare workers (including mycobacteria laboratory personnel) • Children aged < 4 yr or infants, children, and adolescents exposed to high-risk adults • Persons with other high-risk medical factors (e.g, diabetes,silicosis, renal failure, cancer, gastrectomy) • Locally identified high-risk groups
Induration ≥ 15-20 mm	• No Risk factors

ADA (Adenosine deaminase) test

Adenosine deaminase is an enzyme that is found to be increased in the tubercular effusions as well as the sera of tuberculosis patients.
Increased ADA activity may be found in effusions due to a number of causes, including tuberculosis, bacterial infections, rheumatologic disease and lymphoproliferative disorders.
ADA, an enzyme that catalyzes the deamination of adenosine and deoxyadenosine into inosine and deoxyinosine, is found in most cells. ADA has two principal isoenzymes, ADA-1 and ADA-2, It is found in many tissues. The isoenzyme ADA-2 is the major component (73%) of the activity of total ADA in the serum of healthy persons. ADA-2 is found only in macrophages and monocytes. They release it when stimulated in the presence of live micro-organisms in their interior. This explain why ADA-2 increases in biological fluids in the course of infectious disease characterized by micro-organisms infecting the macrophages.
ADA analysis is a simple and inexpensive colorimetric test that can be performed on body fluids.
Although ADA isoenzymes do not detect tuberculosis in all cases, its specificity and sensitivity is much higher than traditional diagnostic tests such as skin test, smear, culture and so on. Difference between ADA levels in different studies is probably due to different methods of ADA measurement, presence of other diseases and TB epidemiology.
Because of the difficulty in isolating the causative organism, pericardial tuberculosis is hardly diagnosed. Recent reports in patients with TB pericarditis have shown that ADA levels in pericardial fluid are diagnostically useful in early diagnosis of TB pericarditis, particularly when the results of other clinical and laboratory tests are negative
Total CSF ADA can differentiate patients with TB meningitis from those with aseptic meningitis or a normal lumbar puncture
Ascitic fluid ADA activity has been proposed as a useful diagnostic test for diagnosis of TB peritonitis

Use of ADA activity for diagnosis of TB depends on prevalence of TB. Where TB is endemic or diagnostic procedures are expensive, ADA appears to be a useful test for early TB diagnosis. Most studies are on the total ADA level, but ADA isoenzymes may be more accurate. Determination of the individual ADA isoenzymes and ratio of the isoenzymes could help in differentiating the various causes of increased ADA activity in body fluids especially in borderline ADA levels. ADA-2 is elevated in TB as compared to other infectious or malignant causes.

ADA IS THE BEST TEST FOR EARLY TB DETECTION WHERE TB IS ENDEMIC OR OTHER DIAGNOSTIC MEANS ARE EXPENSIVE

It is generally accepted that clinical TB eventually occurs in about 10% of persons infected with *M. Tuberculosis*. Half of these clinical cases occur in the first 2 years after infection and the other half occur later. Progression from TB infection to disease is related to such host factors as genetics, nutritional status, and immunocompetence. In addition, other diseases (eg, diabetes, cancer, malnutrition, alcoholism, IV drug abuse, renal insufficiency, HIV) and use of immunosuppressive drugs may compromise the integrity of the immune system. While immunocompetent patients have a 10% lifetime risk for active disease after TB infection, the risk is 8% to 10% *per year* in HIV-infected patients.

To Summarise

The increasing incidence of TB and HIV infection and the emergence of drug resistance worldwide poses a major threat, particularly in developing nations. In an era with an increasing number of individuals living with HIV infection or with immunosuppression associated with chemotherapy or organ transplants, the possibility of primary *M. tuberculosis* and of unusual clinical and radiographic presentations of reactivation disease is becoming more common. The primary care physician plays a crucial role in recognizing high-risk patients and initiating prompt isolation and evaluation. The Laboratarians job has become all the more important to assist the clinicians in their diagnosis followed by apt therapy.

BOUQUET

In Lighter Vein

Teacher: Johnny, you know you can't sleep in my class.
Johnny: I know. But maybe if you were just a little quieter, I could"

"If there are any idiots in the room, will they please stand up" said the sarcastic teacher. After a long silence, one freshman rose to his feet.
"Now then mister, why do you consider yourself an idiot?" enquired the teacher with a sneer.
"Well, actually I don't," said the student, "but I hate to see you standing up there all by yourself."

