Evaluation of the Alere™ HIV Combo point-of-care test

Hitesh Mistry[1], Maximillian S. Habibi[1], and David Muir[1]
[1]Department of Infection and Immunity, Imperial College Healthcare NHS Trust

Imperial College Healthcare NHS
Department of I&I NHS Trust

Background

The Alere™ HIV combo is a CE-marked rapid point-of-care (POCT) test that detects both HIV-1/2 antibodies and free HIV-1 p24 antigen. It therefore has the potential to identify HIV infection earlier than antibody-only tests. The test is a lateral flow assay whereby 50 µL of sample is applied to a sample pad and diffuses along the strip. Results for HIV-1 p24 antigen, HIV-1/2 antibody, and control are read in designated windows (Figure 1). The small strip format is suitable for use with whole blood, serum, or plasma samples, providing results in 20 minutes.

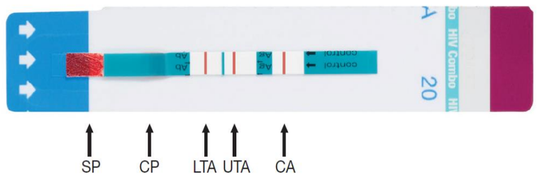

Figure 1 Test strip with sample pad (SP), conjugate pad (CP), lower test area (LTA) for HIV antibody, upper test area (UTA) for HIV-1 p24 antigen, and control area (CA) indicated.

Methods

Stored serum samples from patients undergoing HIV serology at Imperial College Healthcare NHS Trust (ICHNT) Infection & Immunity laboratory were identified. The original result was noted and the samples tested in batches using both the Alere™ HIV combo POCT and the current standard HIV confirmation assay at ICHNT, the bioMérieux VIDAS HIV Duo Ultra 4th generation HIV-1/2 antigen antibody combination assay. A "positive" result on the Alere™ HIV combo POCT is defined as a clearly readable result for the control and either or both of HIV-1 p24 antigen and HIV antibody. A "negative" result was a clearly readable result for the control only. Any other result was declared indeterminate. Results for each method were compared to determine the performance of the Alere™ HIV combo POCT relative to the VIDAS.

Results

120 samples in total were included. 50 originally HIV-1 p24 antigen positive, 47 HIV-1 antibody positive, of which 13 were determined to be recently-acquired infections (incident, acquired within the last 3 months, Lag avidity assay, PHE Colindale), and 34 were determined to be established infections (acquired more than 3 months ago). There were 3 samples from patients with established HIV-2 infection. 20 samples with negative HIV serology from antenatal patients were included. All samples were retested on the VIDAS and the new results recorded, giving 12 discrepant results. One sample originally HIV-1 p24 antigen positive, now gave a negative result for both antigen and antibody and was excluded from subsequent analysis. Four samples that initially were HIV-1 p24 antigen-only positive, now also had detectable HIV-1 antibody (i.e. were antigen / antibody dual positive). Seven samples that were initially HIV-1 p24 antigen-only positive, became HIV-1 antibody-only positive. As the overall result interpretation remained as HIV test "positive", these 11 samples were retained in the subsequent analysis, and all were considered to indicate recently-acquired HIV-1 infection. All subsequent comparisons were made using these new VIDAS test results to ensure comparability between the two methods. The comparative test results were therefore as follows:

Total of 99 HIV seropositive samples
- 62 samples from patients with recently-acquired HIV-1 infection
  - *38 HIV-1 p24 Ag only positive samples*
  - *4 HIV-1 p24 Ag and HIV-1 antibody positive samples (dual Ag/Ab positive)*
  - *7 HIV-1 antibody only positive samples that were originally p24 antigen positive*
  - *13 low avidity HIV-1 antibody positive samples*
- 37 samples from patients with established HIV infection
  - *34 HIV-1 antibody only positive*
  - *3 HIV-2 antibody only positive*

20 HIV seronegative samples

Condition	Total included	Alere Ab		Alere HIV-1 p24 Ag		Alere Ag + Ab		Alere HIV-positive
		N		N		N		
HIV infection, any stage	99	71	72%	11	11%	14	14%	97%
Recently-acquired HIV-1 infection	62	34	55%	11	18%	14	23%	95%
Established HIV-1 infection	34	34	100%	0	0%	0	0%	100%
HIV-2 infection	3	3	100%	0	0%	0	0%	100%
Uninfected	20	0	0%	0	0%	0	0%	0%

