

Laboratory diagnosis of HIV infection in Papua New Guinea

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SUMMARY

In Papua New Guinea, the laboratory diagnosis of HIV infection is based on proof of HIV antibody in the patient's serum. Under the government scheme, the testing is done in 30 laboratories, including the Papua New Guinea HIV Reference Laboratory (NRL), the Red Cross Blood Transfusion Service in Port Moresby, and 19 provincial and 9 district laboratories. An alternative testing strategy was adopted in 1993 based on a WHO recommendation, replacing the classical testing strategy (enzyme immunoassay + Western blot). The alternative testing strategy uses several EIA, rapid or simple HIV antibody assays for the detection and confirmation of the HIV antibody. This approach is faster and cheaper, with the same sensitivity and specificity as the classical testing algorithm. Except for the NRL, the Serodia Fujirebio HIV-1 gelatin particle agglutination assay is used throughout the country as the screening test. The PNG National HIV Reference Laboratory is the only laboratory authorized to perform confirmatory testing and to release positive results. Therefore, all serum samples reactive in the screening assay are sent to the NRL for confirmation by the battery of EIA, rapid or simple assays in accordance with the alternative testing strategy adopted. The paper explains the alternative testing strategy and highlights the principle of each individual test that is employed.

The laboratory diagnosis of HIV (human immunodeficiency virus) infections involves three main domains (Table 1):

1. Tests for detecting HIV infection
2. Tests for monitoring anti-HIV-positive patients
3. Tests for diagnosis of HIV-associated diseases.

Serology is the most definitive method used to detect HIV-infected individuals. Many other technologies, including HIV culture, the recognition and binding of HIV sequences by specific nucleic acid probes and the polymerase chain reaction (PCR or gene amplification), are used to isolate or signal the presence of HIV, but these are performed only in specialized centres with costly containment facilities and staff well-trained in the requisite

precautions for handling large quantities of HIV.

As in most countries, the laboratory diagnosis of HIV infection in Papua New Guinea (PNG) is based on proof of HIV antibodies in the patient's serum. Several different types of laboratory test for detecting HIV antibody in human serum exist today. In Papua New Guinea anti-HIV testing is done in 19 provincial and 9 district laboratories, the PNG HIV reference laboratory at the Central Public Health Laboratory (CPHL), which is the National Reference Laboratory (NRL), and the Port Moresby Red Cross Blood Transfusion Service (PNGRCBTS). Except for the NRL, the Serodia Fujirebio HIV gelatin particle agglutination assay is used throughout the country as the screening test and all positive results are then forwarded to NRL to perform confirmatory testing and release positive results.

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TABLE 1

HIV LABORATORY DIAGNOSIS

| Tests for detecting HIV infection | Tests for monitoring anti-HIV-positive patients | Tests for diagnosis of HIV-associated diseases |
|---|--|--|
| A) Antibody tests – ELISA - indirect, competitive antigen sandwich (Vironostika) – Simple and rapid assays: particle agglutination (Serodia, SimpliRED, Capillus); immunodot assays (Immunocomb, HIVSpot, Genie, Testpack) – Supplemental tests: Western blot, RIPA, IFA | Anti-p24 detection Viral antigen tests (p24) Viral load tests (PCR) CD4 count, CD4/CD8 ratio Beta-2-microglobulin Neopterin | Tuberculosis <i>Pneumocystis carinii</i> pneumonia Kaposi’s sarcoma <i>Cryptococcus neoformans</i> <i>Salmonella</i> Others |
| B) Tests for virus and viral antigens – Culture – EIA for antigens | | |
| C) Tests for viral nucleic acid – PCR (nucleic acid amplification) – Nucleic acid probes | | |
| ELISA, enzyme-linked immunoassay RIPA, radioimmune precipitation assay IFA, immunofluorescence assay EIA, enzyme immunoassay PCR, polymerase chain reaction | | |

Initially when the first local screening for HIV antibodies in PNG was performed in 1987 the classical testing strategy, enzyme immunoassay (EIA) confirmed by Western blot, was adopted. With this strategy, all repeatedly reactive samples in the ELISA, rapid or simple tests in the first screen were then sent to Australia for Western blot confirmation as a secured test (Table 2a).

