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HIV/AIDS
Test and Treat



Atul Kakar
Samiran Nundy

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CHAPTER
2

Laboratory Diagnosis of HIV/AIDS Infection

Sanghamitra Datta

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INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) was first documented amongst homosexual men in America in 1981. A few years later, its causative organism—the human immunodeficiency virus (HIV) was discovered. Today sub-Saharan Africa and parts of Asia are the worst affected areas. According to the National AIDS Control Organization (NACO), the HIV/AIDS epidemic in India started in the year 1986–1987 with the first positive HIV case reported from Chennai, and the first diagnosed AIDS case was from Mumbai. According to the World Health Organization (WHO) data, India with 2.4 million people affected by HIV ranks second in the world only after sub-Saharan Africa.

DETECTION OF HIV

Laboratory tests detect and confirm the HIV status of a person. The choice and sensitivity of laboratory tests depends on the stage of infection. The time of detection of the viral components like the ribonucleic acid (RNA)/complementary deoxyribonucleic acid (cDNA)/p24 antigen and HIV antibodies are variable. Choosing the right laboratory test ensures a correct and confirmed diagnosis.

HIV Detection Window

The time of detection of each of the viral components following infection are 7–10 days after infection for HIV viral RNA, 10–20 days after infection for HIV p24 antigen and 20–30 days for HIV antibody. Figure 2.1 gives a clear picture of the serocon version window.

Stages of HIV

Following infection with the virus the patient goes through an eclipse phase. In this phase, all the viral components are undetectable and all the tests are negative.

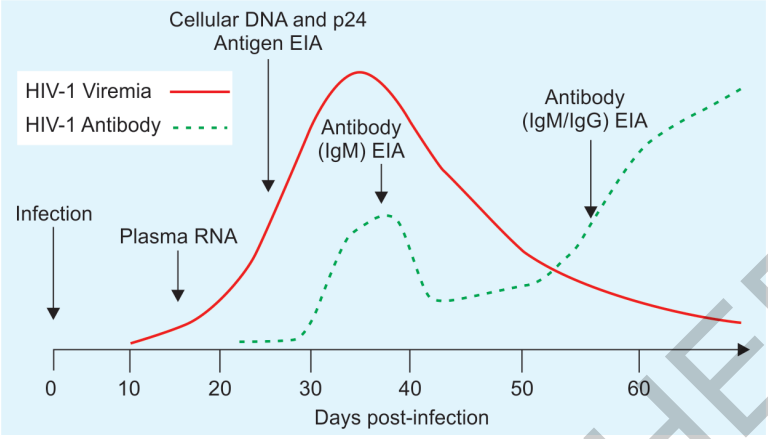

Fig. 2.1: Host response to HIV.

The eclipse stage is followed by three stages of infection: (1) acute stage, (2) chronic asymptomatic stage, (3) AIDS.

Acute HIV Infection Stage

This stage follows the eclipse stage. It is a transient nonspecific clinical syndrome associated with high viral replication which becomes detectable around 10 days after HIV infection. The acute stage usually lasts for a period of 3 weeks to a maximum of 6 weeks. The HIV antibody test is negative at this stage. The infectivity is high due to the absence of neutralizing antibodies and the viral burden is also high in the plasma and genital secretions. As the majority of people at this stage have common symptoms similar to any viral glandular fever, the diagnosis is missed in 80% of them. According to some authors half of HIV transmissions are by those who have a recent history of seroconversion which is less than 6 months. Hence, early detection of HIV infection is important in decreasing its spread.

Chronic Asymptomatic Phase

In this phase, antibodies are present and enzyme-linked immunosorbent assay (ELISA) for HIV antibody is positive. The presence of antibody down regulates viremia. Most of the HIV infections are diagnosed at this stage during screening which lasts for almost 10 years.

Acquired Immunodeficiency Syndrome

This stage lasts for 3 years or even less. The CD4 cells are destroyed steadily by the virus and the count falls below 200 cells/mm^3. The plasma viral counts increases and due to immune paralysis the antibody response gradually goes down and become negative. P24 antigen and the viral load test are positive.

