



An assessment of bioLytical Laboratories INSTI HIV-1 Antibody Test Kit for detection of HIV-2 positive specimens

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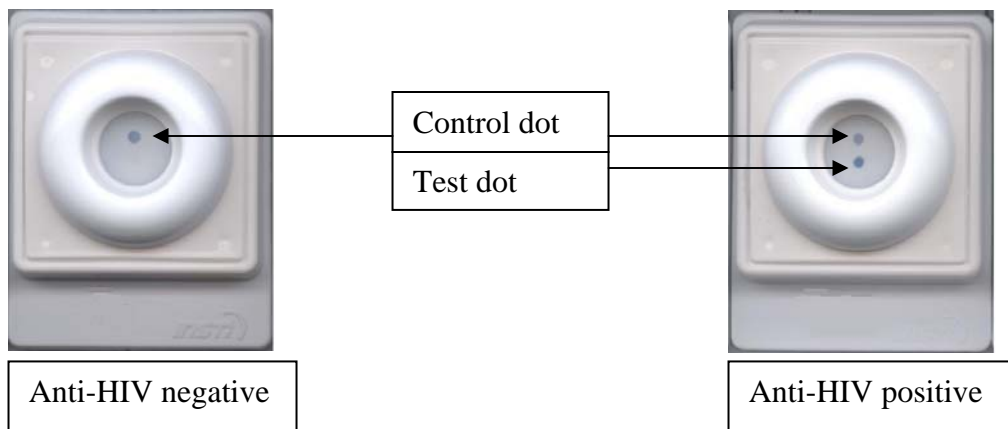
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Study aim

The ability of INSTI HIV-1 Antibody Test Kit to correctly identify anti-HIV-2 positive specimens in previously screened specimens was assessed. The status of the specimens had previously been determined by application of other HIV assays.

Description of the assay

The INSTI HIV-1 Antibody Test Kit is a flow-through rapid test which is currently validated for the qualitative detection of antibodies to HIV-1 in serum, plasma and whole blood. The INSTI HIV-1 Antibody Test Kit test device consists of a synthetic filtration membrane positioned on top of an absorbent material within a plastic cartridge. The device has one test dot which is coated with HIV-1 and HIV-2 recombinant proteins. A blue coloured dot appears in the test dot region if the sample contains antibodies to HIV-1. The assay also has a procedural control dot, capable of detecting IgG in the test sample, to ensure assay validity.



The kit provided for the evaluation (product number 90-1009) included 24 test cartridges and 24 each of three vials containing the agents required to conduct the assay; sample diluent, colour developer and clarifying solution. Product numbers 90-1007 (24 tests) and 90-1008 (individually packaged for single use) also contain the following additional materials: alcohol swab, single-use lancet and single-use pipette, for testing fingerstick whole blood samples.

Evaluation method

The assays were performed according to the manufacturer's instructions. The evaluation panel consisted of three anti-HIV negative specimens and 76 anti-HIV 2 positive specimens. The anti-HIV 2 specimens were previously confirmed to contain anti-HIV in a variety of assays, including the Dade Behring Enzygnost HIV Integral, the Murex HIV Ab-Ag combination EIA and the Genetic systems EIA. The specimens were confirmed to be anti-HIV 2 positive by the ImmunoComb II HIV 1&2 BiSpot assay (Orgenics) and an in-house IgG antibody-capture particle adherence assay, GACPAT HIV 1+2¹ (HPA Virus Reference Department (VRD)). The anti-HIV negative specimens were provided to the Microbiological Diagnostics Assessment Service as screen unreactive. They were further tested by the Genscreen HIV 1/2 ULTRA and Abbott Determine HIV and the ImmunoComb II HIV 1&2 BiSpot. The evaluation was performed using lot number B6G144 of the INSTI HIV-1 Antibody Test

All results were read visually by three readers who independently recorded their scores using separate results sheets. The following scoring system was adhered to:

- 0 = No reactivity
- 1 = Uncertain reactivity (indeterminate)
- 2 = Weak, but definite reaction
- 3 = Medium reactivity
- 4 = Strong reactivity

The consensus of the three readers was taken. A consensus score of 2 or greater equalled a positive reaction, a consensus of 1 equalled an indeterminate reaction and a consensus of 0 was deemed to be a negative reaction. Specimens giving rise to false positive or false negative results were retested in duplicate. The data were entered into a database and checked. Digital images of all rapid tests results were stored for reference.