Teacher: Why are you late, Joseph?
Joseph: Because of a sign down the road.
Teacher: What does a sign have to do with your being late?
Joseph: The sign said, "School Ahead, Go Slow!"

The answer to the problem was "log(1+x)".
A student copied the answer from the student next to him, but didn't want to make it obvious that he was cheating, so he changed the answer slightly, to "timber(1+x)".

Teacher: How can you prevent diseases caused by biting insects?
Jose : Don't bite any.

Wisdom Whispers

If you find it in your heart to care for somebody else, you will have succeeded.

Everyone admits that love is wonderful and necessary, yet no one agrees on just what it is.

Love begins with a smile, grows with a kiss, and ends with a teardrop.

We come to love not by finding a perfect person, but by learning to see an imperfect person perfectly.

Wind is to fire like distance is to love; it extinguishes the small and enflames the great.

Love is a fire. But whether it is going to warm your heart or burn down your house, you can never tell.

Love is shown in your deeds, not in your words.

Brain Teasers

1.What is the normal blood pH?
A. 7.35-7.45 B.7.15-7.25 C. 7.25-7.35 D.7.45-7.55
2. What is the normal anion gap level (in mEq/L)?
A. 10-30 B.30-50 C.60-100 D.8-16
3.What is the upper critical level of blood chloride level for human beings in mEq/L?
A. 106 B.108 C.110 D.115
4.What percentage of band form neutrophils is normal for human beings?
A. 0-5 B.5-10 C.10-15 D.15-20

ANSWERS : 1. A, 2. D, 3. D, 4. A.

INTERPRETATION

DIAGNOSIS AND CLASSIFICATION OF DIABETES MELLITUS

Glycated Hemoglobin (... contd)

The expert committee, however, did not include glycated hemoglobin measurement in the recommendations for international standards for the diagnosis of diabetes mellitus. They noted the lack of standardization and normal ranges among the various tests, making it difficult to dictate a standard cutoff point.

The test for measuring glycated hemoglobin is not widely available in developing countries; consequently, it was not favored for use as an international criterion. There is also some overlap in the levels of glycated hemoglobin in patients with diabetes mellitus and those without it.

Although it was not specifically recommended by the National Diabetes Data Group (US) as a diagnostic test for diabetes mellitus, glycated hemoglobin may, in some cases, be used to diagnose diabetes mellitus.

The diagnosis of diabetes mellitus is made in the following fashion. A glycated hemoglobin level of 1 percent above the reference laboratory's upper range of normal is consistent with diabetes mellitus and has a specificity of 98 percent. People with normal glycated hemoglobin levels (i.e., within the laboratory's published normal range) either do not have diabetes mellitus or have well-controlled diabetes mellitus (i.e., a false-negative test). However, incorrectly diagnosing these persons as normal would not alter their treatment because exercise and diet are adequately controlling their blood glucose levels.

People who are not diagnosed with diabetes mellitus and who have near-normal glycated hemoglobin levels (less than 1 percent above the normal range) may be advised of the high probability that they have diabetes mellitus and may be offered the same treatment as a person with mild diabetes mellitus (i.e., dietary and exercise counseling), followed by repeat testing of glycated hemoglobin several months later.

This method of screening and counseling high-risk persons is easier for many patients and clinicians because the blood specimen can be drawn at the time of the patient visit.

Glycated hemoglobin (also known as glycohemoglobin, glycosylated hemoglobin or HbA_{1c}) is used to monitor treatment in patients with diabetes mellitus;However, it is not recommended for routine diagnosis of this condition because of a lack of standardization of tests and results.

Impact of the New Diagnostic Criteria

Physicians may be concerned that the new diagnostic criteria for diabetes mellitus, including the lower cutoff for fasting plasma glucose levels, may greatly increase the number of people who are diagnosed with diabetes mellitus in their practices.

Concerns about overdiagnosis include the harm created by anxiety, the risks and costs of unnecessary treatment, and possible insurance discrimination, especially if the condition that is being diagnosed is relatively benign or if no effective treatment is available.

On the other hand, underdiagnosing a condition is harmful if early treatment can make a difference in patient outcome, especially if the treatment is relatively benign and inexpensive.

It is true that a rigorous screening program will increase the number of persons who are diagnosed with diabetes mellitus. However, currently one half of the people who have diabetes mellitus according to the old criteria have not been diagnosed and may remain undiagnosed for up to 10 years.