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Patterns of Disease Progression

The progression to AIDS varies depending on the pattern of disease progression which is unique to each patient and should be kept in mind while interpreting the laboratory tests for HIV.

Typical progressors: As per NACO 50–70% of people living with HIV/AIDS (PLHA), suffer initially with an acute flu-like syndrome. This occurs within 3–6 weeks of infection and may last for 9–12 weeks. The viral load during this time is high in the blood. The progression to AIDS from this stage takes almost 8–10 years.

Rapid progressors: These constitute 5–10% of this population. They progress to AIDS within 2–3 years after primary infection.

Long-term nonprogressors: They do not progress to AIDS for a long time even without treatment. Their viral counts remain less than 5,000 HIV RNA copies/mL. About 5% of patients fall into this category. Inherited mutations of the CCR5 receptor of T cell lymphocytes keep the virus under check.

Elite controllers: They are natural controllers of the disease and are a subset of long-term nonprogressors (LTNPs). Despite being infected, they can successfully suppress the virus to levels below 50 copies/mL for many years without therapy.

WHO and NACO have laid down the objectives of HIV testing. These are:

- To ensure safety of blood and blood products: there should be mandatory testing of all donated blood units and blood products
- To screening organ, sperm and tissue donors
- HIV screening in clinically suspected cases
- Antenatal care (ANC)
- Patients with tuberculosis, sexually transmitted diseases, high-risk behavior, hepatitis B and hepatitis C coinfection
- Epidemiological surveillance
- Research.

LABORATORY DIAGNOSIS

The HIV infection can be diagnosed by testing any of the following:

- Antibodies to HIV
- P24 HIV antigen
- HIV nucleic acid (RNA/DNA).

All antibody-based tests are divided into two: (1) screening test and (2) supplemental test.

1. Screening assays:
   - Enzyme-linked immunosorbent assay
   - Rapid screening tests.

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2. Supplemental assays:
   - Immunofluorescence assay (IFA)
   - Western blot assay
   - Line immunoassay (LIA)
   - Radioimmunoprecipitation assay (RIPA).

Other assays apart from screening and supplemental tests are:

1. Molecular assays
2. P24 antigen detection
3. Culture.

Alternate tests and specimens are:

1. Oral fluid/saliva
2. Urine.

Antibody-based Tests

Screening Assays

Enzyme-linked immunosorbent assay: This is a screening test for HIV detection. The HIV antibody can be detected within 6–12 weeks in most cases. The fourth-generation ELISA test which is used today has the ability to detect both p24 antigen and HIV antibody and reduces the window period to 15–20 days.

According to the national HIV testing policy by NACO, two enzyme immunoassays (EIAs) using different principles together with a rapid assay are required to diagnose an HIV infection in a clinical setting.

Factors uniformly known to affect serological test results and are not recommended for testing as per NACO are:

- Hemolyzed samples
- Lipemic samples
- Repeated freezing and thawing
- Improperly stored, expired and deteriorated reagents.

Known common causes false-positive results are:

- Autoimmune diseases
- Alcoholic hepatitis
- Leprosy
- Multiple pregnancies
- Multiple transfusions.

Causes of false-negative results are:

- Window period
- Immunosuppressive therapy
- Immunological dysfunction (B cell disease)
- Last stage of the disease: immune collapse.

Rapid HIV tests: These are visual point-of-care tests and can yield results in less than 30 minutes. Though manufacturers have reported their sensitivities as 99.3–100% and specificity as 99.7–99.9%, many studies have reported lower sensitivities and specificities.

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Rapid tests recommended by NACO:

- Immunoconcentration/dot blot immunoassay (vertical flow), immunochromatographic (lateral flow)
- Particle agglutination (e.g. gelatin or latex test)
- Fluorimetric microparticle technology.

Advantage: Differentiates HIV-1 and HIV-2.

Disadvantage: Subjective interpretation is one of its major disadvantages.

The NACO has recommended that ELISA kits and rapid kits used must have high sensitivity and specificity (>99.5%, >98% respectively).

