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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

ISO 9001

Editorial

We would like to thank all our valued readers for appreciating the previous two efforts. They highlighted two emerging diseases with serious outcomes if not diagnosed in time. This issue highlights a disease that has brought about a hiatus in the living populations of many a nation. It is gradually wiping the working and reproductive age groups. Being an STD, the disease affects both parents and in the process has acquired the dubious distinction of having created the largest number of orphans.

No single disease has evoked so much response and investment from the medical fraternity as HIV/AIDS. Considered so devastating that for almost two decades even "CANCER" has been relegated to the backbench. Labeled as a Bio-hazard 3 disease, HIV/AIDS has no known cure or vaccine available as on date. The "mantra" then is PREVENTION. How does one prevent? Educate the population at large and prevent spread from the already infected individuals. Logic dictates that all infected persons must first be diagnosed. For doing so, we must thoroughly know about the disease process and must be well conversant with the diagnostic tools available. The DISEASE DIAGNOSIS section of this issue discusses all aspects of HIV/AIDS (though in brief) with special reference to internationally accepted diagnostic criteria. The thought of HIV/AIDS evokes suicidal tendencies and attracts the highest degree of social stigma; a disease so horrifying must be reported with utmost caution. Flip this page to be transported to the world of HIV/AIDS diagnosis.

Affliction with tuberculosis has been considered to be a social curse. With the arrival of HIV/AIDS and development of multi-drug-resistant strains, the problem has been compounded manifold. No perfect or foolproof immunological diagnostic tool has been devised as yet, consequently, the dictum "SEEING IS BELIEVING" still holds good. Diagnosis of tuberculosis can be established by actually visualizing the acid-fast bacilli microscopically. Conversely, the disappearance of AFBs heralds the institution of appropriate and effective treatment. The TROUBLE SHOOTING section resolves the problems encountered during this exercise.

Glycosylated hemoglobin has assumed significant importance in the management of diabetes mellitus. The investigation reveals all about previous 90-120 days history vis-à-vis diabetes mellitus. The investigation schedule and interpretation of the GHb. values obtained is clarified in the INTERPRETATION segment of this issue.

BOUQUET has all the shoots it has been coming with thus far. Just the hues and aromas are different

Trust you shall enjoy and appreciate this issue too.

Tulip Group

DISEASE · DIAGNOSIS

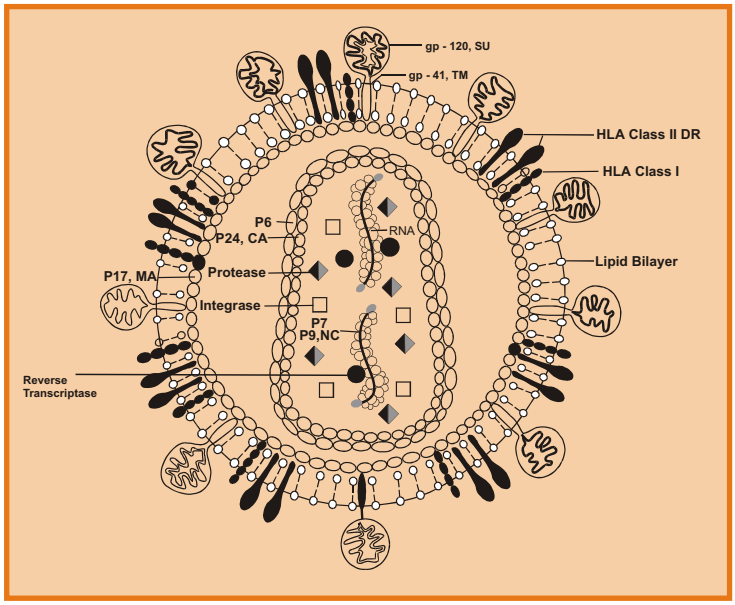
HIV/AIDS

Origin and Historical Aspects

Diagnosed first amongst gays in 1981 in USA, HIV/AIDS has by now disseminated to the entire world. Heterosexual contacts and intravenous drug abusers are the predominant new cases. It is slowly wiping the most productive age group individuals (15-49 years). The first case probably occurred in West or West Central Africa as a Zoonosis from primates/monkeys (affected by SIV or Simian Immunodeficiency Virus). The SIV virus is said to have mutated to the HIV variant. By the year 2001 about 40 million people worldwide were infected, the figure rises by over 5 million people with each passing year. An explosion is in the offing if not curtailed.

Causative Agent: The HIV-1 and HIV-2 belong to the retrovirus family and sub family lentivirinae, having a lipid enveloped positive stranded RNA and possess an enzyme called reverse transcriptase (this transcribes the viral RNA into provirus DNA which is integrated into the host cell genome). They are designated as retroviruses because their genetic information flows from RNA to DNA (opposite direction of the flow as compared to most biological systems).

Variants: HIV virus collectively refers to HIV-1 and HIV-2 retroviruses, which are distinct from each other.

