

## CONTENTS

1 Editorial

2 Disease  
Diagnosis

5 Interpretation

7 Trouble  
Shooting

6 Bouquet

8 Tulip News



## Editorial

Seeing is believing! Does that hold true for HIV as well? Perhaps yes, as most diagnostic methodologies available do have certain limitations. False positive as well as false negative results are known to occur. Seeing HIV virions require electron microscope - not possible to have at every diagnostic setup. Besides being expensive, the process is cumbersome and time consuming. What then? Well, we have to know and understand the limitations of existing methodologies. Malignancies, pregnancy, tuberculosis, other retroviral infections AND MANY MORE do and can give false positive results with the in-use HIV diagnostic systems. The answer is to test multiple samples with multiple methodologies for the same patient. The stigma and the seriousness and certainties associated with HIV should make us think twice before signing out an HIV positive report. False positive HIV reports though are rare. INTERPRETATION section in this issue talks about all existing methods available to diagnose HIV, it very clearly elucidates their limitations or negative aspects. The "GOLD STANDARD" will always be "SEEING IS BELIEVING" i.e., growing the HIV virion and seeing it under the electron microscope. The indirect evidences like existence of antibodies etc will take care of most but the rare cases where interfering antibodies are present. The TROUBLE SHOOTING segment is also devoted to HIV here. The article delves deep into the issues relating to the Rapid HIV Diagnostic formats (RDTs). Where and under what circumstances should one employ them and what are their capabilities is adequately covered under the heading. Until we identify a treatment or a workable vaccine, HIV shall always stay on the 'front burner'. **The C<sup>rux</sup>** team understands the problems faced by laboratarians and constantly strives to inform you about the remedies. The patient must be informed about the test and also about its limitations. This alone can guard us all because false positives just can not be wished away, this because interfering antibodies exist and shall remain so in future too.

DISEASE DIAGNOSIS is again caught up with fever and infection one that usually spares the immunocompetent ones but creates numerous complications for the immunocompromised ones. You have guessed it right! It is toxoplasmosis. Complete clinico-diagnostic details are provided. Serology, PCR and histopathology as related to toxoplasmosis is cited in detail.

Let's see if BOUQUET can motivate you and how much do you remember about the protozoan parasites! At the end of it all, just laugh it off. Forget your worries and straighten the creases on your forehead. After all, life goes on, doesn't it.

## DISEASE DIAGNOSIS

### TOXOPLASMOSIS

#### Description

- Toxoplasmosis is an infectious disease caused by the parasite *Toxoplasma gondii* (*T. gondii*)
- It has a multiform clinical presentation ranging from asymptomatic in immunocompetent patients, to severe and potentially fatal disease in immunocompromised patients and patients with congenital infection
- The type of infection, immune status of the patient, and clinical setting should all be taken into account when making the diagnosis
- Acute infection in immunocompetent patients can result in a self-limiting infectious mononucleosis-type illness. However, in immunocompromised patients and patients with congenital infection, the disease is more serious and can affect a number of different organs or body sites, including the central nervous system (CNS), lungs, or eyes
- The standard treatment of choice is a combination of pyrimethamine, sulfadiazine, and leucovorin (folinic acid) and is generally reserved for immunocompromised patients with acute infection, reactivation of chronic infection or patients with congenital infection

#### Clinical alert

- Pregnant women: prevention of newly acquired infection of the mother if seronegative; treatment to prevent symptomatic infection of baby if the neonate or infant is seropositive at birth or in early infancy
- Immunocompromised patients (e.g. patients with AIDS or transplant recipients): prevention education, prophylactic treatment and treatment if seropositive
- Toxoplasma prophylaxis is indicated in HIV-positive patients if the CD4 count is <100 and *T. gondii* IgG is positive. If the CD4 count is >100 they should only be advised against eating undercooked meat and handling cat litter without gloves

#### Demographics

##### Age

- Seroprevalence of *T. gondii* infection increases with age
- Average figures being: 10% at 10 years, 20% at 20 years, and 50% at 70 years
- Congenital toxoplasmosis in children is more severe than infection acquired postnatally

##### Genetics

The HLA-DQ3 antigen is associated with susceptibility to TE (Toxoplasmic encephalitis) in AIDS patients.

##### Geography

- Lower prevalence in cold, or hot and arid regions and at high elevation
- Infection prevalence varies with geographical location.
- Seroprevalence in women of childbearing age in 1990s: 37-58% in central Europe, north Africa, and Australia; 51-77% in America and sub-Saharan Africa; 4-39% in India, southeast Asia, and China

#### Clinical presentation

Toxoplasmosis has a multiform presentation with signs and symptoms that vary according to the route of infection (congenital or acquired), clinical setting, or the immune status of the patient.

- In immunocompetent adults (including pregnant women) and children, acute acquired *T. gondii* infection is asymptomatic in the majority of cases (80-90%)
- The most common clinical presentation in the 10% of immunocompetent patients who develop symptoms is lymphadenopathy
- In immunocompromised patients (patients with AIDS, hematologic malignancy, autoimmune disease, and bone marrow or organ transplants) toxoplasmosis is a serious disease that most commonly affects the CNS
- Toxoplasmosis in immunocompromised patients can also present as chorioretinitis, myocarditis, pneumonitis, hepatitis, or multiorgan involvement
- The majority of newborns with congenital toxoplasmosis are asymptomatic at birth (70-90%). Symptoms can develop later and include chorioretinitis, visual impairment, and intellectual or neurological impairment
- Symptomatic newborns with congenital toxoplasmosis can present with predominantly neurological disease or generalized disease