Supplemental Tests

Western blot assay: This is a specific test and is the most commonly used of all the supplemental assays. It has a lower positive predictive value when performed alone. HIV-1 Western blot and HIV-1 IFA were previously recommended to make a laboratory diagnosis of HIV-1 infection. These assays are not a part of the recommended algorithm as per the NACO guidelines. The various HIV-specific recombinant or synthetic antigens are electrophoresed onto nitrocellulose paper. Western blot detects the presence of antibodies against specific HIV proteins: Envelope (ENV; gp120, gp160 and gp41), GAG (p55, p24 and p17), POL (p66, p51 and p31). They are seen as bands on the test strip. The criterion for a positive Western blot is different for WHO and the Centers for Disease Control (CDC) in the United States. Table 2.1 gives the details of these.

The WHO criterion is most commonly used.

Interpretation:

Negative: No bands

Positive: Bands as given in Table 2.1.

Intermediate: Bands present that do not satisfy the criteria.

Western blot assay is only used now in case of an equivocal or discordant test (Figs. 2.2 and 2.3).

Line immunoassay: In this assay, the use of "artificial" antigens decreases the presence of contaminating substances derived from cell culture making it more specific.

Immunofluorescence assay and RIPA: IFA and RIPA are other supplemental methods which are not commonly used.

Table 2.1: Positive Western blot criteria.

Organization	*Criteria*
WHO	2 env with or without gag/pol
CDC	Any two p24, gp41, gp120/160

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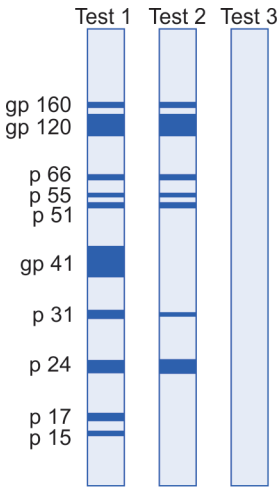
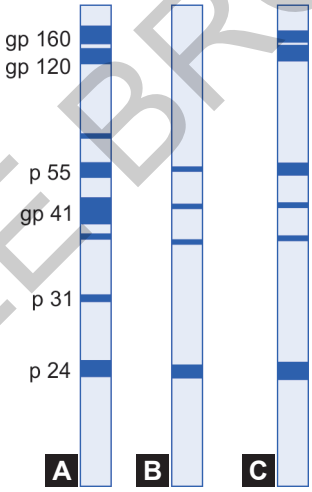


Fig. 2.2: Western blot. (Test 1) Control, as it shows all the proteins tested are present. Controls are crucial to make sure that the test is working correctly; (Test 2) Positive results shows there are antibodies for 7 of the HIV proteins; (Test 3) Negative result.



Figs. 2.3A to C: Western blot. (A) Control strip showing antibody responses to key HIV proteins; (B) Indeterminate response showing antibodies to p24; (C) Positive Western blot showing responses to at least three key proteins.

Other Assays

- HIV p24 antigen detection
- HIV DNA
- HIV RNA polymerase chain reaction (PCR)
- HIV culture.

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Indications are as follows:
- Window period or acute HIV infection
- Indeterminate serology
- Neonatal infection.

P24 Antigen Detection

The test detects free p24 antigen in the serum. Though the detection of the p24 antigen is transient, it shortens the window period and can become positive as early as 2 weeks. The fourth-generation ELISA used nowadays as a screening test incorporates both antibody and p24 antigen detection thus increasing the sensitivity of the assay and decreasing the window period.

Molecular DNA/RNA Assays

These assays are indicated in situations where antibody detection assays cannot be relied upon. Molecular assays amplify both HIV RNA or proviral DNA. They are used for:
- Diagnosis of HIV in children less than 18 months old
- Diagnosis of acute HIV infection
- Resolution of indeterminate results.

Three different techniques have been employed to develop commercial kits. They are: (1) reverse transcriptase PCR or real time-PCR, (2) nucleic acid sequence-based amplification (NASBA) and (3) branched DNA (b-DNA) assay. These kits shorten the window period from infection to detection by 12 days.