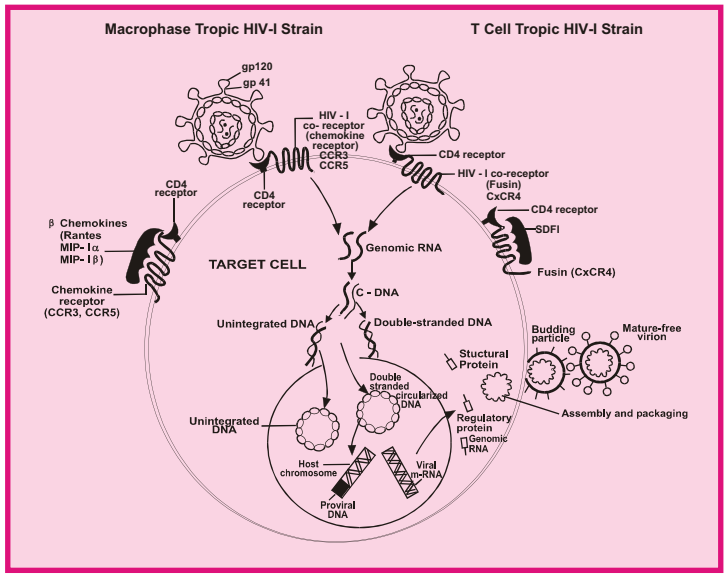


CA- capsid, p-proteins, SU-surface, TM-transmembrane, gp-glycoprotein

Various HIV-1 sub types with their predominant geographic distribution are: - B (USA, Japan, Australia); A, D (Sub Saharan Africa); C (Africa, India); E (Central African Republic, Thailand, South East Asia); F (Brazil, Romania); G, H (Russia, Central Africa); I (Cyprus); and O (Cameroon). Biologically HIV-2 is very similar to HIV-1, it is less easily transmitted and patients tend to live longer. While HIV-1 has a global presence, HIV-2 is primarily found in West Africa.

Life Cycle of the HIV

Like all lipid enveloped viruses, the HIV must remain moist to be infectious and is easily inactivated in a dry atmosphere. Therefore, HIV is primarily transmitted sexually, by blood or its products, or from mother to child transplacentally or by breast-feeding or by sharing infected needles (clinically or by intravenous drug abusers). After initial attachment to lymphoctes and monocytes, a series of events occur which result in multiplication of viral particles (as shown in the figure given next).



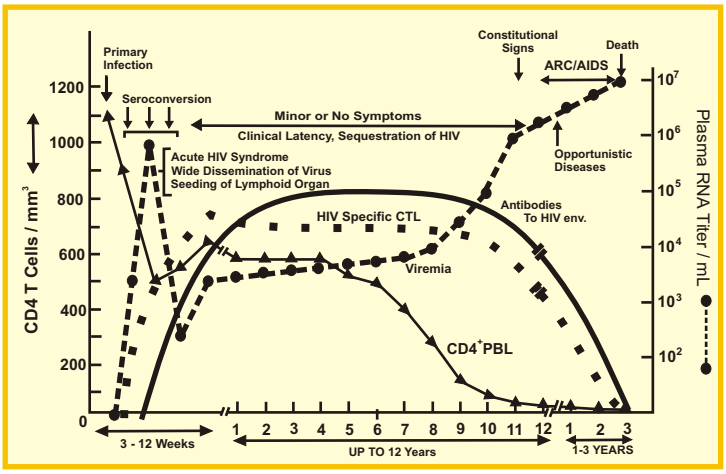
Attachment: The virus attaches to at least two receptors on the T-lymphocytes – CD4 and 7-transmembrane receptor. *Entry:* It fuses with cell membrane and the viral RNA is emptied into the cell's cytoplasm. *Reverse Transcription:* Reverse transcriptase reads the viral nucleic acids' sequence and transcribes the same into a complimentary DNA sequence. Reading of the RNA sequence may be faulty sometimes, leading to development of variants. *Integration of Viral DNA:* The complimentary DNA strand is integrated into the lymphocytes' DNA by using its own enzyme called "integrase" and forms a provirus. *Transcription:* In an activated lymphocyte, the viral DNA produces multiple copies of the viral RNA. This RNA codes for the production of the viral proteins and enzymes (translation) and will also be packaged later as new viruses. *Translation:* There are nine genes in the HIV RNA. Their identification, protein product and function in the life cycle of HIV is given below.

HIV Gene	Protein Product	Function in Life Cycle of HIV
gag	Gag/core protein precursor	Viral core proteins p24: major capsid protein p17: matrix protein p9: binds to viral RNA p7: binds to viral RNA
env	Env./envelope proteins	Viral envelope proteins: gp 120: major envelope protein, mediates virion binding to cell surface receptor (CD4) gp 41: mediates fusion of viral envelope and cell membrane
pol	Reverse transcriptase	Converts single-stranded viral RNA into viral DNA duplex
	Integrase	Integrates viral DNA duplex into host cell genome as provirus DNA
	Protease	Cleaves core precursor polyprotein into functional core proteins
tat	Tat protein	Essential regulatory protein, trans-activates expression of all viral genes
rev	Rev protein	Regulatory protein, activates expression of HIV structural and enzymatic genes
vif	Vif protein	Role in viral budding and infectivity of free virions
vpu	Vpu protein	Promotes release of budding virions from host cell
nef	Nef protein	Regulatory protein (essential for pathogenicity of SIV)
vpr	Vpr protein	Regulatory protein, role uncertain

Assembly and Budding: Finally, viral RNA and associated proteins are packaged and released from the lymphocyte surface as mature free virions, that propagate the cycle. During the cycle mutations occur.