#### Symptoms

Acute infection in immunocompetent patients:

- Pain associated with lymphadenopathy (most frequently cervical), Malaise, Fever, Sore throat, Myalgia, Abdominal pain
- Rare symptoms associated with acute infection in immunocompetent patients:

- Symptoms of chorioretinitis, Symptoms of polymyositis, pericarditis, myocarditis, hepatitis, pneumonitis, or meningoencephalitis
- Symptoms of meningoencephalitis: headache, vomiting, seizures, transitory confusion

Symptoms of acute infection or reactivation of latent infection in immunocompromised patients may involve the brain, eyes, and spinal cord, or be generalized. Congenital toxoplasmosis may cause generalized and CNS/neurological disease. Ocular toxoplasmosis can occur in immunocompetent and immunocompromised patients infected with *T. gondii*, but occurs most commonly as a result of congenital infection.

Toxoplasmic encephalitis:

- Headache, Confusion, Mental status changes, Seizures, Focal motor deficits, Hemiparesis, Speech abnormalities, Cranial nerve disturbances, Sensory abnormalities, Movement disorders

Spinal cord toxoplasmosis:

- Motor or sensory disturbances of single or multiple limbs, Bladder or bowel dysfunction, Local pain

Additional symptoms can include:

- Fever, Malaise, Symptoms of chorioretinitis, pneumonitis, hepatitis, carditis, and myositis

Congenital toxoplasmosis: CNS/neurological disease:

- Symptoms of chorioretinitis, Blindness, Psychomotor or mental retardation, Seizures, Hearing loss

Congenital toxoplasmosis; generalized disease:

- Hemorrhage, Vomiting, Diarrhea, Feeding problems

Ocular toxoplasmosis:

- Sudden onset of floaters, Blurred vision, Visual loss, Pain, Photophobia

#### Signs

Signs that can be associated with acute infection in immunocompetent patients:

- Lymphadenopathy
- Mononucleosis-like syndrome: maculopapular rash; hepatosplenomegaly

Rare signs in immunocompetent patients:

- Signs of polymyositis, pericarditis, myocarditis, hepatitis, pneumonitis, or meningoencephalitis
- Cerebellar signs, Neuropsychiatric findings, CNS lesions, Focal abscesses, Diffuse encephalitis

Additional signs:

- Signs of chorioretinitis, pneumonitis, hepatitis, carditis, and myositis
- On ophthalmoscopic examination *Toxoplasma* chorioretinitis manifests as raised, yellow or white cottony exudates in a multifocal distribution, whereas the lesions of CMV retinitis are distributed adjacent to major vessels
- Hemodynamic abnormalities
- Acute respiratory failure

Congenital toxoplasmosis: CNS/neurological disease:

- Most infants with congenital toxoplasmosis are without apparent abnormalities at birth, Hydrocephalus, Intracerebral calcification, Microcephaly, Strabismus
- Classic triad of chorioretinitis, hydrocephalus, and cerebral calcifications resulting in mental retardation, seizures, and impaired vision is highly suggestive of congenital toxoplasmosis but is rarely observed today

Congenital toxoplasmosis; generalized disease:

- Rashes (e.g. maculopapular), Poor temperature regulation with hypothermia, Prematurity, Lymphadenopathy, Hepatosplenomegaly, Jaundice, Cyanosis, and Edema secondary to hepatic, pulmonary, myocardial, and renal involvement, Petechiae due to anemia, and thrombocytopenia, Neutropenia, Ecchymoses, Endocrine abnormalities: hypothyroidism, diabetes insipidus, sexual precocity, and partial anterior hypopituitarism, CSF abnormalities: lymphocytic pleocytosis, hypoglycorrhachia, and elevated protein level

Ocular toxoplasmosis:

- Focal lesions in the retina and choroids, Vitritis, Iridocyclitis, edema, cellular infiltration

- On ophthalmoscopic examination toxoplasma chorioretinitis manifests as raised, yellow or white cottony exudates in a multifocal distribution, whereas the lesions of CMV retinitis are distributed adjacent to major vessels

### Differential diagnosis

Acute toxoplasmosis:

- Epstein-Barr virus mononucleosis, cytomegalovirus infection, tularemia, cat-scratch disease

Toxoplasma brain lesions:

- Lymphoma, Nocardia infection, sarcoidosis, tuberculosis, histoplasmosis, bacterial abscess

Congenital toxoplasmosis:

- Congenital herpes simplex virus, syphilis, or rubella infection; erythroblastosis fetalis

### Workup

#### Diagnostic decision

- Diagnosis of toxoplasmosis cannot be made on clinical grounds alone, as patients present with symptoms and signs that are generally nonspecific
- Diagnostic decisions are based on results from a combination of laboratory tests including serology, tissue biopsy, and PCR amplification of *T. gondii* DNA in body fluids suspected to be infected
- Diagnostic tests and their interpretation vary according to the clinical setting and category of infection (i.e. acute infection in the immunocompetent and during pregnancy, acute infection or reactivation of latent infection in the immunocompromised, or congenital infection)
- Distinguishing between recently acquired and chronic infection is particularly important in pregnant women, as congenital toxoplasmosis almost always results from women who acquire the infection during gestation
- Congenital infection should be suspected based on demonstration of maternal seroconversion during pregnancy, and can be confirmed by appropriate laboratory tests prenatally or during the first 2 weeks of life
- Early diagnosis of acute or reactivated *T. gondii* infection in immunocompromised patients (AIDS patients, patients with hematological malignancies, bone marrow, or solid organ transplants) is critical, as without treatment, toxoplasmosis can be fatal in these patient populations
- In immunocompromised patients (except heart transplant patients), reactivation of chronic, latent infection is the most common cause of toxoplasmosis. For this reason, the anti-*T. gondii* antibody (Ab) status of these patients should be determined at their initial routine assessment, before onset of any symptoms