Diagnosis of pediatric HIV infection (<18 months): Transplacental transfer of maternal HIV immunoglobulin G (IgG) from infected mothers to babies during pregnancy results in a false-reactive test in babies born to HIV-positive mothers. However, the infection is transmitted in less than 50% of such babies. Maternal antibodies can persist in an infant's blood for 18 months after birth. Therefore, diagnosis of HIV infection till this age cannot be done by serological tests. In this situation, NACO recommends the use of the test called qualitative HIV-1 DNA PCR.

Detection of acute HIV infection: Molecular tests are the tests of choice for diagnosing of infection during the "window period" also called acute HIV infection. A positive test confirms the diagnosis, but a negative test does not rule it out.

Resolution of indeterminate results: Specimens that are reactive on initial immunoassay and nonreactive or indeterminate on the antibody differentiation assay should undergo HIV-1 nucleic acid testing for resolution of the discordant result.

The RNA quantitative assays used these days have a low detection limit and are very sensitive. They are of great importance in monitoring the viral load especially in patients on antiretroviral therapy.

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Alternate to Classical Tests

These tests have recently come to the fore. The samples are saliva and urine instead of blood that detect HIV antibodies in saliva and urine.

Advantages:

- Noninvasive methods of sample collection
- Appropriate for people who object to giving blood
- Safer to handle urine and saliva which contains less virus that is insufficient for transmission.

Oral fluid: Detects antibody and not virus in the saliva. OraSure is the only oral fluid test approved by the US Food and Drug Administration. Lower sensitivity than blood-based tests.

Urine: Detects HIV antibodies but not the virus in urine. Sensitivity is less than blood and oral fluid.

Disadvantage: There is no approved urine-based confirmatory assay necessitating collection of blood when results are reactive.

Social Aspects of HIV Testing

Legal aspects of HIV testing: NACO guidelines should be followed.

1. *Pre-test counseling*: The pre-test counseling is an integral part of HIV testing services.
2. *Confidentiality*: The confidentiality of HIV test results should be maintained for both positive and negative reports. The reports should be dispatched to the individual concerned or person authorized by the individual or to the clinician in a sealed envelope who requested the test. The records must be kept secure.
   Reports are not to be communicated via the electronic media.
3. *Mandatory testing*: Mandatory testing is usually not recommended in India. However, it can be done *only* for:
   - Screening of units of blood for donations
   - Blood products
   - Organs, tissues and semen donors to prevent the transmission of HIV to the recipient.

The national HIV testing policy as per NACO further reiterates the following:

- No person should be tested mandatorily for HIV
- No mandatory HIV testing should be imposed as a precondition for employment or for providing healthcare services and facilities
- Any HIV testing must be accompanied by pre-test and post-test counseling services and informed consent
- Confidentiality of the result should be maintained.

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NATIONAL STRATEGIES AND ALGORITHM FOR HIV TESTING (NACO GUIDELINES)

In India, the objectives for HIV screening and its diagnosis are guided by NACO guidelines.

Three strategies for HIV diagnosis have been laid down by NACO for (1) blood/organ donation safety, (2) surveillance and (3) disease diagnosis.

The laboratory diagnosis is based on serial testing. This includes repeat testing on initially reactive samples (e.g. ELISA/ELFA).

The first, second, and third tests (A1, A2, and A3) are based on different principles and use different antigenic composition (e.g. different generations of ELISA, i.e. 3rd/4th generation).

Serial Testing Strategy

- Serial testing strategy refers to the use of one screening test followed by one or more other ELISA/Rapid (E/R) tests, depending on the results of the first.
- A nonreactive result from the initial screening test completes the testing, i.e. report is given out as HIV negative.
- If the initial test is reactive, it is followed by a single test (in a two-test algorithm), or two more tests (in a three-test algorithm) for resolution.

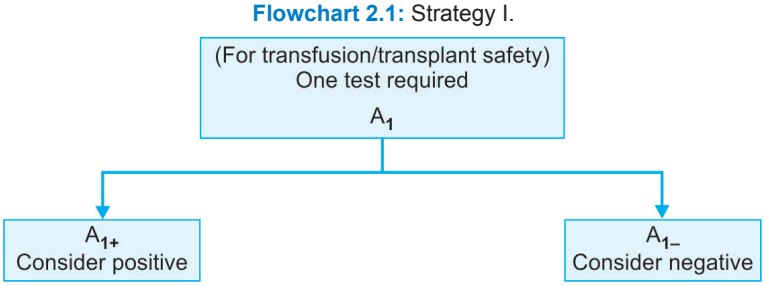
Screening Strategies

The following strategies have coined by NACO and should be followed.