Natural History of HIV Infection: Primary HIV infection —3-6 weeks→ Acute HIV syndrome (mononucleosis-like, plasma viremia present) —1 week- 3 months→ HIV-specific immune response (serum antibody detectable) —1-2 weeks→ Clinical latency (curtailment of viremia, decline of CD4 T-cell count) —10 years, median→ Clinically apparent disease or AIDS-defining illness (deterioration of immune system,increase in plasma viremia) —2 years avg.→ Death from AIDS.

Immunopathogenesis of HIV infection



CTL-cytotoxic T lymphocytes, PBL- peripheral blood lymphocytes. ARC- AIDS related complex

Clinical Aspects

A reducing CD4-T cell count and increasing plasma viremia with p24 antigenemia herald deterioration of the immune system which signifies the onset of complications. Symptomatic stage arrives when CD4-T cell count continues downward to the range of 200-400 cells/ cubic mm. A CD4 T-cell count of less than 200 cells/cubic mm implies the onset of AIDS. The plethora of clinical presentations that ensue is given below.

(CD4 T-cell count 200-400/ cubic mm).

Constitutional symptoms	Infections	Other manifestations
• Fever • Weight loss • Fatigue • Night Sweats • Diarrhoea • Persistent generalized lymphadenopathy	• Oral or vaginal candidiasis • Oral hairy leukoplakia • Herpes zoster (shingles) • Herpes simplex • Listeriosis	• Cervical dysplasia • Cervical carcinoma in situ • Idiopathic thrombocytopenic purpura (ITP) • Neuropathy • Seborrhea

	List of conditions in the 1993 AIDS Surveillance Case Definition issued by the Centers for Disease Control and Prevention
1	**CD4 T-cell count 200/cubic mm**
2	Opportunistic infections Candidiasis of bronchi, trachea or lungs Candidiasis, esophageal Coccidiodomycosis, disseminated or extrapulmonary Cyptococcosis, extrapulmonary Cryptosporidiosis disease (other than liver, spleen or nodes) Cytomegalovirus retinitis (with loss of vision) Herpes simplex : chronic ulcer (s) (1 month duration) ; or bronchitis, pneumonitis or esophagitis Histoplasmosis, disseminated or extrapulmonary Isopsorasis, chronic intestinal (1 month duration) Mycobacterium avium complex or M. kansasii, disseminated or extrapulmonary Mycobacterium tuberculosis, any site Mycobacterium, other species or unidentified species, disseminated or extrapulmonary Pneumocystis carinii pneumonia Pneumonia, recurrent Salmonella septicemia, recurrent Toxoplasmosis of brain
3	**Neoplastic disease** Cervical carcinoma, invasive Kaposi's sarcoma Lymphoma, Burkitt's (or equivalent term) Lymphoma, immunoblastic (or equivalent term) Lymphoma, primary in brain
4	**HIV encephalopathy (AIDS dementia complex)**
5	**Wasting syndrome due to HIV**
6	**Progressive multifocal leukoencephalopathy (PML)**

HIV Prognostic Markers

Specific: Several can be used but declining levels of antibody to p24 and increasing concentrations of p24 HIV antigen is associated with disease progression.

Non specific: a) CD4 cell count as has been explained earlier (normal range is 600-1700 cells/cubic mm).

b) CD4:CD8 ratio (normal adult ratio being 1.2 to 3.5).

Recent studies have stressed the importance of combining measurements of the levels of serum β2-microglobulin or neopterin along with the CD4 cell counts.

Seroconversion and Window Period: Seroconversion (antibody production) occurs in most cases by 4-8 weeks after exposure although in some cases it may take 6-9 months. This delay between infection and appearance of antibodies is referred to as the "Window Period". At seroconversion IgM class antibodies are detectable and later IgG antibodies take over. These antibodies are formed against principal structural proteins, core proteins and the polygene proteins. Eventually, as the immune system is destroyed, antibodies may not be detectable in some patients.

HIV DIAGNOSIS

Screening and diagnostic tests rely upon the detection of antibodies to HIV. During part of the window period IgM anti-HIV and HIV Ag may be present. In the earlier years Western Blot (WB) was devised as a confirmatory technique but as the epidemic spread the need to have rapid, simple and inexpensive assays was felt. WB is expensive and prone to producing large numbers of indeterminate results. Specific formats for screening and confirmation of HIV have been developed.

Enzyme Immunoassays (EIA): These can broadly be defined as binding assays that depend upon the antigen-antibody reaction as the basis and the enzyme reaction as a marker for the proof of reaction. Performing these tests require specialized laboratories and trained manpower.