#### Never miss!

- Regardless of initial presentation, congenitally infected babies should be monitored further for learning disabilities, vision, psychomotor development and hearing, because late sequelae are not uncommon
- Most important factor in preventing severe, potentially fatal disease in immunocompromised patients is early detection of *T. gondii* infection
- Prophylactic therapy should be administered in chronically infected immunocompromised patients, particularly transplant recipients and those with AIDS, who are at high risk of reactivation and severe disease

### Investigations

#### 1. Complete blood count (CBC) with differential

- CBC with differential and smear examination is important in the diagnosis of all serious diseases, especially infectious diseases

#### Normal

- Total white blood cells (age >2 years): 5000-10,000/mm<sup>3</sup> (5.0-10.0x10<sup>9</sup>/L)
- Lymphocytes: 20-40% or 1500-4000/mm<sup>3</sup> (1.5-4.0x10<sup>9</sup>/L)
- Platelets: 130-400x10<sup>9</sup>/mm<sup>3</sup> (130-400x10<sup>9</sup>/L)
- Red cell count: 4.3-5.9x10<sup>6</sup>/mm<sup>3</sup> (4.3-5.9x10<sup>12</sup>/L) (males); 3.5-5.0x10<sup>6</sup>/mm<sup>3</sup> (3.5-5.0x10<sup>12</sup>/L) (females)
- Hemoglobin: 13.6-17.7g/dL (males); 12.0-15.0g/dL (females)
- Hematocrit: 39-49% (males); 33-43% (females)

#### Abnormal

- Lymphocytosis with presence of atypical lymphocytes
- Leukopenia
- Anemia
- Thrombocytopenia

### Cause of abnormal result

Acute or reactivated *T. gondii* infection.

Medications, disorders and other factors that may alter results

- Age may influence results; normal infants have higher white blood cell (WBC) counts than adults, while the elderly may have decreased counts in response to infection
- Stress or vigorous physical activity may increase WBC counts
- WBC counts are normally elevated during last months of pregnancy
- Epinephrine, allopurinol, aspirin, chloroform, heparin, quinine, steroids, and triamterene can cause increased WBC counts
- Antibiotics, anticonvulsants, antihistamines, antimetabolites, antithyroid drugs, arsenicals, barbiturates, diuretics, sulfonamides, and some chemotherapeutic agents may decrease WBC counts

### 2. Serologic tests: Detection of anti-*T. gondii* antibodies in serum

- Standard tests for IgG, IgM, IgA, IgE, IgG avidity, and Western blot analysis of mother-infant paired serum samples
- IgG antibodies appear within 1-2 weeks of infection, peak in 6-8 weeks and decline over the subsequent 1-2 years; however they remain detectable for life
- IgM antibodies appear in the first week of infection and decline over a few months. In some subjects they may remain persistently elevated, therefore an elevated IgM is not conclusive evidence of acute infection
- IgA assays may be more sensitive than IgM assays for diagnosing congenital infections
- Seroconversion or a two-fold increase in the antibody titer confirms the diagnosis of acute infection

### Advantages/disadvantages

Advantages:

- A combination of serological tests [e.g. IgM, IgG, IgA, IgE ELISA, IgG avidity test, AC/HS test(differential agglutination test)] can differentiate between a recently acquired infection and chronic infection; particularly important in pregnant women
- Rapid, inexpensive results
- Western blots for IgG antibodies of mother-infant pairs is a sensitive method for diagnosing congenital toxoplasmosis (more sensitive than IgM tests)

Disadvantages:

- IgM tests have low specificity and are often misinterpreted
- Interpretation of a positive IgM test (indicative of acute infection) is complicated by false-positives and persistence of positive titers years after infection in some patients; additional diagnostic tests are required to confirm acute infection
- In immunocompromised patients chronically infected with *T. gondii*, serological testing is of little diagnostic value and can be misleading - IgM antibodies are not found in reactivation disease in severely immunosuppressed patients
- Interpretation of IgM and IgA test results in newborns with suspected congenital toxoplasmosis can be complicated by transfer of maternal IgA during birth and false positive antibodies may occur

### Normal

- Seronegative: no antibodies to *T. gondii* present in venous blood (indicates no previous infection)
- High avidity IgG and no IgM to *T. gondii* present in venous blood (usually indicates previous or chronic infection)

### Abnormal

- Detection of *T. gondii* IgM in venous blood (indicative of acute infection)
- Detection of IgA and IgE in venous blood (indicative of acute infection)
- Four-fold rise in IgG titer in sera run in parallel
- Detection of IgM and IgA in newborn venous blood indicates congenital infection
- Keep in mind the possibility of a false-positive result, particularly with IgM test

### Cause of abnormal result

- Acute *T. gondii* infection [IgM, IgA, IgE, a 4-fold increase in low avidity IgG, and an acute pattern in the AC/HS test(differential agglutination test)]
- False-positive IgM results are common

Medications, disorders and other factors that may alter results

- Immunosuppressive drugs or disorders inhibit antibody production
- Patients with rheumatoid arthritis or antinuclear antibodies may give false positive results