In 1993 a new testing strategy, first implemented in 1992 and based on World Health Organization (WHO) recommendations (1), was adopted with some modification, replacing the classical testing strategy. This was called the alternative testing strategy (Table 2b). It involved two different additional ELISA, rapid or simple assays to test the repeatedly

reactive samples in the first screen. It is obvious that positive results must be unequivocally reactive in all three tests used. Samples giving discrepant results are further investigated and sent to the Australian National Reference Laboratory at Fairfield for confirmation by either antigen assays or Western blot.

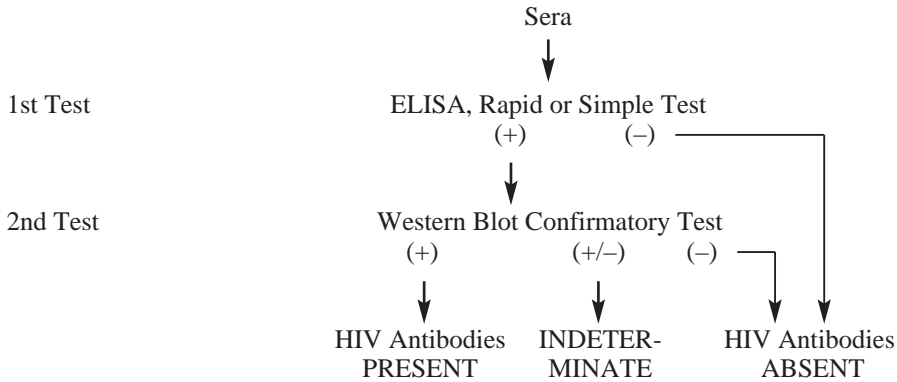
The selection of the most appropriate test or combination of tests to use (i.e. the testing strategy) depends on 3 criteria:

1. The objective of the test, out of four main objectives: transfusion, surveillance, diagnosis and research.
2. The sensitivity and specificity of the test(s) being used.

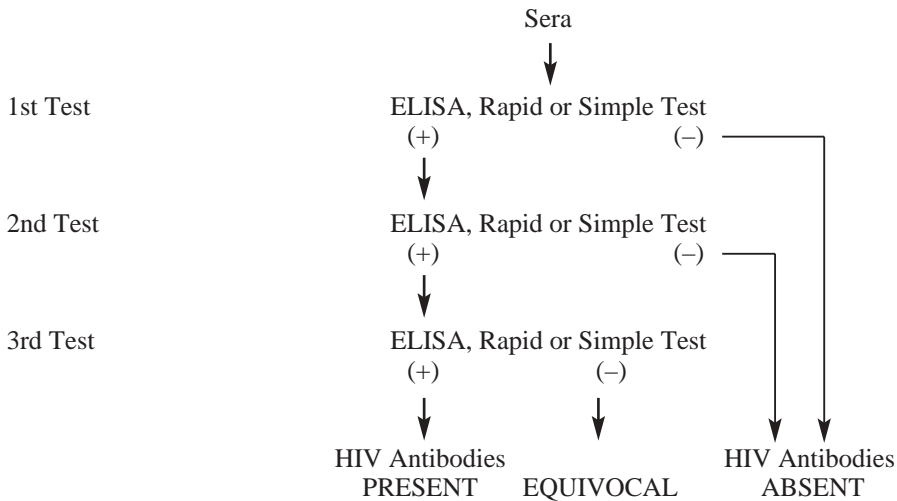
TABLE 2

CLASSICAL AND ALTERNATIVE TESTING STRATEGIES FOR HIV DIAGNOSIS

(a) Classical Testing Strategy



(b) Alternative Testing Strategy



3. The prevalence of HIV infection in the population being tested.

According to the above criteria, the simplicity of the test procedure used, the rapidity of the test performance and the cost-benefit ratio, the national HIV testing scheme has adopted the following assays to fulfil the alternative strategy. All laboratories throughout the country use the Serodia method as the first screening test and any repeatedly reactive