Generation of Anti HIV Assays

First Generation	Native viral antigens from lymphocyte culture. These lacked specificity.
Second Generation	Expression products of recombinant HIV cDNA
Third Generation	Chemically synthesized oligopeptides (about 15-40 amino acids) Double antigen sandwich assays
Fourth Generation	Simultaneously detect p24 antigen and anti HIV antibodies

(Except first generation assays all others are more specific and sensitive, safer and easier to use and have better reproducibility). By EIAs one can detect HIV antibodies or HIV antigens

Western Blot: The assay is based on the detection of the reaction of specific antibodies in the sample with viral antigens that have been electrophoretically separated and transferred ("blotted") onto a nitrocellulose sheet on a backing. Antibodies, which react with antigens on the membrane, are visualized with an enzyme labelled anti-human immunoglobulin and an enzyme substrate, which gives an insoluble coloured product at the site of reaction. Where antigen-antibody reaction takes place eventually appears as band. Recently, immunoblots, which resemble WB but utilize a selection of synthetic HIV antigens instead of natural ones, have been developed. The third generation tests now pick up seroconversions early and are gradually closing down the "window phase", consequently one comes across cases that are WB negative and positive by other formats. In this context, slowly, the Western Blots may be losing their original relevance.

Criteria for Interpretation of Western Blot

Recommending Organisation	Criteria	
	Positive	Negative
WHO	Two env. Bands	No HIV specific bands
C.D.C	Any two of p24; gp41; gp 120/160	No bands at all
Consortium for Retrovirus Serology standardisation	p24 or p32 and gp 41 or gp 120/160	No bands at all
American Red Cross	At least one from each of gag, pol and env	No bands at all
FDA (USA)	p24, p32 and gp 41 or gp 160	No bands at all

Results that are neither positive nor negative as per the aforesaid criteria are classified as "indeterminate"

Various agencies have differing set of criteria that create diagnostic problems. A case positive by criteria from one agency may be negative by employing criteria prescribed by another.

Indirect Immunofluorescence: Detect HIV antibodies.

Radioimmunoprecipitation Assay: Very few laboratories use RIPA.

Virus Detection Methods: Patient's peripheral blood lymphocytes are cultured with uninfected stimulated lymphocytes. Cytopathic changes may be confirmed by immunofluorescence or the culture medium may be assayed for HIV p24 antigen or reverse transcriptase activity.

P24 Antigen and Nucleic Acid Technologies: p24 antigen is detected by ELISA while nucleic acid sequences are detected by PCR.

The methods mentioned above are expensive, time consuming, cumbersome and require highly trained manpower.

Rapid Tests for Detection of anti-HIV Antibodies: Rapid HIV antibody assays based on agglutination, immunodot, immunochromatographic (ICT) and immunoconcentration / filtration techniques are now available. These tests should, even in window period or otherwise, pick up HIV-1 (O and M variants included) with a sensitivity and specificity in excess of 99%. They should employ recombinant antigens or peptides from the conserved immunodominant regions of the HIV-1 and HIV-2 genomes. Third generation rapid tests now utilize the principle of double antigen sandwich immunoassay technique. Rapid tests are available in Flow Through and lateral flow formats. Flow through formats are multicomponent, multistep based systems that require cold storage. ICTs are easier to perform and are useful tools even in field settings.

There are three main diagnostic applications of anti HIV testing:

(a) To confirm that a patient presenting with symptoms associated with HIV infection is infected with HIV

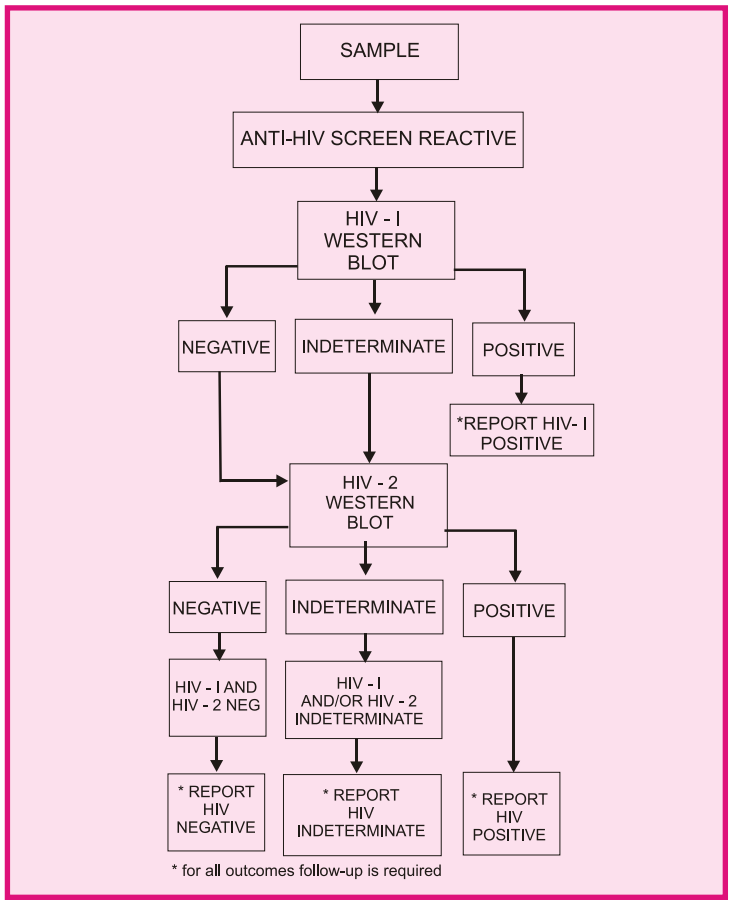
(b) To investigate a person who has been exposed to infection because of exposure to body fluids from a known HIV-infected individual, perhaps by sexual intercourse or by inoculation

(c) And to test those who belong to one of the recognized risk groups, are healthy and have no definite history of exposure, but are anxious and present themselves for testing in the hope of gaining the reassurance of a negative result.