### 3. Histologic diagnosis

Description

- Tissue sections or body fluid smears [e.g. BAL( Bronchoalveolar lavage), CSF,

or amniotic fluid] suspected of being infected

- The immunoperoxidase stain enables specific staining of *T. gondii*

#### Advantages/disadvantages

##### Advantages:

- Toxoplasmic lymphadenopathy has characteristic histologic features
- Immunoperoxidase technique is sensitive and specific
- Tissues can be unfixed or formalin-fixed paraffin-embedded tissue sections
- Wright-Giemsa staining of air-dried slides is rapid and simple
- Can distinguish acute infection or reactivation of latent infection (tachyzoites in tissue sections or smears, or multiple tissue cysts near an inflammatory necrotic lesion indicate active infection)
- Can link histopathological changes to presence of *T. gondii*

##### Disadvantages:

- Presence of tissue cysts does not distinguish between acute and chronic infection (except with placental tissue or tissue from newborns)
- Can be invasive (biopsy)
- Can take several days
- Labor intensive

##### Normal

Tissue section or smear exhibits normal cellular appearance for type examined.

##### Abnormal

- In toxoplasmic lymphadenopathy there is a characteristic triad of reactive follicular hyperplasia, with irregular clusters of epithelioid histiocytes and monocytoid B cells that distend the subcapsular and trabecular lymph node sinuses
- Presence of tachyzoites in tissue or body fluid
- Presence of multiple tissue cysts close to inflammatory necrotic lesions in tissue sections

##### Cause of abnormal result

Acute *T. gondii* infection or reactivation of latent infection.

Medications, disorders and other factors that may alter results

Inadequate tissue sampling may cause a false-negative result.

#### 4. Polymerase chain reaction (PCR)

##### Description

- Immunocompromised: blood, affected body fluids [bronchoalveolar lavage (BAL), cerebrospinal, pleural, ascitic, peritoneal, or ocular fluids], bone-marrow aspirate, or tissues
- Newborn: peripheral blood, CSF, and urine
- Prenatal diagnosis of congenital toxoplasmosis: amniotic fluid
- Ocular toxoplasmosis: vitreous or aqueous fluid

#### Advantages/disadvantages

##### Advantages:

- Very sensitive
- High specificity and predictive value
- Samples can be stored almost indefinitely before testing
- Enables early prenatal diagnosis (amniotic fluid PCR) and avoids the need for more invasive procedures on the fetus
- More rapid, sensitive and safe than conventional fetal blood sampling methods for prenatal diagnosis of congenital toxoplasmosis
- Real time PCR has a fast turnaround with same day results
- Real time PCR and automated DNA extraction techniques can reduce the contamination risk and interlaboratory variability associated with PCR
- Real time PCR is quantitative, so can be used to identify high-risk patients and monitor efficacy of treatment
- PCR of CSF is useful for evaluating CNS involvement in AIDS patients
- PCR of ocular fluids is useful if retinitis presents atypically

##### Disadvantages:

- Very sensitive (a low positive test may be seen in ill patients who show no clinical signs of toxoplasmosis)
- Not standardized; interlaboratory variation makes comparisons of quantitative data between laboratories difficult
- Sensitivity of prenatal diagnosis with PCR can vary according to gestational age at which maternal infection was acquired
- Risk of contamination
- Sensitivity of PCR can be affected by sample handling, shipping and storage conditions, amplification and detection techniques, and previous use of anti-

*T. gondii* specific drugs

##### Normal

No detection of *T. gondii* DNA.

##### Abnormal

- Detection of *T. gondii* DNA
- Keep in mind the possibility of a false-positive result

##### Cause of abnormal result

Acute *T. gondii* infection or reactivation of latent *T. gondii* infection

Medications, disorders and other factors that may alter results

- Sample contamination
- Sensitivity of PCR can be affected by sample handling, shipping and storage conditions, amplification and detection techniques, and previous use of anti-*T. gondii* specific drugs

#### 5. Parasite isolation

##### Description

Inoculation of mice or cell cultures with any body fluid or human tissue suspected to be infected.

#### Advantages/disadvantages

##### Advantages:

- Can be used to confirm acute infection (isolation of *T. gondii* from blood or body fluids)
- Allows accurate typing of *T. gondii* strains
- Versatile (can be run on virtually any human tissue or body fluid)

##### Disadvantages:

- Isolation techniques require live parasites
- Technique is not very sensitive
- Tissue culture inoculation is less sensitive than inoculation in mice
- Results may take several weeks
- Specimen should be processed immediately; freezing or formalin treatment kills the organism
- Isolation of *T. gondii* from tissue homogenates does not necessarily distinguish between acute or chronic infection
- This test is not available in clinical diagnostic laboratories and must be performed in reference laboratories

##### Normal

No isolation of *T. gondii*.

##### Abnormal

- Tachyzoites in peritoneal fluid of inoculated mice
- IgG to *T. gondii* in sera of inoculated mice
- Tissue cysts in brains of inoculated mice
- Plaques containing necrotic cells and replicating tachyzoites in inoculated tissue culture cells

##### Cause of abnormal result

Acute *T. gondii* infection or reactivation of latent infection.

Medications, disorders and other factors that may alter results

- Conditions and duration of specimen transport can affect results
- *T. gondii* tissue cysts may be isolated from tissue in the absence of an acute infection

#### 6. CT or MRI scan of brain or spinal cord

##### Abnormal

- Single or multiple ring or nodular-enhancing mass lesions that often involve the basal ganglia and brain stem
- Calcified brain tissue (congenital toxoplasmosis)

##### Cause of abnormal result

Acute *T. Gondii* infection or reactivation of latent infection in the CNS.

Medications, disorders and other factors that may alter results

Use of steroids may diminish the ring enhancement seen with cerebral toxoplasmosis.