Table 1 Sensitivity of Alere™ HIV combo POCT in various stages of HIV infection

No sample tested gave an indeterminate result using the Alere™ HIV combo POCT. 96 out of 99 known HIV seropositive samples gave a positive result using the Alere™ HIV combo POCT, yielding an overall sensitivity for HIV infection of 98% (Table 1). The 3 samples that gave a "false" negative result on Alere™ HIV combo POCT had originally, as well as on repeat VIDAS testing, been HIV-1 p24 antigen-only positive. No HIV-negative sample gave a positive result using the Alere™ HIV combo POCT, yielding a specificity of 100%.

Condition	Total included	Alere Ab		Alere HIV-1 p24 Ag		Alere Ag + Ab		Alere HIV-positive
		N		N		N		
HIV-1 p24 antigenaemia	42	18	43%	10	24%	11	26%	93%
Low avidity HIV-1 antibody*	20	16	80%	1	5%	3	15%	100%
Established HIV-1 infection	34	34	100%	0	0%	0	0%	100%

Table 2 Results of Alere™ HIV combo POCT by antigen and antibody for HIV-1. * includes 7 samples that were originally HIV-1 p24 antigen only positive that were found to be HIV-1 antibody only positive on retesting.

HIV-1 p24 antigen positive samples

Of the 38 HIV-1 p24 antigen-only positive and 4 HIV-1 p24 antigen and antibody positive samples, eighteen (43%) gave a result of antibody only detected using the Alere™ HIV combo POCT, 10 (24%) gave a result of antigen only detected, 11 (26%) gave a result of both antibody and antigen detected, and 3 (7%) were negative, yielding a sensitivity of 93% (Table 2).

Discussion

The Alere™ HIV combo POCT yielded an overall sensitivity for HIV infection of 97% and a specificity of 100%. A positive test on the Alere™ HIV combo POCT had a PPV for HIV infection of 100%. Although a negative result had a NPV of only 87%, this was largely accounted for by the very high prevalence of HIV infection in the sample set. It is therefore likely that the NPV is underestimated compared to a real-life setting in the UK, where HIV prevalence rates of <10% would be expected. Given the observed specificity of 100%, the PPV would not be similarly affected.

The sensitivity of the Alere™ HIV combo POCT for detecting early HIV infection was 95%. This was accounted for by a reduced sensitivity for HIV-1 p24 antigen (93%, Table 2), and laboratory testing remains imperative for patients with suspected recent HIV infection. Whilst not as good as 4th generation laboratory assays however, the sensitivity of the Alere™ HIV combo POCT for very early HIV infection is expected to be greater than that of the standard 3rd generation POCT assays currently available. Therefore, providing this level of sensitivity can be maintained in the clinical setting using fresh whole blood obtained by finger-prick (further evaluation required), in addition to its role in the diagnosis of established infection the Alere™ HIV combo POCT would represent a useful adjunct to laboratory testing in cases where there is a clinical suspicion of recent HIV infection: performance of this assay at the point of care would provide an earlier indication of infection in the majority of cases, allow earlier implementation of preventive measures, and thereby contribute to limiting onward transmission of HIV, as well as facilitating inclusion of the patient into care pathways where delays might otherwise result in loss to follow up.

Reproducibility of test results was not assessed in this study. Furthermore, this study has a number of limitations that may affect the applicability of the findings to clinical practice. Firstly, only serum samples were tested. It is possible that performance with other types of samples may differ and validation should ideally involve whole blood. Secondly, the testing was carried out in a laboratory by trained and experienced personnel under controlled conditions, and therefore further evaluation of the use of this assay in a real-life clinic setting is still required. Finally, only 3 samples were from HIV-2 infected patients. This type of HIV is generally uncommon in the UK at present, but the prevalence may be higher in some communities.

Conclusion

The Alere™ HIV combo POCT is a simple to use, rapid test capable of reliably identifying established HIV infection. Providing the assay performance can be replicated in the clinical setting, its ability to detect p24 antigen could also make it a useful adjunct to laboratory testing for recently acquired infection, potentially allowing more rapid implementation of interventions