Many diagnostic laboratories now take the precaution of running two screening tests (usually different manufacturers kits) on each sample to decrease the possibility of false negative findings, whether due to a technical or clerical error or some inherent fault in an assay.

Confirmatory Supplementary Tests: The best of the currently available anti-HIV screening assays will give rise to less than 1 in 1,000 repeatable false positive reactions. It is generally accepted that only positive and unexpected negative results in screening assays need to be reconfirmed. So that unnecessary confirmatory testing is not undertaken, most laboratories first retest reactive samples twice or more in the screening assay. Unrepeatable reactions are reported as negative. Whatever confirmatory strategy is employed, it is good practice to seek a follow-up sample from any individuals found to be reactive. This is to primarily safeguard against a false diagnosis due to technical or clerical errors, but also to check that an uncomfirmable positive reaction on a screening assay is not a result of sampling too early during seroconversion. Cases of dual infectivity with HIV-1 and HIV-2 have been reported and thus in some instances this may require confirmation.

The most widely accepted "confirmatory" test is Western Blot. It provides additional information specificity, in terms of reactivity with particular HIV proteins. Nucleic Acid Technologies or NAT-PCR based assays detect the nucleic acids of HIV directly in an infected patient's sample and are employed as confirmatory tests too. A protocol for approach to confirmatory tests is presented below.



Alternative Approach to Confirmatory Testing: This approach seeks to determine if a sample reaction in a screening test will also give a positive signal in at least two further 'independent' screening assays. "Independent" here implies - of a different type. But this concept has recently been broadened to incorporate assays using antigens from different sources, eg. viral, recombinant DNA expression products and synthetic peptides. It has recently been shown that a combination of rapid screening assays can also give diagnosis as accurate as WB, and more cheaply. This option should be particularly useful for anti-HIV confirmation especially in the developing world, as a cost effective solution.

W.H.O. / UNAIDS Strategies for HIV Antibody Screening

These strategies take into account the objective of testing (i.e. whether for transfusion safety, surveillance or diagnosis) and the local seroprevalence rate. Depending on these factors, three different algorithms for repeat and confirmatory testing have been proposed to confirm initial screening results.

They avoid the use of the Western Blot for confirmation of positive screening test result; although Western Blot confirmation is still the rule in the USA, Germany and other countries, it can be replaced by more economical testing algorithms using ELISA-type assays and / or rapid / simple test devices.

Strategy 1 requires only one test. It is to be used for:

- Transfusion screening (if no results are to be provided)
- Surveillance (if prevalence>10% and no results are to be provided)
- Diagnosis (only if patient is symptomatic and population HIV prevalence>30%)

  NB: This is not normally adequate for diagnosis!

Strategy 2 requires up to two tests and is to be used for:

- Surveillance (if prevalence < 10% and no results are to be provided)
- Diagnosis if patient symptomatic and population prevalence < 30%, or if patient asymptomatic and population prevalence > 10%.

Strategy 3 requires up to three tests for:

- Diagnosis if patient asymptomatic and population HIV prevalence < 10%.

TROUBLE SHOOTING

Staining Mycobacteria

Poverty (nutritional deprivation), industrialization (crowded habitation), and HIV (diminished immunity) have all contributed to an exponential spread of tuberculosis. Efforts spent on HIV have pushed tuberculosis on the backburner. Each year, India adds about 1.5 million new cases; of which about 25% are sputum positive. Emergence of multidrug resistant strains due to poorly administered therapeutic measures and patient non-compliance are further complicating the issue.

Mycobacteria are slow growing, aerobic, non-motile, slightly curved or straight, "acid fast" organisms. They are gram positive but stain poorly with gram's stain. The mycobacterial cell wall is lipid rich, which includes true waxes and glycolipids. 60-90 carbon, long chain Mycolic acids, unique to the mycobacterial cell wall are responsible for their: a) Acid fastness, b) Failure to react with Gram stain, and c) Resistance to the action of antibodies and complement.

Diagnosis is usually based on clinical symptoms, radiographic images and detection of AFB in the material provided. Systems based on immunological principles for M. tuberculosis have yet to overcome problems of poor sensitivity and specificity.

Recommendations for Sample Collection for Mycobacterial Isolation and Acid Fast Staining.