#### 7. Fetal ultrasound

##### Abnormal

- CNS abnormalities, e.g. cerebral calcifications
- Ventricular dilation
- Hepatic enlargement
- Fetal ascites
- Increased placental thickness

##### Cause of abnormal result

Acute *T. gondii* infection or reactivation of latent infection in the CNS.

## INTERPRETATION

### HIV TESTS AND ISSUES RELATED TO THEIR ACCURACY

#### HIV TESTING

The ELISA, Western Blot and PCR viral load are the most frequently used tests to confirm HIV infections. The ELISA and Western Blot tests detect HIV antibodies in the serum of patients, whereas the PCR Viral Load test is a genetic test that detects small HIV nucleic acid fragments in whole blood. The veracity and reliability of these tests are key to the validity, reliability, quality and accuracy of epidemiological data used by any country. The ELISA test is mainly used to screen for HIV infection in blood donors and for general surveillance, whereas the Western Blot and PCR are generally used as confirmatory tests and in the context of research. All these tests, individually or in combination, are considered by the proponents of the HIV/AIDS theory as important indicators of infection by HIV. The CD4 count is an additional laboratory test used in combination with ELISA to make a diagnosis of AIDS; and with the Viral Load Test to determine the clinical progression of the AIDS disease and the monitoring of the effectiveness of anti-retroviral treatment. The Western Blot test is more expensive and requires a well-developed laboratory infrastructure; it is therefore not affordable for many developing countries. The Western Blot test is not accepted in the United Kingdom as a confirmatory test for HIV infection due to its unreliability.

Co-culturing of virus is used to isolate the virus from the blood of infected AIDS patients. This method is generally used as a research tool as it is too expensive and time-consuming to conduct as a routine surveillance and screening method. It also requires highly specialised staff and infrastructure.

The value of HIV testing and making a diagnosis of HIV infection on the basis of the antibody tests can not be denied. AIDS surveillance was first based on the clinical case definition, and AIDS was initially regarded as just a cluster of diseases. The subsequent discovery of HIV led to the incorporation in the definition of the disease of various clinical and immunological patterns, and, as recently as 1993, the CDC AIDS definition was widened to include a wider spectrum of clinical disease also utilising CD4 counts. European countries do not include the CD4 count in the definition of AIDS.

There is a general lack of standardisation of the definition of AIDS throughout the world. This is because it is possible to diagnose HIV infection by means of non-standardised laboratory tests, as well as by the verification of the presence of clinical symptoms. Since data are compared across countries, there is a need to standardise the definition of AIDS.

After 15 years of research, there is the lack of a 'gold standard' against which to measure the accuracy and reliability of the data generated from the commonly used methods to diagnose HIV infection.

#### ELISA test

The ELISA test is the most commonly used test for screening blood from donors. Its specificity and high sensitivity make it widely acceptable since it is able to detect all the possible HIV infections. It has also been found to be useful in surveillance. It is generally accepted that a single test cannot be regarded as proof of HIV infection. However, in order to improve the reliability and validity of ELISA, the CDC testing guidelines state that "a test for HIV antibody is considered positive when a sequence of tests, starting with a repeatedly reactive enzyme immunoassay (EIA) and including an additional, more specific assay, such as a Western Blot, are consistently reactive". Similarly, the WHO testing guidelines require confirmation of samples that are repeatedly reactive by ELISA using the same blood sample but a different ELISA kit. Both testing regimes call for repeated ELISA testing of a single blood sample rather than ELISA testing of more than one blood sample. However, the UNAIDSWHO recommendations state: "An additional blood sample should be obtained and tested from all persons newly diagnosed as seropositive on the basis of their first sample. This will help eliminate any possible technical or clerical error". Major concerns surrounding the ELISA test, however, include its specificity, reliability and reproducibility, as well as the lack of a comparative 'gold standard'. Some researchers claim that the HIV ELISA tests are not specific for HIV. They cite the fact that four repeat ELISA tests plus a Western Blot are required for a diagnosis of HIV disease in the USA. Furthermore, be elucidated that there is no standard by which to establish the specificity and sensitivity of the ELISA test. It has also been

pointed out that the some PCR kits specifically states that it must not be used as a screening test for HIV nor to diagnose HIV infection .

It has also been pointed out that WHO, in its description of the 34 HIV antibody ELISA tests on the market, uses one antibody test as a gold standard for another It is also opined that mycobacterial and fungal antigens can cross react with HIV ELISAs causing false positives.

It has been argued that no test is perfect and, moreover, that the current generation of ELISA tests is much more sensitive and more specific than in 1984. The current ELISA test uses recombinant proteins made from clones and consequently minimises cross-reactivity by other proteins from the plasma. Immunoassays based on recombinant viral proteins are more specific than tests used previously. All HIV diagnosis should be supported by laboratory tests "and HIV screening should ideally be always followed by two confirmatory tests. All immunoassays should be designed, calibrated, optimised and standardised to the level of their discriminating power which is the power to discriminate between negative and positive cases (ideal being 100%, but high confidence results of 99.9% should be acceptable).

The specificity, reliability and validity of ELISA. based platforms has been questioned from time to time.