People who are asymptomatic and undiagnosed continue to develop the complications of diabetes mellitus.

Screening Recommendations

The expert committee provided guidelines governing the selection of patients to be tested for diabetes and the frequency of that testing *(Table shown below)*. Testing should be considered for all persons who are 45 years or older and should be repeated at three-year intervals.

Testing should be considered at a younger age and be performed more frequently in persons who are obese (120 percent of desirable body weight or greater or a body mass index of 27 kg per m^2 or greater); who have a first-degree relative with diabetes mellitus; who have delivered a baby weighing more than 4,032 g (9 lb), or who were diagnosed with gestational diabetes mellitus during pregnancy; are hypertensive; or have a high-density lipoprotein level of 35 mg per dL (0.90 mmol per L) or lower and/or a triglyceride level of 250 mg per dL (2.83 mmol per L) or higher. In addition, any patient with impaired glucohomeostasis should be reevaluated on a more frequent basis.

The expert committee recommended that screening for gestational diabetes mellitus be reserved for use in women who meet one or more of the following criteria: 25 years of age or older, obese (defined as more than 120 percent above their desirable body weight), a family history of a first-degree relative with diabetes mellitus, and belong to a high-risk ethnic population.

Recommendations for Diabetes Screening of Asymptomatic Persons

Timing of the first test and repeat tests

- *Test at age 45:*
  Repeat every three years (Patients 45 years of age or older)
- *Test before age 45:*
  Repeat more frequently than every three years if patient has one or more of the following risk factors:

  a) Obesity ≥ 120% of desirable body weight or BMI ≥ 27 kg per m^2

  b) First-degree relative with diabetes mellitus

  c) Member of high risk-ethnic group (Black, Hispanic, Native American, Asian)

  d) History of gestational diabetes mellitus or delivering a baby weighing more than 4,032 g (9 lb)

  e) Hypertensive (≥ 140/90 mm Hg)

  f) HDL cholesterol level ≥ 35 mg per dL (0.90 mmol per L) and/or triglyceride level ≥ 250 mg per dL (2.83 mmol per L)

  g) History of IGT or IFG on prior testing

BMI = body mass index; HDL= high density lipoprotein;
IGT = impaired glucose tolerance; IFG = impaired fasting glucose

Final Comment

The changes recommended by the expert committee for the diagnosis of diabetes mellitus should prove beneficial to patients. Measurement of fasting plasma glucose levels should be more acceptable to patients than the oral glucose tolerance test and can be readily incorporated with fasting lipid determinations. Identifying asymptomatic persons earlier in the disease process will allow earlier institution of lifestyle changes and medical therapy that may decrease the complications of hyperglycemia. The National Diabetes Data Group (US) emphasizes that these changes in diagnostic criteria have not changed the treatment goals in patients with diabetes mellitus. These goals include maintaining a fasting plasma glucose level of less than 120 mg per dL (6.65 mmol per L) and a glucose hemoglobin measurement of less than 7.0 percent.

TROUBLE SHOOTING

PRINCIPLES OF QUALITY ASSURANCE AND STANDARDS FOR CLINICAL CHEMISTRY (...contd)

II. Analytical Factors Important in Clinical Chemistry

A. Monitoring

1. Internal monitoring should include the following

(a) Quality of water (as specified by instrumentation and assays).

(b) Stability of electrical power (as specified by instrumentation).

(c) Temperatures of water bath, refrigerator, and freeze(recommended at least monthly).

(d) Regular calibration of analytical balances and pipettes (recommended annually).

(e) Maintenance of up-to-date procedure manuals with clearly stated dates when procedures are first implemented and when any changes are made and implemented.

(e) Maintenance of adequate inventory, with proper storage and handling.

(g) Maintenance of a log of changes in any procedures, problems or other factors affecting methods, as well as actions that resolved the problem. All entries should be clearly dated and signed by laboratory personnel.

2. External monitoring should include participation in an external proficiency program

(a) All participating laboratories should analyze the same materials.

(b) Results should be tabulated regularly (monthly, quarterly) and distributed to participants with statistical summaries and comparison of participating laboratories with mean indices expressing the closeness of individual laboratory results to the group mean.

(c) Means should be calculated and analyzed based on identification of the method (same methods compared).

(d) Each laboratory should carefully assess the validity of their reported performance and consider any changes indicated by the proficiency program.