Specimen type.	Specimen requirements.	Special instructions.
Abscess contents, aspirated fluid.	As much as possible in syringe with Luer tip cap.	Cleanse skin with alcohol before aspirating sample. Laboratory may provide 7H9 broth / Kirchner medium for transport of small volumes of aspirates.
Blood.	10 ml SPS (yellow top) blood collection tube or 10 ml Isolator tube.	Disinfect site as for routine blood culture. Mix tube contents immediately after collection. SPS is preferred anticoagulant. Heparinized blood is also acceptable.
Body fluids (pleural, pericardial, peritoneal).	As much as possible (10-15 ml min.) in sterile container or syringe with Luer tip cap. Collect bloody specimens into SPS blood collection tubes.	Disinfect site with alcohol if collecting by needle and syringe.
Bone.	Bone in sterile container without fixative or preservative.	----
Bone marrow.	As much as possible in SPS blood collection tube or 1.5 ml in pediatric Isolator tube.	Collect aseptically. Mix SPS tube contents immediately following collection.
Bronchoalveolar lavage or bronchial washings.	≥ 5 ml in sterile container.	Avoid contaminating bronchoscope with tap water. Saprophytic mycobacteria may produce false-positive culture or smear results.
Bronchial brushings.	Sterile container or Middlebrook 7H9 broth, or Kirchner medium.	----
CSF	≥2 ml in sterile container.	Use maximum volume attainable.
Gastric lavage fluid.	≥5-10 ml in sterile container. Collect in the morning soon after the patient awakens in order to obtain sputum swallowed during sleep.	Collect fasting early morning specimen on three consecutive days. Use sterile saline. Adjust to neutral pH with 100 mg of sodium carbonate immediately following collection. Laboratory should provide collection tube containing sodium carbonate.
Lymph node.	Node or portion on sterile container without fixative or preservative.	Collect aseptically, and avoid indigenous microbiota. Select caseous portion if available. Do not immerse in saline or other fluid or wrap in gauze.
Skin lesion.	Submit biopsy specimen in sterile container without fixative or preservative. Submit aspirate in syringe with Luer tip cap.	Swabs in transport medium (Amies or Stuarts) are acceptable only if biopsy sample or aspirate is not obtainable. For cutaneous ulcer,collect biopsy sample from periphery of lesion, or aspirate material from under margin or lesion.
Smear on slides.	Smear specimen over 1.5 by 1.5 cm area of clear slide.	Heat fix smears. Transport in slide container taped closed and labeled BIOHAZARD.
Sputum.	5-10 ml in sterile, wax-free disposable container. Collect an early morning specimen from deep, productive cough on at least three consecutive days. Do not pool specimens. For follow-up of patients on therapy, collect at weekly intervals beginning three weeks after initiation of therapy.	For expectorated sputum, instruct patient on how to produce sputum specimen as distinct from saliva or nasopharyngeal discharge. Have patient rinse mouth with water before collecting sputum to avoid contaminating specimen with food particles, mouthwash, or oral drugs, which may inhibit the growth of mycobacteria. For induced sputum, use sterile hypertonic saline. Indicate on request if specimen is induced sputum.
Stool.	≥1 g in sterile, wax-free, disposable container.	Collect specimen directly into container, or transfer from bedpan or plastic wrap stretched over toilet bowl. Wax from container may produce false positive smear.

Sample Concentration and Decontamination

Specimens obtained from sterile body spaces (CSF, pleural, peritoneal, and pericardial fluid) do not require decontamination. However, samples obtained from the respiratory tract contain a mixed microbial flora and hence make concentration and decontamination necessary. The sample is treated with 2% NaOH (decontaminant) and 0.5% N-acetyl-L-cysteine (mucolytic). This is followed with sequential buffered wash of the concentrated sample as high or low pH can interfere with staining and culture. Buffered wash, additionally, reduces the specific gravity of the specimen and sediments the Mycobacteria effectively. For pelleting the mycobacteria a relative centrifugal force of about 3800 G is ideal as is clear from the table given below:

Specimen	Relative Centrifugal Force (*g*)		
	1260	3000	3800
Positive smears	1.8%	4.5%	9.6%
Positive cultures	7.1%	11.2%	11.6%
Correlation of positive smears/ cultures	25%	40%	82%

The AFB Smear

The sensitivity of AFB smear for extrapulmonary specimens is lower than for sputa. The carbolfuchsin binds so tightly to the lipid-rich mycobacterial cell wall, so much so, that it resists decolourisation even with strong acids or alcohols. On microscopy, they appear as beaded and slightly curved rods. Carbolfuchsin stains both, viable and degenerating bacteria. Dead bacteria are, however, not stained. AFB staining is a simple and inexpensive tool to diagnose and monitor treatment of tuberculosis. Two types of acid-fast stains are most often employed:
1.Carbolfuchsin based stains and 2. Fluorochrome based stains.

Carbolfuchsin stain: The reagent consists of Basic Fuchsin and a disinfectant Phenol (carbolic acid). Carbolfuchsin stained Mycobacteria appear bright red/ pink against a green or blue counterstaining background. Three kinds of Carbolfuchsin based systems are available.
1.Three components Ziehl-Neelsen or "hot stain".
2.Three component Kinyoun or "cold stain" and
3.Two component modified Kinyoun cold stain (the decolouriser is incorporated into the counterstain).
The two-component stain saves time, labor and money and besides being more user-friendly it is also easy to standardize. Carbolfuchsin stains require 40 x - 100x objectives for visualization.

Fluorochrome based stains: For AFB Auramine O alone or in conjunction with Rhodamine is used. This technique requires a fluorescent microscope for screening. The method requires 25 x objective only. Mycobacteria appear bright yellow against a dark background obtained by counterstaining with potassium permanganate. The method increases screening speed and larger areas can be screened with a lower magnification.
The crucial factors in maximizing smear sensitivity and specificity are:
- Centrifugation of digested fluid specimen at a minimum of 300 G;
- The smear should be prepared on a new clean undamaged glass slide;
- Scanning of at least 300 fields per slide;
- The reporting of the AFB smear should preferably be done according to the C.D.C., USA method, or as per the National Reference Institution norms.