The antibody ELISA test is based on the reaction between the unique viral protein (the p24 proteins 'supposedly' from HIV) and serum antibodies from a blood sample. Independent data show that p24 proteins, the basis for the ELISA antibody test, have been found to cross react with a wide variety of uninfected human tissue and blood samples from other disease states. For example, antibodies to candidiasis and mycobacterium infections cross react with p24. Furthermore a warning in the manufacturer's inserts suggests that the ELISA should not be used on its own for HIV diagnosis. It has also been found that many other disease conditions such as leprosy, malaria, leishmaniasis and other viral infections give rise to false positive results in the ELISA test without the concomitant HIV infection. Furthermore, many of the conditions that cause a false positive result in the ELISA test are conditions that are also prevalent in many of the recognised AIDS risk groups. A great deal of scientific data indicates widespread non-specific interactions between what are considered retroviral antigens and unrelated antibodies. A positive HIV ELISA test may also indicate previous antigenic stimulation by other retroviral infection. Another concern is that there is no precedent for the diagnostic utilisation of the ELISA test for other viral diseases. In general, the presence of antibodies specific to a particular disease is a major indicator of potential immune protection by the body, which is not the case with HIV infection, since antibodies to HIV fail to confer any immunoprotection against HIV. The ELISA test may therefore not be a true indicator of infection but an artefact arising from cross reactivity of other naturally occurring viral proteins.

The lack of standardisation of ELISA results, occurs across countries is a source of major concern to some diagnosticians. Results of ELISA tests may be interpreted differently within a single laboratory, between laboratories within one country, and between countries. This may mean that a person that tests positive at one laboratory in say country "A" may test negative at a different laboratory in the same country. Moreover, the lack of standardisation across countries could result in an individual's testing positive in one country and negative in another.

#### Western Blot

The Western Blot is an antibody test, which, is one of the tests used to confirm the diagnosis of HIV infection in most countries. A positive Western Blot result is synonymous with HIV infection and the attendant risk of developing AIDS. Most clinicians and diagnosticians agree that there was general agreement on the correlation between Western Blot and AIDS and patients that were suffering from AIDS always reacted positively to the Western Blot test.

However, a number of concerns were raised around the specificity, reliability and reproducibility of the Western Blot test.

Some specialists believe that the Western Blot should not be used to confirm and validate the results of the ELISA test since the Western Blot and ELISA tests are based on the same antibody reaction mechanism. As with the ELISA test, another concern over the use of the Western Blot test is its non-specific positive reaction to a number of diseases (including tuberculosis, a variety of parasitic infections and other viral infections) in the absence of HIV infection. The antigens used in the Western Blot test may be similar or identical to other

human proteins, and hence the results of the Western Blot may thus not provide an indication of HIV infection.

Some researchers have reported cross reactivity of a Western Blot test with a number of samples from leprosy, TB and AIDS patients. It appears that the Western Blot results from the different samples are indistinguishable from one another, showing the Western Blot test to be non-specific and unreliable. Many samples test positive, even those from leprosy and TB patients. Furthermore indeterminate results from Western Blot are a definite possibility. The above underlines the fact that the Western Blot test cannot be used as a determinate diagnostic tool.

#### PCR test for viral load

The PCR viral load test is also used as a confirmatory test. It is based on the amplification of tiny HIV viral particles that are supposed to originate from HIV in the blood. This test is virus specific and specifically detects HIV RNA. It is used to determine the level of viral load in the blood. It is mainly used in the tracking of the clinical progression of advanced HIV infection to AIDS disease, the monitoring of the effect of anti-retroviral treatment and the monitoring of mother-to-child transmission. There is a high correlation between clinical disease progression and the viral load. A high viral load is associated with an increased risk of transmission and the clinical progression to AIDS. Also the level of virus in the blood is directly related to the degree of risk of transmission to uninfected individuals. People with undetectable levels of virus in their blood do not transmit to uninfected partners. Mothers with high viral loads have the highest chance of transmitting the virus to their infants.

Arguments against the use of PCR are that this test is characterised by high variability and lack of reproducibility. In addition, the very wide variability may lead to the erroneous interpretation of results, thus compromising the accuracy and validity of the PCR results. It has been pointed out that the PCR viral load test might not be a legitimate measure of infectious virus. It demonstrates a high level of fluctuation, and the viral load can be increased non-specifically by other viral and bacterial infections (opportunistic infections may also increase viral load). Research results indicate that the viral load test may not always be an indicator for the clinical progression of HIV to AIDS.

Another point of concern that has been raised is the fact that the PCR test was developed for the non-C-clade virus, whereas many countries may have the prevalence of the clade-C virus.

#### CD4 count

The CD4 count is a determination of the concentration of CD4 T-lymphocytes in the blood. The associated immune deficiency leading to infection by opportunistic infections is ascribed mainly to the depletion of CD4 T-cells. The CD4 count can therefore be regarded as an accurate determination of the robustness and functionality of the immune capability and status to effectively protect the body against general infections. HIV infects and destroys CD4 cells (though some dispute this), rendering the immune system incapable of protecting the body against general infections, hence the resultant immunodeficiency in HIV infection and AIDS. This immunological test is used to monitor the progression of HIV infection to clinical AIDS disease and to monitor the effectiveness of anti-retroviral therapy. The CD4 count can be inversely correlated with the viral load. The higher the viral load, the lower the CD4 count will be. Intermediate progressors (patients who take longer than 10 years to progress from HIV infection to AIDS) consistently maintain the concentration of CD4 within normal range. When the CD4 count drops, it predicts the onset of opportunistic infections. In rapid progressors (those who developed AIDS within 2-4 years after infection), the CD4 drops precipitously, coinciding with the onset of infections and clinical progression to AIDS.

The improvement of the concentration of CD4 during anti-retroviral therapy is used as a surrogate marker for the effectiveness of the treatment.

