Full work up of HIV infected person begins with confirmation of HIV infection. HIV infection can be confirmed by direct tests and indirect tests. The direct tests detect the presence of HIV by identifying its presence by viral DNA or by viral proteins. The indirect tests are directed at detecting presence of anti HIV antibodies.

**DIRECT TESTS:**

1. **DNA PCR** – This is also called as a qualitative test. Using a PCR technique the proviral DNA is detected in peripheral blood mononuclear cells. It is a very sensitive and the earliest test however, it also has a ‘window period’. After exposure to HIV infection, it is detected by DNA PCR after a small window period of 5-7 days. It is useful in anxious patients who had unprotected sex with professional partner, in cases of sexual assault, in cases of needle stick injuries and for the earliest diagnosis in an infant born to HIV infected mother. But, if therapeutic interventions like post exposure prophylaxis is used then the test results will be difficult to interpret.

2. The detection of viral proteins – The core protein or P24 antigen can be detected within 7-10 days of the exposure.

The direct tests are expensive and only indicate presence of HIV infection in a person.

**INDIRECT TEST:**

The infection can be identified by presence of antibodies. These tests are less expensive, sensitive and are used on a large scale.

Anti HIV antibodies are detected by Ag + Ab reaction indicated by a colour code or by measuring optical density of eliza band. Ideally, three tests using three different antigens with three separate blood samples should be carried out. But now with improved sensitivity and specificity of these testing kits, a single sample is tested as follows –

The three tests increase the specificity of the investigation thereby minimizing the chance of false positivity. Hence, in diagnosing a seropositivity in a patient, as per WHO and our National Guidelines, 3 tests are essential in an asymptomatic individual and two tests in a symptomatic case.

Alternatively, a single reactive test can be confirmed by Western Blot test which increases the specificity. But, Western Blot technique is expensive, time consuming, needs a technical expertise hence in resource limited countries it is used only in specific indications.

There is a cross reactivity in HIV-1 and HIV-2 antibodies. Therefore many a times reports are received as Abs against HIV-1 & HIV-2 are detected. These cases are to be treated as HIV-1 infection. But there are cases where Abs are detected only against HIV-2. This is to be confirmed by HIV-2 Western Blot because in case of HIV-2, NNRTI therapy is not indicated.

**IMMUNOLOGICAL PARAMETERS:**

Once HIV infection is confirmed, the immunological assessment is done by CD4 and CD8 estimation. It is necessary to get a complete report of total leukocytic count, percentage of CD4+ cells, absolute CD4+ and CD8 cells numbers. The absolute numbers may vary based on leukocytic count and percentage of lymphocytes. In adults, one often considers absolute CD4 cell count but in paediatric age group, the decision about antiretroviral therapy is based on percentage of these cells.

If CD4 is more than 500 cells, the patient is followed up by six
monthly CD4 counts.
If CD4 is less than 500 cells, quarterly monitoring will show the trend of CD4 decline and will help in initiation of therapy. If CD4 cell count is less than 350 cells per microlitre, antiretroviral therapy is indicated.

**VIRAL PARAMETERS:**

There are various techniques to estimate viral load in a given case. Although our National guidelines do not take the decision to initiate ART on the basis of viral load, most of the international guidelines initiate ART on the basis of CD4 as well as viral load. Viral load estimation is necessary not only to initiate ART but also to monitor therapeutic response as well as to detect virological failure which guides the switch of ART.

With effective and successful ART, the viral load is expected to decline rapidly and become undetectable within 24 weeks. Six monthly monitoring of viral load also helps in detecting the virological failure at the earliest so that, the therapy can be switched from the first line to the second line. However, in resource limited countries, the expertise and expenses may not be available to carry out this test.

Once a decision is taken to initiate Art on the basis of National/Rational guidelines, a detailed work up of the patient will guide the selection of anti-HIV drugs.