Strategy 1 (Flowchart 2.1)

- Sample subjected to screening test
- If negative: Free of HIV
- If positive: Reactive unit is discarded and donor is not recalled
- Used for donation safety (blood, blood products, organ, tissues and sperm).

Flowchart 2.1: Strategy I.



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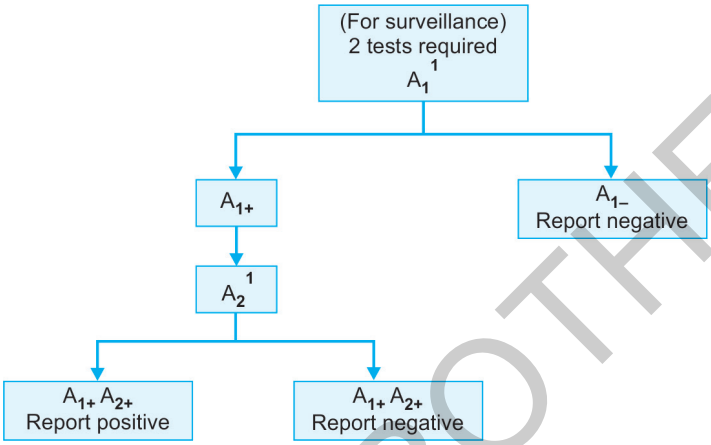
Strategy 2A (Flowchart 2.2)

- Used for surveillance A sample is negative if ELISA negative
- If reactive, second assay utilizing a different system from first.

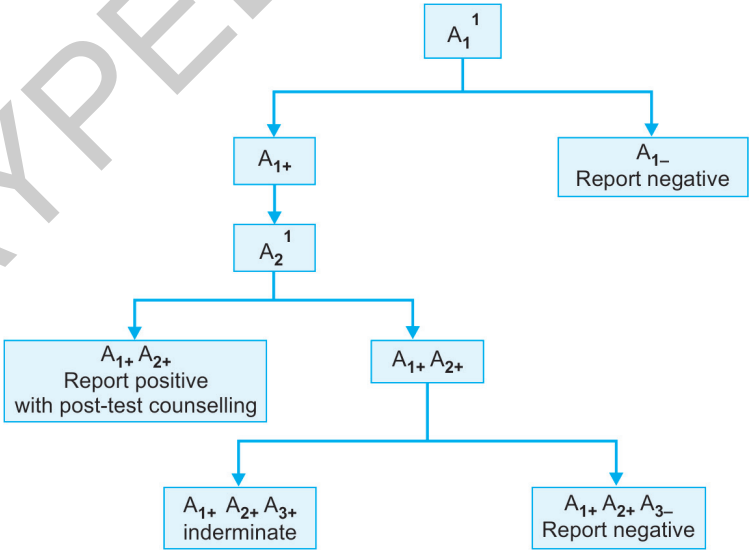
Flowchart 2.2: Strategy 2A.



Strategy 2B (Flowchart 2.3)

- For diagnosis of individuals with AIDS-related disease and symptoms
- In case of indeterminate sample, repeat after 14 days.

Flowchart 2.3: Strategy 2B.