#### General recommendations on testing

1. The case definition of AIDS should be standardised for clinical practice.
2. Any positive HIV ELISA result to be repeated with at least two additional blood samples before an HIV diagnosis is confirmed in order to improve the reliability and validity of ELISA.
3. Apply a series of HIV tests of increasing stringency in order to establish the validity, veracity, rigour, reliability and concordance of ELISA, PCR and viral isolation.

## BOUQUET

### IN LIGHTER VEIN

- A doctor vacationing on the Riviera met an old lawyer friend and asked him what he was doing there. The lawyer replied, "Remember that lousy real estate I bought? Well, it caught fire, so here I am with the fire insurance proceeds. What are you doing here?" The doctor replied, "Remember that lousy real estate I had in Mississippi? Well, the river overflowed, and here I am with the flood insurance proceeds." The lawyer looked puzzled. "Gee," he asked, "how did you start the flood?"
- A doctor and a lawyer were talking at a party. Their conversation was constantly interrupted by people describing their ailments and asking the doctor for free medical advice. After an hour of this, the exasperated doctor asked the lawyer, "What do you do to stop people from asking you for legal advice when you're out of the office?" "I give it to them," replied the lawyer, "and then I send them a bill." The doctor was shocked, but agreed to give it a try. The next day, still feeling slightly guilty, the doctor prepared the bills. When he went to place them in his mailbox, he found a bill from the lawyer.
- A man went to apply for a job. After filling out all of his applications, he waited anxiously for the outcome. The employer read all his applications and said, "We have an opening for people like you." "Oh, great," he said, "What is it?" "It's called the door!"

## WISDOM WHISPERS

- Motivation is what gets you started. Habit is what keeps you going.
- When you make a mistake, don't look back at it long. Take the reason of the thing into your mind and then look forward. Mistakes are lessons of wisdom. The past cannot be changed. The future is yet in your power
- Our lives are not determined by what happens to us but by how we react to what happens, not by what life brings to us, but by the attitude we bring to life. A positive attitude causes a chain reaction of positive thoughts, events, and outcomes. It is a catalyst, a spark that creates extraordinary results.

## BRAIN TEASERS

1. What is the maximum no. of nuclei that an *Entamoeba histolytica* cyst can have?  
A. 2 B. 4 C. 8 D. 16
2. Of the following which parasite is a ciliate?  
A. *Giardia lamblia* B. *Chilomastix mesnili* C. *Trichomonas vaginalis* D. *Balantidium coli*
3. Microgametocyte of which malarial parasite is kidney or bean shaped?  
A. *P. vivax* B. *P. ovale* C. *P. malariae* D. *P. falciparum*
4. Which species causes Oriental sore, Chiclero's disease and Uta?  
A. *L. donovani* B. *L. tropica* C. *T. gambiense* D. *T. cruzi*
5. Which disease does Tsetse fly spread?  
A. Kalaazar B. Sleeping sickness C. Chaga's disease D. Espundia
6. Which of the following does not have a cystic form?  
A. *Trichomonas vaginalis* B. *E. histolytica* C. *G. lamblia* D. *B. coli*

ANSWERS: 1.B, 2.D, 3.D, 4.B, 5.B, 6.A

## TROUBLE SHOOTING

### RAPID HIV TESTING ISSUES AND USAGE

Wait time reduced from days to minutes

#### Objectives of this article

- To know the capabilities of rapid HIV testing
- To understand the significance of reactive and nonreactive rapid HIV test results
- To recognize the clinical indications for rapid HIV testing

#### Introduction

Recent breakthroughs in technology have produced tests for HIV antibody that are highly accurate and easy to use and can give a preliminary result in 20 minutes or less. These rapid HIV tests will be used increasingly in labor and delivery wards, emergency departments, urgent care centers, and the primary care office. They have unique applications for healthcare worker exposures, military operations, public health venues, and developing countries. In this article, the advantages and limitations of rapid HIV testing in various settings are presented. Though based on US (CDC) guidelines, the presentation that ensues may not be acceptable as it is in many different nations, as each nation has set its own diagnostic protocols. By and large, the matter presented can not be refuted by most learned authorities. The conventional HIV testing algorithm starts with a sensitive enzyme immunoassay (EIA). The EIA can be performed with serum, plasma, urine, or oral fluid, and the result is typically available after 3 to 4 days. If the EIA is negative, the result is considered definitive, and no further testing is indicated. A limitation is that HIV antibodies can take up to 3 months to develop after infection occurs. During this window period, antibody tests may remain negative. If the EIA is repeatedly positive, more specific testing, using the Western blot technique, is done for confirmation. The testing process—from the time a specimen is submitted until a final result is available—can take a week or longer.

With rapid HIV antibody testing, a preliminary result is available in 10 to 20 minutes, depending on the brand of test used. The result is comparable in clinical significance to a sensitive EIA result. Patients who have nonreactive rapid tests can be counseled that they are negative for HIV, just as with a negative EIA (including the caveat about the window period). Those who have a reactive rapid HIV test must be advised that this is a preliminary result: it could indicate HIV infection or could be falsely reactive. Confirmation with a Western blot test must be performed before a diagnosis of HIV can be made. This confirmation can take 5 to 7 days. Importantly, the EIA lacks the specificity to be a confirmatory test. Good pretest counseling is critical with rapid testing, and the patient needs to understand that a false-positive test is a possibility.

Conventional testing has two advantages over rapid testing. First, by the time a patient learns the HIV test result, it is definitely positive or negative (except for the occasional indeterminate result). Second, in the case of a positive test, the physician usually has the result a day or two in advance and can be better prepared to discuss it with the patient. A disadvantage of conventional testing is that many patients, especially those in transient care and public health settings, fail to return for test results.