Each case has to be evaluated with baseline investigations as follows :-

1. **Complete Blood count including Hb.**
   - The toxicity of ZDV results into macrocytic anemia and bone marrow suppression. To differentiate the same from HIV induced anemia and BM suppression it is desirable to have complete blood count with Hb, RBC indices, WBC, total and differential and platelet count.
   - In presence of Anemia, one needs to select the NRTI drugs properly. RBC indices are necessary to monitor ZDV toxicity as the first sign of the same is increased MCV.
   - HIV infection per se can lead to anemia, bone marrow suppression, or thrombocytopenia therefore, it is necessary to have this information before starting ART. Otherwise post ART it may be confused with ART toxicity.

2. **Biochemistry :-**
   - a. **Blood sugar –** Use of protease inhibitors may cause hyperglycemia or can aggravate pre-existing diabetes. Therefore, Pre-ART blood sugar levels are necessary.
   - b. **Serum Creatinine –** HIV nephropathy can results into increased creatinine level.
     - In such a condition creatinine clearance is to be measured to adjust the dosage of ARVs particularly NRTI except Abacavir. Certain ARVs like Tenofovir, Indinavir are known to cause Nephrotoxicity resulting into proteinuria and rise in serum creatinine. To avoid the confusion, between nephropathy & renal toxicity of ART baseline creatinine is desirable.
   - c. **Liver function Tests –** NNRTI and Protease inhibitors are known to cause hepatotoxicity. As against, there are many factors which affect liver functions in ART naïve cases. Large number of these patients are alcoholics. Malnutrition can lead to steatosis and rise in transaminases. Many OIs in liver may remain undiagnosed and affect liver functions. Similarly, coinfections with HBV and HCV affect the liver functions. Therefore, to avoid dilemma after ART, it is necessary to monitor liver functions before initiating ART.

**X-RAY CHEST AND ABDOMINAL SONOGRAPHY –**

TB is the commonest OI in presence of HIV. In large number of cases in presence of immune deficiency TB may not have clinically manifested e.g. abdominal lymphadenopathy or splenic micro-abscesses. Therefore, each case should be subjected to these investigations. It has a direct bearing on selection of ARVs in view of drug interactions between ATT and NNRTI or PI.

**INVESTIGATIONS FOR CO-INFECTIONS :**

Considering the risk behavior of HIV infected cases, these cases are liable to get co-infections like Hepatitis B which shares the same routes of transmission as HIV. Although the prevalence of HBV in India is low, in general population, the HIV infected case should be investigated for HbsAg. If HbsAg is positive, patient should be further subjected to Hb ‘e’ Ag. Based on these results the NRTI/NtRTI drugs are selected which can also suppress HBV viral load.

HCV infection is more common in IDUs. A few HIV infected cases giving a history of blood transfusion in the past when HCV testing of blood unit was not introduced, may also be carriers of HCV; hence should be tested for HCV co-infection.

**BLOOD VDRL –**

Although the incidence of syphilis has declined in many states, HIV infected case with H/O unprotected multi-partner sex may be subjected to Blood VDRL as both HIV and syphilis are affecting each other in rapid progression of either diseases.

**INVESTIGATIONS FOR OIs :**

OIs are diagnosed as and when they occur. After the treatment of OI, the patients are continued on suppressive therapy, The search for dormant/latent OIs is futile. As such chemoprophylaxis with Septran offers the protection against PCP, toxoplasmosis, infective diarrheas, and infective pneumonia. Search for dormant fungal e.g. cryptococcal infection, CMV and other infections, is not cost effective because prophylaxis against them is not possible because of very narrow therapeutic window.
Drug sensitivity tests:

In clinical practice, one carries out culture and drug sensitivity tests in case of Bacterial Infections. Can we do the same in HIV infection? First of all, viral culture is time consuming and very expensive.

Therefore, ARV resistance is detected by Genotypic and Phenotypic assays. The RT and Prot genes are sequenced. The mutations detected in the sequence are compared with the standard resistance algorithms (Stanford, IAS, aNRS). This gives the ARV resistance pattern. The Phenotypic assays are expensive and are carried out only in specialized centers. Therefore, from Genotypic assay one can determine the drug resistance using virtual Phenotypic resistance.

With increasing use of ARVs in India, with rampant inadvertent prescriptions, with no prescription auditing, it may become necessary to carry out Genotypic study of a patient before initiating ART.