(positive) samples are then sent to the national reference laboratory (NRL) at CPHL in Port Moresby for confirmation using two or more additional assays. At CPHL the second test used is the ELISA and the third method is the ImmunoComb (Table 3); HIVSpot or Capillus may also be used for test 3. Although only three different tests are used in routine practice, discrepant samples may be examined with up to three other assays listed as test 4 in Table 3. The reason is to evaluate the performance of

TABLE 3

SEQUENCE OF ASSAYS USED IN THE NATIONAL TESTING STRATEGY

| | Assays used in the CPHL | Other laboratories in PNG |
|--------|---|----------------------------------|
| Test 1 | Vironostika | Serodia |
| Test 2 | Serodia Vironostika (referred samples) | |
| Test 3 | Immunocomb | |
| Test 4 | HIVSpot, Capillus, Western blot | |

CPHL, Central Public Health Laboratory

different assays in PNG conditions and to decide the serostatus of discrepant samples.

Table 4 shows the assays used in the general algorithm for HIV antibody testing according to the national testing strategy. It highlights the principles of each test, the antigens used, time to perform each test, and the cost, sensitivity and specificity of each assay. In a few cases experience in PNG has provided somewhat different values for the sensitivity and specificity of tests from those given by the manufacturers. These discrepancies are indicated in Table 4. According to NRL experience, Serodia Fujirebio performs much better in Papua New Guinean conditions than the manufacturer's data show: the specificity has been consistently greater than 99% (2-5). In contrast, false reactive results have been experienced with Immunocomb and HIVSpot, rendering the specificity of these tests less than 100%.

This new testing strategy described here for HIV diagnosis in PNG has proved to be suitable and effective and has provided rapid results with a favourable cost-benefit ratio. Moreover, in the case of clearly positive samples it makes the PNG reference laboratory

independent of the Australian NRL. Only discrepant and indeterminate samples are now sent to Australia for further investigation by antigen assays or Western blot. This enables the PNG NRL to send out clearly positive and negative results in a short time: 7 days for the alternative strategy compared to 2 months for the classical strategy (since these all had to be sent overseas for confirmation).

REFERENCES

- 1 **World Health Organization.** Recommendations for the selection and use of HIV antibody tests. *Wkly Epidemiol Rec* 1992;No 20:145-149.
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- 3 **Papua New Guinea National HIV Reference Laboratory.** Annual Report 1995. Port Moresby: Department of Health and World Health Organization, Feb 1996.
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TABLE 4
ASSAYS USED FOR HIV ANTIBODY TESTING IN THE PNG NATIONAL TESTING STRATEGY

| Assay | Principle | Antigen used | Time to perform | Cost USD | Sensitivity % | Specificity % |
|--------------------------|--------------------------------------|--|--------------------|----------|---------------|---------------|
| Serodia Fujirebio | Particle agglutination (gelatin) | Virus lysate (HIV-1) | 3.5 hours/90 tests | 0.65 | 100 | 96.9(?) |
| Vironostika Mixt HIV 1/2 | Indirect ELISA | HIV-1 - lysate HIV-2 - synthetic | 2.5 hours/90 tests | 0.70 | 100 | 100 |
| Vironostika UniForm 1/2 | Double antigen sandwich | HIV-1 - recombinant HIV-2 - synthetic | 2 hours/90 tests | 0.50 | 100 | 99.7 |
| Immunocomb HIV 1/2 | Comb immunodot assay | HIV-1/2 - synthetic | 50 min/1-12 tests | 2.00 | 98.5 | 100(?) |
| HIVSpot HIV 1/2 | Cartridge immunodot assay | HIV-1/2 - synthetic | 5 min/test | 2.00 | 98.8 | 100(?) |
| Capillus HIV 1/2 | Particle agglutination (latex) | HIV-1/2 - recombinant | 10 min/1-5 tests | 2.00 | >99 | >99 |
| SimpliRED Agen | Particle agglutination (erythrocyte) | HIV-1 - synthetic | 4 min/test | 3.00 | 100(?) | >99 |
| Western blot | Commercial immunoblot assay | HIV-1 - lysate | 3 hours/1-7 tests | 15.00 | 100 | 100 |

Note: ? refers to a discrepancy between the manufacturer's data and the experience of use in PNG (see text for details)