The readers also scored the kit for ease of result interpretation. The scoring was on a sliding scale of one to ten, with a score of 1 as 'easy to read' and a score of 10 being 'very difficult', ie a great deal of deliberation was necessary to determine the test result.

Evaluation panel

The evaluation panel consisted of 82 specimens, Table 1.

Seventy-six of the 82 samples were anti-HIV 2 positive. The samples were collected over a number of years from various sources including samples referred to the VRD for confirmatory testing and samples from commercial sources. According to the VRD testing algorithm for the differentiation of anti-HIV 1 and anti-HIV 2 reactivity, the samples were positive for anti-HIV 2 and negative for anti-HIV 1.

Three anti-HIV negative samples were provided by Kings College Hospital and were included in the panel as a small check on the behaviour of the kit against negative samples. Three anti-HIV 2 quality control samples were also included in the panel.

The HPA HIV 2 QC2 and QC3 are samples which are available from the Quality Control Reference Unit, HPA. The HIV 2 Monitor Sample, product code 99/674-007, is available from the National Institute of Biological Standards and Control, Potters Bar, Hertfordshire, UK.

Table 1. Evaluation panel

Category	Number of specimens
Anti-HIV 2 Positive	76
HPA HIV 2 QC2	1
HPA HIV 2 QC3	1
NIBSC HIV 2 Monitor Sample	1
Anti-HIV Negative	3
TOTAL	82

Results

Seventy-two of the 76 anti-HIV-2 positive specimens were reactive by the INSTI HIV-1 Antibody Test Kit to give an initial sensitivity of 94.7% (95% confidence interval 87.1-98.6%) (Table 2). Sixty-nine were medium or strongly reactive and three were weakly reactive. Of the four remaining anti-HIV-2 positive samples, two were indeterminate and two were unreactive by the INSTI HIV-1 Antibody Test Kit.

Three anti-HIV negative samples were tested by the INSTI HIV-1 Antibody Test. Two of these samples were unreactive and one was weakly reactive on initial testing in the INSTI HIV-1 Antibody Test Kit. Due to the limited number of anti-HIV 2 negative samples tested, a specificity value is not given.

One sample gave a weakly reactive result in the control dot, the remaining samples were of medium reactivity or were strongly reactive. Therefore, all test results were valid.

Table 2. Initial results for 76 anti-HIV 2 positive and three anti-HIV 1 and 2 negative specimens tested by the INSTI HIV-1 Antibody Test Kit

Specimen status	Consensus initial results				
	Number tested	Medium/strong reactive	Weak Reactive	Indeterminate	Negative
HIV 2 Positive	76	69	3	2	2
HIV 1 & HIV 2 Negative	3	0	1	0	2

Note: All control dots were valid

On repeat testing of the two initially false negative samples, one was still unreactive and the second was indeterminate. Of the two anti-HIV 2 positive samples that were initially indeterminate in the INSTI HIV-1 Antibody Test Kit, one still gave an indeterminate result after retesting. This sample gave a high background colour which may have affected the ability of the readers to differentiate the test dot from the background. The second indeterminate sample was weakly reactive after retesting. Therefore a total of 73 of the 76 anti-HIV 2 positive samples were reactive after retesting of discordant samples to give a final sensitivity of 96.1% (95% confidence interval 88.9-99.2%). A summary of final results is shown in Table 3 and additional test results are shown in Table 4.

The anti-HIV negative sample that was falsely positive on initial testing was again weakly reactive after retesting.

All control dots were reactive in the repeat tests and therefore all tests were valid.

Table 3. Final results for 76 anti-HIV 2 positive and three anti-HIV negative specimens tested by the INSTI HIV-1 Antibody Test Kit

Specimen status	Consensus repeat results				
	Number tested	Medium/strong reactive	Weak Reactive	Indeterminate	Negative
HIV 2 Positive	76	69	4	2	1
HIV 1 & HIV 2 Negative	3	0	1	0	2

Note: All control dots were valid

Table 4. Test results for five discordant specimens

Sample ID	Origin	INSTI HIV-1 Antibody Test Kit				Immunocomb II HIV 1&2 Bispot ¹		VRD GACPAT ¹		Abbott Determine ¹	Genscreen ULTRA ¹	Genetic Systems EIA ²	IAF Biochem EIA ²
		Initial consensus		Repeat consensus		HIV 1	HIV 2	HIV 1	HIV 2	Consensus result	OD/CO	OD/CO	OD/CO
		Control	Test	Control	Test								
99-44547	BBI	4	1	4	1	0	4	0.194	10.00	4	NT	NT	19.108
99-44553	BBI	3	0	3	1	0	4	0.130	11.11	4	NT	NT	11.484
99-44562	BBI	3	1	4	2	1	4	0.156	10.00	4	NT	NT	16.646
05-H0108	BBI	4	0	4	0	0	4	0.162	6.667	4	NT	11.114	NT
03u0001	EU / KCH	4	2	4	2	0	0	0.193	0.182	0	0.296	NT	NT