C.D.C., USA criteria

Carbolfuchsin (X 1,000)	Fluorochrome (X 250)	Quantity Reported
No AFB/300 fields	No AFB/30 fields	No AFB seen
1-2 AFB/300 fields	1-2 AFB/30 fields	Doubtful; repeat test
1-9 AFB/100 fields	1-9 AFB/10 fields	Rare (1+)
1-9 AFB/10 fields	1-9 AFB/ field	Few (2+)
1-9 AFB/ field	10-90 AFB/ field	Moderate (3+)
>9 AFB/ field	>90 AFB/ field	Numerous (4+)

Indian Reference Institutions criteria

Number of Acid Fast Bacilli observed	Report
No Acid Fast Bacilli	Negative
1-10 Acid Fast Bacilli	Actual Number
>10 Acid Fast Bacili	+
Masses of Acid Fast Bacilli in several fields	++

Factors Influencing Sensitivity and Specificity of AFB Smears.·
Acid-fast particles other than tubercle bacilli. Food particles (e.g., waxes, oils), stain precipitates, other micro-organisms (including saprophytic or commensal AFB as Mycobacteria smegmatis or Mycobacteria kansasii; Nocardia species; spores of Bacillus subtilis,), inorganic materials, fibres and pollens and artifacts (scratches on the slide) may be mistaken for pathogenic AFB.*Contamination through the transfer of bacilli from one smear to another.* To prevent this, immediately discard a slide that has been used once.·*False negative results can occur because of:-*
- Inadequate sputum collection. Guide the patient. Saliva and nasopharyngeal secretions do not constitute a specimen. Early morning bronchial sputum from the "depths of the chest" is ideal. If necessary forced expectoration may be tried.
- Improper storage of sputum specimens and stained smears. AFB may loose their acid-fastness on exposure to sunlight, radiation, excessive heat, or storage for over a week in hot and dry conditions. Immersion oil decolourises AFB, if re- examination is considered, clean oil with xylol. Fluorochrome stained smears lose fluorescence with storage.
- Failure to select suitable sputum particles for smear preparation. Tubercle bacilli are most likely to be found in little blobs ("lentils") of greenish-grey or yellowish matter of thick, creamy consistency sputum.
- Inadequate preparation of smear or staining of slides. Too little material; too thick smear; over heated smear; insufficiently fixed smear; excessive staining with carbolfuchsin; intensive counterstaining; and inappropriate stain timings, all can lead to false negative reports.
- Colour blind and short of time examiners are likely to miss acid-fast bacilli.

BOUQUET

In Lighter Vein

- A father was worried about his son's ability to pass his English essay test. So he made him cram one on 'My best friend' which he was sure would be the one of the choices. Instead of that the subject on the examination paper was 'My Father'. Undaunted, the boy utilized his memorized text to his best advantage by substituting friend with father. This is how the essay went "I have many fathers. Ravi Prasad is my best father. He lives next door to us. He comes to visit us everyday. My mother loves him very much. A father in need is a father indeed."
- A young lady went to a hospital and told the receptionist that she wanted to see an upturn. "You mean an intern, don't you dear?" asked the kindly nurse. "Well, whatever you call it, I want a contamination," replied the girl. "You mean an examination," corrected the nurse. "Maybe so," allowed the girl. "I want to go to the Fraternity ward." "Maternity ward," said the nurse with a slight smile. "Look," insisted the girl, "I don't know much about your big words, but I do know that I haven't demonstrated for two months, and I think I'm stagnant."

Wisdom Whispers

- Give to every other human being every right you claim for yourself.
- If you do not stand for something you will fall for everything.
- Empty sacks will never stand upright.
- Silence is one great art of conversation.
- One man with courage is a majority.
- Enthusiasm is caught, not taught.

Brain Teasers

1. In which condition does one find raised urobilinogen in stool?
   A) Hemolytic anemia B) Severe liver disease C) Aplastic anemia
   D) Complete biliary obstruction.
2. Which diarrhoeal stool would not show you polymorphonuclear leucocytes on microscopy?
   A) Yersinia infection B) Shigellosis C) Ulcerative colitis D) Cholera.
3. What is the etiology of Curling's ulcer of stomach?
   A) H. pylori B) Drug induced C) Idiopathic D) Acute burns.
4. In which of the following syndromes is Serum Gastrin level very high?
   A) Marfan's syndrome B) Zollinger Ellison syndrome
   C) Loefller's syndrome D) Gilbert's syndrome.
5. In which of the following conditions can Serum Alkaline Phosphatase be diminished?
   A) Osteomalacia B) Scurvy C) Paget's disease D) Healing fractures.
6. In which of the following is proteinuria predominantly albumin?
   A) Acute glomerulonephritis B) Multiple myeloma
   C) Macroglobulinemia D) Primary amyloidosis
7. In Plummer Vinson Syndrome, which of the following investigations will be useful?
   A) Serum iron B) Serum magnesium
   C) Serum calcium D) Serum bilirubin.
8. In ataxia telangiectasia, which of the following marker's values are raised?
   A) CEA B) AFP C) HCG D) PSA

ANSWERS 1) A, 2) D, 3) D, 4) B, 5) B, 6) A, 7) A, 8) B.