B. Method Validation

Method validation should be performed before a test procedure is placed into routine use. Validation may be accomplished by thoroughly testing reference materials or by comparison of results of tests performed by an alternative method. For each method, the laboratory should verify the manufacturer's claims and any adjustments before initiating patient testing. Method validation should provide evidence of the following:

1. Accuracy Perform either (a) or (b)

(a) Run known value substance and compare results to expected value.

(b) Perform split sample patient comparison between existing method of known accuracy and new method.

2. Precision Perform either (a) or (b)

(a) Run 10 replicates of 2 levels of quality control (QC) samples.

(b) Gather 21 results; 7 results in each of 3 separate runs (better estimate of day -to-day precision, as well as within-run precision).

With results from (a) or (b) determine mean, standard deviation (SD) and coefficient of variation (CV). Determine whether within-run SD is acceptable.

3. Sensitivity Perform (a), (b) or (c)

(a) Assess manufacturer's claims.

(b) Use concentration of low calibrator or another sample or fluid low levels of analyte.

(c) Run a series of dilutions and assess acceptability of performance.

4. Specificity Perform (a) or (b)

(a) Use published list of interfering substances, check with manufacturer.

(b) Assess known or suspected interfering substances by spiking specimens or use patient material with known conditions.

5. Linear reportable range

(a) Establish upper and lower limits for reporting patient values based on calibration materials.

(b) For the lower limit, there should be confirmation of the discriminatory ability of the test.

(c) The highest calibration point is the maximum upper limit and the lowest calibration point or zero should be the minimum lower limit for reporting patient results.

6. Linearity Perform either (a) or (b)

(a) Determine by analyzing multiple dilutions of either a high calibrator, control or patient samples with increased levels of analyte.

(b) Analyze calibrators of variable, known concentrations.

(c) Linearity should be established at the time of validation and whenever new or altered reagents are used.

7. Reference intervals

(a) The laboratory should establish or validate existing reference intervals for each method before reporting results.

(b) Parallel tests should be run to confirm reference intervals for controls when changing reagents or QC lot number.

C. Instrumentation

1. Instrument performance

The equipment and instrument used must be capable of providing test results within the laboratory's stated performance characteristics. These include: detection limits, precision, accuracy, specificity, sensitivity, freedom from interferences and related test variables (refer to previous section on method validation)

Additional points to consider: Instruments with adjustable setting for different substances and/or species should be carefully checked for compliance performance characteristics as defined, Compare and make adjustments for performance characteristics as defined by the laboratory and the manufacturer, Make sure certain species differences are accommodated.

2. Functional checks

(a) Appropriate function checks should be made on all instruments. These are critical operating characteristics of an instrument, i.e., stray light, zeroing, electrical levels ,optical alignment, background checks, etc.

(b) Laboratory personnel should recheck and /or calibrate each instrument daily or once per shift, prior to patient testing, to ensure that it is

functioning correctly and is properly calibrated. This includes QC.

3. Calibration

(a) Instruments should be calibrated every 6 months or more frequently if indicated by: manufacturer's recommendation, after major service, QC outside limits or troubleshooting indicates need, laboratory determination that volume, equipment performance or reagent stability indicate a need for more frequent calibration.

(b) After calibration, controls should be run.

4. Laboratory personnel knowledge of equipment and its use, including, but not limited to:

(a) Linearity differences from possible manufacturer's range (human) to animal.

(b) Effects of hemolysis, lipemia, icterus, caretenoid pigments (especially large animals), and different anticoagulants on each assay.

(c) Reportable ranges.

(d) Species-specific ranges and reference intervals.

(e) Expected abnormal ranges..

(f) Common problems encountered with veterinary samples.

(g) Regular instrument maintenance schedule.

(h) Replacement of inadequate or faulty equipment.

(i) Problem-solving procedures, troubleshooting.

D. Quality Control

1. For each run, at least 2 controls should be assayed. Use of 'high' and 'low' abnormal controls is recommended.
2. Maximum length of a run is 24 hours. If the instrument manufacturer requires more frequent controls, observe the recommended frequency (i.e, some blood gas instruments).
3. Verify that the instrument is stable over the "run time". During a validation check, controls are assayed more frequently to establish run time.
4. Establish QC frequency; consider the following:

(a) Test volume (number performed each run or day) and frequency.

