HIV infection is commonly diagnosed by detection of antibodies (anti-HIV) by ELISA or agglutination. Reactive results are confirmed by western blot (immunoblot) or further specific tests such as competitive ELISA, which, when evaluated quantitatively, allow the differentiation of HIV types and partially subtypes. Detection of infection of newborn babies, characterisation of individual strains for subtyping and forensic identification, and therapeutic monitoring are the domain of nucleic-acid-based assays. Nucleic-acid-based assays narrow the serological diagnostic window period in early HIV infection and, when quantified, give some indication of clinical status.

**HIV series**

**Difficulties and strategies of HIV diagnosis**

Lutz Gürtler

HIV infection is diagnosed by detecting antibodies specific to the virus, or by detecting the virus itself, either through the p24-antigen, by nucleic acid-based tests, or if necessary by culture and virus isolation. Currently, two types of HIV are known, HIV-1 and HIV-2, with 40–60% aminoacid homology between the various genes. HIV-1 is subdivided into several subtypes (from A to I) with an additional subtype O; subtype O shows an aminoacid homology of 55–70% with the other HIV-1 subtypes, and is regarded by some researchers as a new subgroup.1,2 Similarly, and to reflect the close relation of subtypes A–I, these are grouped together as subgroup M (for major).3

**Serology**

Antibodies to HIV usually begin to be detectable 6–8 weeks after infection with the virus. This period—known as the diagnostic window or serological latency—may be somewhat shorter than 6 weeks or several weeks longer. Inability to detect antibodies 3 months after infection is unusual,4,5 and reports of serological latency lasting for years need confirmation; if such protracted latency occurs, it is extremely rare. Antibodies to HIV (anti-HIV) persist for life, with antibodies specific for the env-proteins, and particularly the immunodominant domain of gp41,6 persisting and for gag frequently declining. Antibodies to the integrase (p32) can be detected after antibodies to the reverse transcriptase (p51 and p66) and the other viral proteins appear and, like the env antibodies, they persist for life.

**Screening assays**

Enzyme-linked immunosorbent assays (ELISA) and agglutination assays are commonly used for screening. The sensitivity of these methods was increased by replacing whole-virus lysate with recombinant proteins and peptides in assays based on the indirect ELISA principle and on the double-antigen assay sandwich principle (figure 1).8 Double-antigen assays are more sensitive in the early phase of seroconversion7 but have a lower crossreactivity with antibodies directed against variant HIVs. Consequently, subtypes other than B may be missed during early seroconversion. In particular, infections with subtype O strains may be missed even at later stages of infection.10 The false-negative results obtained with selected anti-HIV subtype O specimens7,9 led to modification of assays and/or inclusion of subtype O antigens.

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**Figure 1:** ELISAs in common use

Within the well of a microtitre plate the patient’s antibody (Y) binds to antigen (●). Patient’s antibody is subsequently detected either by an enzyme-labelled anti-human-antibody (Y dashed), or an enzyme-labelled antigen (●-E) or by the competition with enzyme-labelled human antibody of the test. Enzymatic action is visualised by the conversion of the colourless of a substrate (❍) to its coloured derivative (❍).
HIV subtype O isolates are themselves highly heterogeneous, as illustrated by an aminoacid alignment (figure 2) of an immunodomina region in the transmembrane protein which represents an important diagnostic epitope (Gnann epitope). This heterogeneity explains the low antibody cross-reactivity between HIV-1 subtype O sera and corresponding peptides from HIV-1 subgroup M or HIV-2 strains.

Confirmatory assay

Radioimmunoprecipitation, which yields a strong reaction with the glycoproteins, was used as an early confirmatory assay in HIV diagnosis. However, since this assay is very laborious, the reaction profiles of recent HIV variants are not available. The indirect immunofluorescence assay on HIV-producing cells allows the detection of antibodies to all viral antigens. Many HIV-2-positive sera react with HIV-1-infected cells and vice versa; the same is true for anti-subtype O with HIV-1 subtype B infected cells. So far, no anti-subtype O specimens have failed to react with HIV-1-infected cells, in contrast to failure to react with HIV-2-infected cells.

The most commonly used confirmatory assay is the immunoblot (western blot), which has become the gold standard for investigating reactive results of ELISA screening. This test is very reliable for the detection of antibodies against isolates from the same HIV-1 subgroup (commercially available western blots are prepared with subtype B strains) but may miss sera obtained during the early stages of seroconversion. When an HIV-1 western blot is used to confirm HIV-2-positive sera, about 20% of the specimens make false-negative results, especially when the antibody titre is low. An HIV-1 subtype B western blot also misses subtype O infection in about 10% of the specimens. Commercially available immunoblots have not yet been modified to include subtype O antigen, as has been done for ELISA since 1994, so the risk of a false-negative immunoblot result remains. However, since infection with HIV-1 subtype O is rare outside Africa, this is not a frequent diagnostic problem. The easiest way to improve existing immunoblot strips would be to include highly immunogenic subtype-O-specific peptides (figure 2).