INTERPRETATION

Glycohemoglobin

Glycohemoglobin (GHb.): While blood glucose estimation reveals current situation of carbohydrate metabolism in the diabetic patient at the time of blood collection. GHb and fructosamine in contrast permit retrospective assessment of glycemia. Glucose and other monosaccharides react, depending on their concentration, with free amino groups of proteins. This irreversible, nonenzymatic reaction is referred to as glycation (depends essentially on the degree and duration of the blood glucose elevation). It happens with hemoglobin also. As half life of hemoglobin is 100-120 days. So, GHb can be used as a parameter to assess carbohydrate metabolism for the same period retrospectively. Glycation is irreversible and enzymatic systems for the degradation of glycation sites of hemoglobin are not known.

Assays for the determination of total glycated serum proteins, also referred to as *fructosamine* because of the ketoamine form of the attached glucose-protein complex, as well as for the determination of single glycated proteins, mainly albumin are commercially available. In a few cases where GHb is of a limited value, determination of fructosamine is an alternative for long-term glycemia monitoring, within a shorter time frame.

Nomenclature of glycohemoglobins

HbA	95-97%	Hemoglobin A
HbA_0	90%	Nonglycated HbA fraction
HbA_1	5-7%	Glycated Hemoglobin A_0 ($\alpha_2\beta_2$)
- HbA_{1a}	HbA_{1a1}	Glycation with fructose-1-6 – biphosphate
	HbA_{1a2}	Glycation with glucose-6 phosphate
-HbA_{1b}		HbA_1 with unknown reaction partner
-HbA_{1c}		75-80% of HbA_1, glycation with D-glucose at the N-terminal valine of the β-chain
l-HbA_{1c}		Labile HbA_{1c} (aldimine)
s-HbA_{1c}		Stable HbA_{1c} (ketoamine)
HbA_2	<3%	Hemoglobin A_2 ($\alpha_2\delta_2$)
HbF	<1%	Hemoglobin F ($\alpha_2\gamma_2$)

(Glycated hemoglobin determined by affinity chromatography is referred to as total glycohemoglobin)

Recommended time frequency for HbA_{1c} testing in DM

Type of DM/treatment	Recommended frequency
Type I DM, minimal or conventional therapy	3-4 times a year (quarterly)
Type I DM	Every 1-2 months
Intensive therapy type II DM	2 times per year in stable metabolic conditions
Diabetic pregnant	Every 1-2 months
Gestational DM	Every 1-2 months

Many methods are available for determining HbA_{1c}; commonest among these is based upon Ion Exchange Chromatography (column chromatography, microcolumn, HPLC, FPLC fall under this category). Other methods utilize Thiobarbituric acid, Electrophoresis, Isoelectric focusing, Affinity chromatography, and Immunochemical techniques.

Anticoagulated blood (EDTA or heparinised) is the sample of choice. Relationship between age or gender and HbA_{1c} is not clear. The reference interval is dependent on the analytical method and/or the reagents.

Clinical Significance

Two different approaches are used to assess the quality of blood glucose control in diabetics.

- In the first approach the mean blood glucose of the preceding 6-8 weeks is correlated with the corresponding GHb value. By using an algorithm, the mean blood glucose of the preceding 6-8 weeks can be derived from the GHb value. These algorithms are valid for individual methods and may not be universally applied

Mean blood glucose concentration (mg/dL) = 33.3 x HbA_{1c} (%) – 86 OR

HbA_{1c} (%) = 2.07 x mean blood glucose concentration (mmol/L) OR

HbA_{1c} (%) = 0.038 x mean blood glucose concentration (mmol/L) + 3.78.

- The other approach combines clinical experience with the extrapolation of borderline values based on the reference interval. According to the St. Vincent Declaration of the WHO, for example, a good blood glucose control is connected with GHb. values lower than three standard deviations above the reference population (99.9% of the values in the reference population are below this limit). A poor blood glucose control is connected with GHb values, which are higher than five standard deviations above the mean of the reference population. GHb values between these values are connected with borderline good blood glucose control.

Parameter	Good (< x + 3SD)	Borderline (x + 3-5 SD)	Poor (> x + 5 SD)
HbA_1 (%)	< 8	8-9.5	>9.5
HbA_{1c}	< 6.5	6.5-7.5	>7.5

GHb values are increased in: Diabetes mellitus, glycosuria, and hyperglycemia. Falsely high values may be obtained due to fetal-maternal transfusion, hemodialysis, hereditary persistence of fetal hemoglobin, neonates and pregnancy.

GHb values are decreased in: Falsely decreased values may be obtained in anemias (hemolytic, pernicious, sickle cell); chronic loss of blood; effects of splenectomy; renal failure (chronic), and thalassemias. Any condition that shortens RBC lifespan will give falsely low GHb values.

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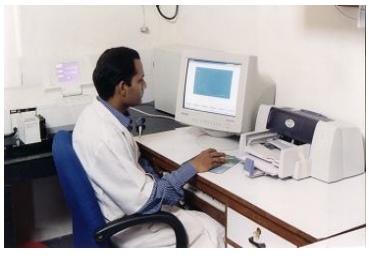

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