GUIDELINES FOR INTERPRETING WESTERN BLOT PROFILES

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Introduction – historic

In 1984, the western blot was shown to have greater specificity for detecting HIV antibodies [in AIDS patients] than any other assay available at the time. Not only could it distinguish between individuals with and without HIV antibodies, but it could actually demonstrate the antibody reactivity to individual antigens of HIV. Because of this, the western blot has been widely used as a supplemental test to determine whether samples which repeatedly react on screening assays contain HIV antibodies or not.

In January 1985, the U.S. Centres for Disease Control issued the first guidelines for interpreting western blot profiles. These guidelines stated that HIV antibodies were detected by western blot if there was at least reactivity with p24 and/or gp41. Since a number of samples from uninfected individuals showed reactivity to p24, it soon became clear that these guidelines were unreliable for confirming serodiagnosis of HIV infection.

In August 1986, NRL issued new guidelines for interpreting and reporting western blot results. These guidelines were based on analysis of western blot profiles of routine specimens taken from individuals with reactivity on screening tests and tested between January 1985 and July 1986. The stringency of the guidelines ensured the specificity of the western blot without compromising its sensitivity. Specimens showing western blot reactivity that did not fulfil the positive criteria were regarded as “indeterminate”. A prospective study on subjects whose samples showed indeterminate reactivity was instituted.

By August 1987, it had become clear that while some indeterminate profiles were never associated with HIV infection, others occasionally were. This led to all indeterminate band patterns being divided into four groups on the basis of their probable true HIV antibody status.
Establishment of Positive and Negative Criteria for Interpreting Western Blot Reactivity

In August 1986, the reactivity profiles required for reporting a western blot as positive or negative were recommended by NRL as follows:

**Positive:** Reactivity to at least one envelope glycoprotein (gp41, gp120 or gp160) and at least three other viral viral-specific proteins of the *gag* or *pol* series.

**Negative:** No reactivity to any viral specific proteins.

Western blot reactivity which did not fulfil the positive criteria was termed INDETERMINATE, and subjects with such profiles were followed up for as long as possible.

Clarification of the Indeterminate Profiles

In August 1987, the indeterminates were separated on the basis of both their profile and their probable true anti-HIV status. They were grouped as follows (Figure 1):

**Group 1:** reactivity to viral bands, but not to p18, p24, or any envelope glycoproteins.

**Group 2:** reactivity to viral bands including p18, but not to p24 or any envelope glycoproteins.

**Group 3:** reactivity to viral bands including p24, but not to any envelope glycoproteins.

**Group 4:** reactivity to envelope glycoproteins but with less than three other viral proteins.

Interpretation of Indeterminate Western Blot Profiles

Follow-up of *uninfected* subjects whose samples produced indeterminate profiles showed that the banding patterns did not change significantly over time. By contrast, HIV *infected* subjects developed positive profiles on follow-up. During seroconversion, western blot reactivity progressed from either a negative or indeterminate profile to a group 3 or 4 indeterminate profile, then to positive. Since the implementation of early anti-retroviral therapy, this transition in reactivity from negative to indeterminate to positive can be significantly delayed or, in some cases, never lead to development of a full positive profile on western blot[1].
Presently, NRL’s documentation concerning interpretation of its in-house western blot requires that reactivity to gp41 is present, along with reactivity to three other viral-specific bands, for it to be called “positive”. In other words, we stipulate that the gp41 env band is present instead of simply requiring reactivity to any one env protein. This changed over time because NRL no longer scores the high molecular weight (MW) glycoprotein bands on its in-house western blot despite their being visible. The absence of the high MW gp bands from NRL’s interpretation criteria came about because of an historical modification to the viral lysate electrophoresis protocol; the high MW glycoproteins were not sufficiently separated from each other during electrophoresis to enable their distinction.

Some, if not all reference laboratories in Australia, use NRL criteria to interpret reactivity in commercial western blots. However, it is important to note that genuinely uninfected individuals with indeterminate group 4 western blot profiles do occur infrequently, especially if gp160 is the only env protein to which reactivity is detected. NRL is aware of at least one occasion when a commercial western blot was falsely interpreted as positive, using NRL criteria, based on the presence of gp160 and three other viral specific bands. In this case, the manufacturer’s instructions for the relevant commercial western blot required two env bands for a positive result. In other words, when there are multiple env bands available for scoring on a commercial western blot, laboratories need to be aware of the risk of reporting a false positive result if only one of the env bands is present, especially when the blot manufacturer requires two for a positive result.

Reference laboratories should also take note that once the new IVD framework in Australia is fully implemented, a commercial western blot will become a Class 4 in-house IVD if interpretation criteria other than those of the manufacturer are used to report a result.
Figure 1: Profiles of indeterminate groups 1-4 on NRL's in-house western blot$^2$
References

1. C. Bradley Hare et al. Seroreversion in subjects receiving antiretroviral therapy during acute / early HIV infection. Clinical Infectious Diseases 2006; 42:700-708