Notes: Score 0 = negative, 1 = indeterminate, 2 = weak positive, 3 = medium positive, 4 = strong positive. NT = not tested.
 Samples 99-44547, 44553 & 44562 were also anti-HIV positive by Access HIV 1/2, AxSYM HIV 1/2 and Ag-Ab Combo, Enzygnost Integral, Genscreen Ag-Ab, Murex Ag-Ab and PRISM. Data collected during kit evaluations.
 BBI = Boston Biomedica Inc. EU / KCH = Antenatal sample provided to the Microbiological Diagnostics Assessment Service by Kings College London.
¹ Results generated at HPA. ² Results provided by BBI.

Three quality control samples were included in the evaluation panel. All 3 controls are produced by dilution such that they generate weak to moderate reactions in a range of HIV EIA screening tests. The INSTI HIV-1 Antibody Test Kit did not detect anti-HIV 2 in either the HPA HIV 2 QC2 or QC3, although in both cases the control dot was scored as medium positive and therefore the tests were valid. The INSTI HIV-1 Antibody Test Kit was not reactive in either the test or control dot for the NIBSC HIV 2 Monitor Sample, therefore the test was invalid. It should be noted that the latter sample is diluted in PBS and therefore there is probably insufficient IgG in the sample to allow detection in the control dot. Overall, none of the QC controls tested were suitable for use with the INSTI HIV-1 Antibody Test Kit and therefore other suitable controls need to be identified or specifically manufactured.

Technical appraisal

The INSTI HIV-1 Antibody Test Kit was easy to use and, like other RTDs, is appropriate for use in small laboratories, GUM clinics and emergency care settings. It is particularly useful in situations that require no electricity, ancillary reagents and equipment. bioLytical Laboratories produce three versions of the kit, two of which contain all the materials required to carry out the test (product numbers 90-1007 and 90-1008), see Description of the Assay section above. The version of the kit (product number 90-1009) provided for this evaluation contained only test devices and reagents therefore a micropipette was used to measure the required 50µL of each sample.

The readers reported the INSTI HIV-1 Antibody Test Kit device 'easy to read', all readers gave a score of two of a scale of 1 to 10 (1=easy, 10=difficult). On initial testing, the readers found the control dot easy to read in all 79 samples (78 showed medium or strong reactivity and one showed a weak, but clear, reactivity). The readers found the test dot easy to read in the majority (72/76) of anti-HIV 2 positive samples (69 showed medium or strong reactivity and 3 showed weak, but clear, reactivity). For two samples, visibility of colour in the test dots was uncertain therefore readers were unable to discern whether a dot was present, one of which had a high background colour on the device membrane. For the anti-HIV negative samples, 2 of 3 were clearly negative and one showed a weak, but clear, reactivity.

One sample had a slow flow-through time; however the sample gave a clear test result with no background colour. The remaining samples had rapid flow-through rates.

It was noted in one device (after testing) that a small area of the test membrane was detached from the surrounding frame, although this was not considered to have affected the testing of the specimen. Of the remaining devices used, on opening, two devices were found to have the membrane detached from the surrounding frame and were considered to be defective. These devices were not used for testing.

Conclusions

The INSTI HIV-1 Antibody Test Kit was found, on initial testing, to detect anti-HIV 2 in 72 (94.7%) of the 76 anti-HIV-2 positive specimens included in the panel. On repeat testing of the discordant samples, the INSTI HIV-1 Antibody Test Kit detected 73 (96.1%) of the 76 anti-HIV-2 positive specimens.

One of the three anti-HIV negative samples was repeatedly weakly reactive in the INSTI HIV-1 Antibody Test Kit. However it would not be appropriate to make any conclusions in this study from such a small number of samples.

None of the three quality control samples included in the evaluation panel would be suitable for internal quality control purposes with this assay.

Reference:

1. Parry JV, Connell JA, Garcia AB, Avillez F, Mortimer PP. GACPAT HIV 1+2: a simple, inexpensive assay to screen for, and discriminate between, anti-HIV 1 and anti-HIV 2. *Journal of Medical Virology* (1995) 45: 10-16.