Rapid testing has the obvious advantage of a short turnaround time for obtaining negative or preliminary positive results. This time savings can be critical in clinical situations that require prompt initiation of antiretroviral therapy.

#### When is a rapid test indicated?

Rapid HIV tests may be indicated and used most often in obstetric wards, healthcare worker occupational exposures, urgent care clinics and emergency departments, military medicine, public health settings, developing countries, and the primary care office.

**Obstetric wards:** In women with untreated HIV infection, mother-to-child transmission occurs in about 25% of pregnancies. Much of this transmission (60% to 70%) is thought to occur during the birthing process. Knowledge of maternal serostatus is the first step in all measures to decrease mother-to-child transmission.

In urban hospitals many women who present to the labor and delivery ward may

have unknown HIV status. The patient has either received no prenatal care or received prenatal care but had no record of an HIV test. An added concern is that patients without prenatal care often have risk factors, such as illicit drug use or history of unsafe sex, that puts them at higher risk for HIV infection. These women can now be offered rapid HIV testing. In the event of a reactive test, the woman can be advised of the possibility of HIV infection and started on prophylaxis to prevent mother-to-child transmission. Fortunately, serious short- or medium-term adverse effects of antiretroviral therapy for the mother or neonate have not been detected. Studies of long-term adverse effects are pending. Pretest counseling of women having a rapid HIV test in labor must be thorough. The patient needs to understand that a false-positive test is a possibility and that HIV therapy will likely be initiated on the basis of a preliminary positive test. Post-test counseling for a reactive test is stressful for both patient and physician. The physician should be able to explain, again, the possibility of a falsely reactive test, the need for confirmatory testing, and the rationale for urgent antiretroviral therapy. In the obstetric setting, the physician should use the most specific test possible to minimize false positives.

**Healthcare worker occupational exposure:** The number of healthcare workers having a documented HIV seroconversion from occupational exposure is rising steadily. Healthcare exposures can be stressful for everybody involved, including the treating physician. Often, an emergency department physician is called on in the middle of the night to make the decision to treat or not to treat. Confirmation of the source patient's HIV status can be crucial. With a rapid HIV test, the source patient's HIV status can be determined within 20 minutes. If the source patient does not have high-risk behavior and the rapid test is negative, the physician, along with the healthcare worker, might elect not to begin prophylactic therapy. With non-rapid EIA, results might not be available for 3 to 4 anxiety-filled days. An emergency department study has showed that rapid HIV testing is cost-effective in treatment of healthcare worker exposures.

**Urgent care clinics and emergency departments:** Chart reviews have shown that most patients with newly diagnosed HIV or AIDS have had missed opportunities for earlier diagnosis. Often, these missed opportunities occurred in visits to an urgent care clinic or emergency department. The CDC has long recommended routine HIV testing in urgent care clinics and in emergency departments in areas where HIV prevalence is greater than 1%. Persons who access these episodic care settings are at increased risk for HIV infection because of underinsurance, lack of primary care, and acute medical concerns.

**Military medicine:** In battle, occupational exposures can be extensive and unavoidable for soldiers and medics. The technical simplicity and portability of rapid HIV testing make it convenient for frontline use. A negative rapid test in the source patient could resolve anxiety and prevent unnecessary post-exposure prophylaxis; a reactive test would be an indication for prophylaxis in the exposed person and for confirmatory testing in the source patient. Rapid testing is not approved by the FDA for screening donated blood, but it could be used for screening emergency blood donations in combat.

**Public health settings:** A huge problem with publicly funded HIV testing is that 30% to 40% of patients do not return for their test result. Groups least likely to return include adolescents and persons tested at a clinic for sexually transmitted disease. As a consequence, valuable HIV testing resources are squandered and opportunities for timely treatment and prevention counseling are lost.

Rapid HIV testing greatly improves the percentage of patients who learn their test result. The average interval between doing the testing and learning the test result is usually about 30 minutes. With this short turnaround time, 99.7% of patients can learn their test result on the spot.

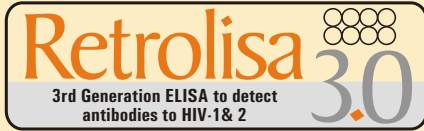
**Developing countries:** Rapid HIV tests are already used widely in resource-poor countries because the tests are technically simple, accurate, and cost-effective. Also, they can accurately be done by non-laboratorians and do not require refrigeration or expensive laboratory equipment. A definitive diagnosis of HIV infection can be achieved by using two or three different rapid HIV tests in combination. These protocols yield sensitivity and specificity equal to those of standard EIA and Western blot methodologies and are recommended by the World Health Organization.

**Primary health centers:** There are clinical situations where rapid HIV testing makes sense in the primary care setting. A rapid HIV test is recommended for patients with a high probability of not returning (eg, sex workers).

## TULIP NEWS

Moving ahead with technology, introducing advanced assay systems into the diagnostic market has been a continuous endeavour of Tulip Group of Companies.

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FEATURES	BENEFITS
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Recombinant antigens with highly immunodominant & conserved epitopes used - gp41, p24(O) & gp36	The use of both envelop & core antigens enables detection at all stages of infection  Ensures sensitive detection of all subtypes of HIV-1 including HIV-1 group "O"  Ensures HIV-2 detection
DHS3 Technology	High detectability particularly in low-titre samples  High thermal stability  Longer shelf-life
Excellent Performance	Sensitivity : 100% Specificity : 99.8%
Extremely Reliable	Evaluated by NIB

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