Assay combinations

The usual combination of diagnostic assays is an initial screen with an ELISA followed by testing of reactive specimens with an immunoblot (figure 3). This procedure is generally reliable and safe but expensive. Sometimes a final diagnosis of HIV infection cannot be achieved—eg, when the western blot only shows reactivity with HIV-core-derived bands (p24, p55), since this pattern can be non-specific. Such indeterminate profiles in immunoblots gained a new perspective after the identification of subtype O infections: whereas an HIV-2 infection detected by an HIV-1 immunoblot has a common profile of p24,55, the subtype O crossreactivity is strongest with p32 (integrase) and p51,66 (reverse transcriptase). WHO has proposed the use of two or three different ELISAs, each based on a set of individually selected antigens, to facilitate confirmation of HIV antibodies. The combined use of different ELISAs allows a clear distinction between HIV-infected and uninfected specimens. This method is also suitable for distinguishing between HIV-1 and HIV-2 infection,
except for the 30% of specimens that are doubly reactive.19–21

Another helpful approach is a two-ELISA strategy—an ELISA detecting anti-HIV-1 and anti-HIV-2 is combined with a competitive ELISA based on HIV-1 lysate. This combination is also suitable for differentiating between HIV-1 subtype O and HIV-2 infection and, when used quantitatively, for eliminating some of the false-positives and confirming negatives. The pattern obtained with various specimens is shown in figure 4. An extinction/cut-off of greater than 4 in an indirect or double-antigen ELISA and of less than 0·2 in a competitive ELISA leads to a 100% predictive value of true positivity. Anti-HIV-1 positive specimens cluster in the right lower corner, anti-HIV-2 positive samples cluster in the area above the anti-HIV-1 specimens, and anti-subtype O samples lie in a borderline position of the cut off of the competitive ELISA. Anti-HIV-negative samples, and those reactive in the first ELISA screening, cluster together at the left side at the ordinate. When this strategy is used in countries where only HIV-1 is prevalent, there are very distinct clusters of anti-HIV-1 negative and positive samples, as can be seen for Malawi and Paraguay.

**Nucleic-acid-based assays**

To achieve genomic level testing for HIV, tests such as polymerase chain reaction (PCR)22 isothermic amplification,23 and a multiprobe branched signal amplification assay24 have been developed. These assays amplify fragments of the genome. All assays use primers/probes for HIV-1 subtype B and may therefore miss, or detect with only low sensitivity, other HIV-1 subtypes, in particular subtype O, and HIV-2. For the amplification of subtype O, valid primer combinations have been selected by Simon9 and manufacturers are currently changing primers to allow amplification of all HIV-1 subtypes.

After 5 years’ experience, the need for nucleic-acid-based assays such as PCR is limited to investigations such as:

- determining the infection status of newborn babies of infected mothers
- subtyping HIV variants and identification of HIV strains for forensic reasons—eg, by sequencing regions of the envelope gene25
- sequencing of reverse transcriptase or protease genome fragments for monitoring drug resistance and therapeutic efficacy.26

Figure 4: Two-dimensional graphs of the optical absorbances obtained in a double-antigen ELISA (abscissa) and a competitive ELISA (ordinate) testing various specimens from Ivory Coast (HIV-1 and HIV-2 prevalence) and Cameroon (HIV-1 and subtype O prevalence), and from countries where only HIV-1 is prevalent (Malawi, HIV-1 subtypes B, C, and D; and Paraguay, HIV-1 subtype B) in HIV-1+2 ELISAs the cut-off has a value of 0·2–0·3; in the competitive ELISA of 0·7–0·8. Positive samples in the competitive ELISA have extinctions below the cut off. Presence and absence of anti-HIV had been confirmed in all specimens by immunoblotting.

Data were obtained in Ivory Coast together with Dr G M Gershy-Damet and Dr A Sangaré; in Cameroon with Dr L Zekeng and Prof L Kaptue; in Malawi with S Kraemer and Dr G N Liomba; and in Paraguay with Dr A Cabello and Dr M Vera-Antola.
HIV infection in young children can be confirmed serologically only after 15–21 months, depending on the amount of maternal antibodies transferred. A few children lose antibodies despite being infected with HIV, and these children show signs of severe immunodeficiency, p24-antigenemia, and positive PCR. In HIV-exposed children, detectability of HIV infection by PCR after the first month of life is nearly 100% when breastfed infants are excluded. Failure to detect HIV variants by PCR can be overcome by analysing blood samples from the child and the mother simultaneously and by comparing the amplificates quantitatively with the positive control of the assay, and with the mother’s clinical status.

Prospects

Although serological assays for HIV have been refined over the past few years to unprecedented levels of sensitivity and specificity, identification of new variants has emphasised the limits of sensitivity of existing assays. These HIV variants demand the development of assays to detect all HIV strains, thereby further reducing the risk of HIV transmission by blood transfusion and rendering the serological diagnosis of HIV infection even more reliable. In addition, as recent events have shown, even sensitive automated commercial assays are affected by minor amplificates quantitatively with the positive control of the assay, and with the mother’s clinical status.

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