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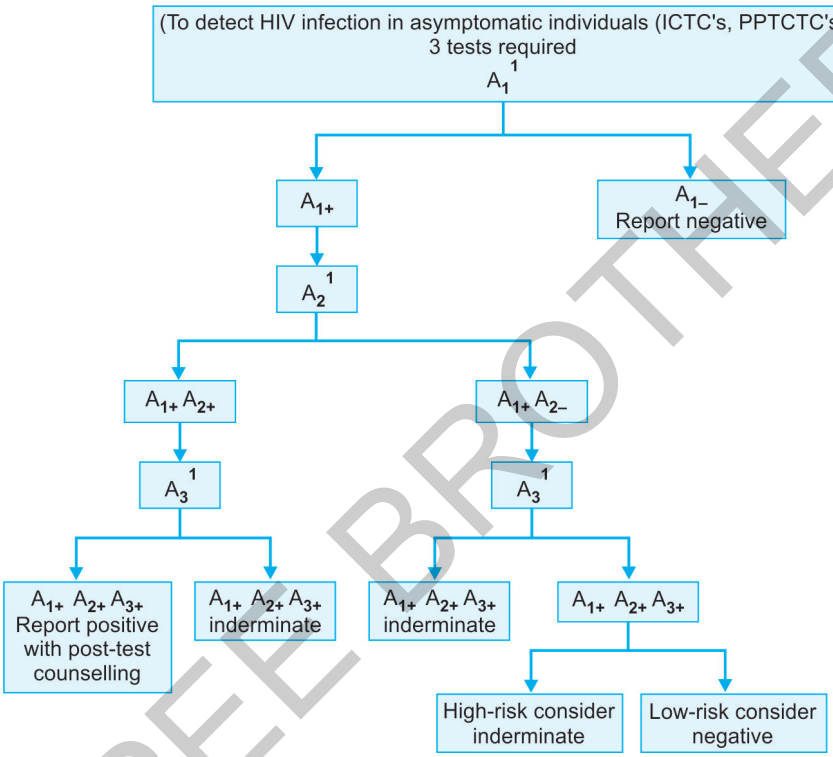
Strategy 3 (Flowchart 2.4)

- Same as strategy 2, with added confirmation of third reactive ELISA
- Used to diagnose HIV infection in asymptomatic individuals indulging in high-risk behavior.

Flowchart 2.4: Strategy 3.



DIAGNOSIS OF HIV-2

HIV-2 was first detected from patients of AIDS living in West Africa. In India, it is detected sporadically. Though the modes of transmission are the same, it runs a much milder course in terms of infectivity. The progress to AIDS of HIV-2 is slower than HIV-1. The diagnosis of HIV-2 is important as some antiretroviral drugs used for HIV-1 are not effective for HIV-2. There is no exclusive algorithm for the diagnosis of HIV-2. The highly sensitive serological screening tests used these days for detecting HIV-1 also detect HIV-2. The supplemental Western blot assay is type specific and is available separately for HIV-1 and HIV-2 detection.

Since there are no molecular qualitative or quantitative licensed assays available for the confirmation of the diagnosis of HIV-2, Western blot is often

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used as a confirmatory test following a screening assay positive for HIV-2. Sometimes the detection of HIV-2 may be challenging in case of a repeated "indeterminate report" in a screening assay and a negative molecular test for HIV-1. In such cases, Western blot should be done to rule out HIV-2 infection as there are no recommended molecular assays for HIV-2 diagnosis in patients and molecular assays available for HIV-1 do not detect HIV-2.

CONCLUSION

- Early detection of HIV is of paramount importance to prevent further transmission of the disease as the infectivity is highest during the early stages.
- The strategies for diagnosis of HIV laid down by NACO should be strictly followed.
- Newer and improved screening assays have reduced the window period and hastened early detection. Legal aspects of HIV testing should be followed diligently.
- The pretest consent form should always accompany the sample to be tested, duly signed by both the doctor advising the test and the patient who is to be tested. In case of a minor, it should be signed by either the parent or guardian. The test results should be kept strictly confidential and positive results should be released with proper post-test counseling. In case of a high degree of suspicion, a negative result may not rule out infection and may be conveyed to the patient during counseling and the patient should be advised retesting after 4–6 weeks.

SUGGESTED READING

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HIV/AIDS
Test and Treat

This book on HIV/AIDS is a comprehensive manual which deals with various aspects of its diagnosis and treatment. It discusses the antiretroviral drugs available, pre- and post-exposure prophylaxis and the effect of HIV on various important organs of the body like the kidneys, bones and liver. Because the body's immune system is compromised it is also vulnerable to what are also known as 'opportunistic' infections with tuberculosis, cryptococcal meningitis and pneumocystis carinii.

There have been remarkable advances in the management of this previously near fatal disease and this publication describes them in some detail and gives reasons for hope.

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He has published around 24 medical books and published 256 articles.

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