(b) Technique dependence of the method.

(c) Analyte or reagent stability.

(d) Frequency of QC failures.

(e) Training and experience of personnel.

(f) Cost of QC (increasing frequency adds to cost-per-test).

5. Quality control parameters

(a) Mean, SD and CV should be calculated (minimum number = 20).

(b) Controls should be assayed in the same manner as patient specimens.

(c) A mechanism should be in place to determine whether testing personnel follow policies and procedures correctly.

(d) Use of Westgard multirule procedures or other rules based on QC validation is recommended.

(e) Policies and procedures should be written and available in a laboratory. Standard Operating Procedures (SOP) manual to ensure accurate and reliable test results .

(f) An SOP manual should have clearly marked and dated entries of current procedures (manufacturer package inserts are sufficient as long as verified) and when any changes are made and implemented.

(g) QC records should be reviewed frequently to ensure that when QC values fail to meet the criteria for acceptability, suitable action is taken.

(h) Control products should be purchased commercially, if possible. If using calibrators as controls, use a different lot as QC material. If patient pooled samples are used, establish the mean value of all analytes (minimum n = 10 to establish a mean).

(i) Monitor results of clinical specimens for various sources of error by use of parameters such as anion gap, comparison of test results with previous submissions from same patient (delta checks), and investigation of markedly abnormal results (limit checks).

E. Procedures Manual

All procedures currently in use should be included. Protocols may be organized in manuals and/or stored in computers, and be in written form They should contain such information as: patient preparation, specimen collection, processing and handling, criteria for rejection of specimens, limitations and things that interfere with the method in use, step-by-step procedures, reagent preparation, manufacturer reference interval, reportable range, literature references, reagent labeling: content, storage requirements, expiration and laboratory-specific information, such as identification of instrument used, result reporting method, actions to take when system is down, criteria for specimen referrals to outside laboratories ("send outs"),quality control procedures, documentation of critical values, clearly stated and dated entries of procedure implementation or change

F. Comparison of Test Results

If the laboratory performs the same test by more than one method or a more than one test site, or the test is sometimes also sent to a referral laboratory, comparisons should be run at least twice annually to define the relationships between methods and sites. Comparison of different test methods for the same analyte within the laboratory or between laboratories (if samples are tested in-house and at a referral laboratory) is recommended. This should be done every 6 months or at a frequency determined by the laboratory manager. The following steps should be included:

1. Perform a 20-sample or greater comparison using specimens covering the analytical range:(a) group data in an x-y comparison plot,(b) calculate slope and intercept by a least squares method
2. Laboratory director or qualified personnel should define acceptable performance limits
3. If individual test results performed on the same patient or material do not correlate with each other (ie, BUN/creatinine, electrolyte balance), the cause should be investigated and corrective action taken.

Postanalytical Factors Important in Clinical Chemistry

A. Computer Entry of Data
B. Report Generation
C. Report Delivery
E. Specimen Disposal
F. Personnel Safety
G. Laboratory Environment
H Personnel Training Requirements

TULIP NEWS

Tulip Group puts up a spectacular display of its products at the MEDICA this year again

This year too, Tulip Group participated in MEDICA 2005 - The 37th International Trade Fair with Congress World Forum for Medicine held from 16th -19th November 2005 at Dusseldorf, Germany.

The Tulip Group's stall *(Inset Picture)* and the CE marked products on display were commendable and appreciated by the huge crowd of visitors to the stall. The excellent response received at this year's MEDICA foresee Tulip Group's plans of achieving global heights in the field of diagnostics as one of the WORLD's leading manufacturer and marketeer of *in vitro* diagnostic reagents and instruments.

More than 137,000 trade visitors travelled to Dusseldorf to attend the world's largest medical fair

The medical sector is among the key innovation and growth drivers – as was impressively confirmed by the world's largest medical trade fair MEDICA. On the four days of the trade fair a total of 137,000 trade visitors from nearly 100 countries came to Dusseldorf (the figure for the previous year being 136,152). Especially for companies with their sights on new markets MEDICA 2005 offered a second-to-none basis for business due to the events' high degree of internationality. Almost 40 % of visitors came from abroad, a particularly high percentage also came from overseas markets such as the USA, India, China or Taiwan and increasingly also from Arab countries. All in all, people from more than 100 nations attended MEDICA, thereby once again underscoring its outstanding importance as the World Forum for Medicine.

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