

EDITORIAL

Issue Fifteen, August 2003

The featured article in this edition is *Laboratory diagnosis for infection with Human Immunodeficiency virus (HIV)* by Dr John Vivian Wells and Richard Allen. The authors combined experience in testing for HIV makes the article essential reading for those wanting to be up to date with current trends in HIV diagnosis.

Pathology FAQ features NHMRC recommendations for the use of Rh D Immunoglobulin. An article giving more detail of the rationale for these guidelines will appear in future issues of InfoLink.

Ensuring Patient Safety and providing Quality Health Care is often a team effort. On page seven is an article which address just this topic as it relates to one area of pathology.

PaLMS was a sponsor of the recent Australian Institute of Medical Scientists NSW Conference. A number of PaLMS staff participated in the conference in a variety of ways. This included being a member of the organising committee, presenting papers, poster presentations and as delegates. The program was diverse and of high quality. PaLMS was proud to be able to support an organisation that fosters the professional development of medical scientists.

Please contact the Editorial Team if there are topics you would like to see featured in InfoLink.

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Haemochromatosis Community Screening Project

What is it?

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Or phone Dr Lisa Koe on 9926 7453

Laboratory diagnosis for infection with Human Immunodeficiency virus (HIV)

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Introduction

The clinical syndrome of AIDS (Acquired Immune Deficiency Syndrome) was first reported in the USA in 1982. The responsible virus was isolated and sequenced in 1983 and subsequently named HIV (Human Immunodeficiency Virus). A diagram of HIV is shown in Fig 1.¹

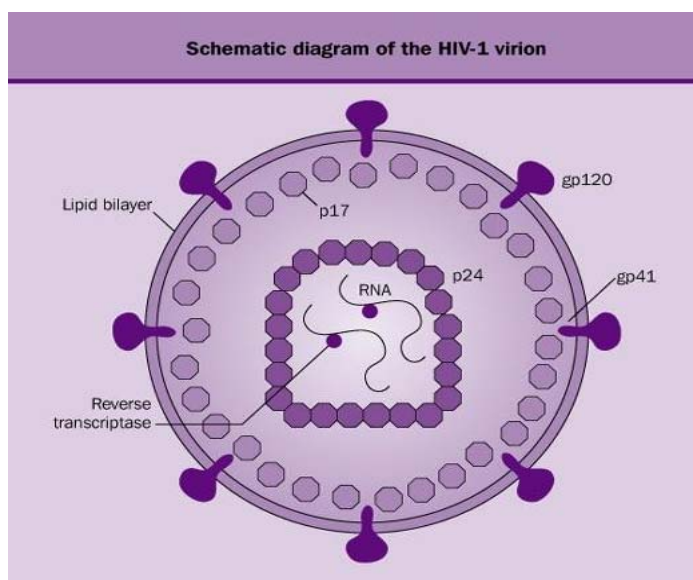


Fig 1. Diagram of HIV. Note p24 core protein and membrane proteins (gp41 etc).

Tests for the detection of antibodies to HIV were developed in 1984 and testing for anti-HIV antibody was introduced across Australia in May 1985. The AIDS Laboratory in the Clinical Immunology Department at Royal North Shore Hospital began routine anti-HIV antibody testing at that time (HIV-antibody screening) and after its incorporation into PaLMS was authorised as a Reference Laboratory. An HIV Reference Laboratory performs additional antibody tests; Western Blot for confirmation of a positive screening test, test for HIV p24 antigen, and measurement of HIV viral load by PCR (Polymerase Chain Reaction) assays.

There have been major changes in the past 18 years in testing for HIV:

- Increase in sensitivity: this is especially important in areas of low prevalence eg NSAHS.
- Decrease in false positive rate: the initial false positive rate was unacceptably high at around 25%, but this was quickly reduced and the current quoted rate is

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- 0.3%.
- Routine tests are mainly based on the ELISA method (Enzyme Linked ImmunoSorbent Assay).
 - The ELISA tests have been automated: PaLMS uses the Abbott AxSYM automated analyser for anti-HIV testing.
 - The tests were adapted to detect both IgM and IgG class antibodies to the 2 main types HIV-1 & HIV-2.
 - Abbott developed a “Combo” assay and since April 2003 we have performed routine anti-HIV testing with this assay which, in a single assay, detects both antibody to HIV and HIV core p24 antigen.

Clinical Status

The general principle that the result of a pathology test should be considered in the clinical setting of the individual patient applies equally to anti-HIV testing.

For many patients the test is now requested as routine in a clinical protocol or trial, eg pre-surgery. In other patients with high-risk activity with possible exposure to HIV, eg unprotected sex, or needlestick injury, it is important to note the time interval between that exposure and the date of collection of the blood specimen. Follow-up tests with counselling are required in such settings, as there may have been insufficient time to develop anti-HIV antibodies (the so called “window period” – see below).

There are 2 important situations in which a negative antibody test may be found in a patient with HIV: initial HIV infection, and terminal AIDS. Terminal AIDS patients should be quite obvious clinically as they are clearly quite ill with opportunistic infections, cancer, and clear evidence of illness. Detection of primary HIV infection has always presented special problems. A primary infection with HIV is generally, but not always, associated with a defined clinical HIV sero-conversion syndrome with fever, sore throat, rash, lymphadenopathy and, frequently, neurological symptoms. Some patients however may remain basically asymptomatic.² The patient is undergoing a primary immune response to HIV at this time and tests for anti-HIV antibody are usually negative for a period of several days to 2 weeks. This period is referred to as the “lag” or “window” period, where antibody is not yet detected but other tests for HIV may be positive, eg HIV p24 antigen, PCR tests, and virus isolation. Specialised tests such as HIV virus isolation are not routinely performed and other approaches have been used to detect such patients. It is important that we detect such patients as blood taken from a patient with primary HIV infection and a negative anti-HIV antibody test is capable of transmitting HIV infection. Tests for anti-HIV in blood donors have been directed to detecting such donors by newer methods, and by increasing sensitivity to shorten the effective “lag period”, ie detect smaller amounts of the “early” IgM-class anti-HIV antibody.

Principles of Anti-HIV Testing in NSAHS

1. All subjects shall receive appropriate pre-and post-test counselling.
2. Relevant clinical information should be included on the request form.
3. The identification details on the request form and the blood specimen must be identical.
4. Each specimen shall be tested according to the current protocol (see Fig 2).
5. The result of the initial screening test shall not be released to the patient, only the final result.
6. The result of the initial screening test may be discussed with the requesting physician if indicated by the clinical setting.

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7. The final report shall include results of all tests performed and the final conclusion.
8. The results shall not be released over the telephone to unauthorised persons.
9. Copies of the final Report shall be sent to other doctor(s) as authorised/requested on the original Test Request Form.
10. A copy of the final Report shall not be sent to any other doctor without specific written

Screening Protocol

We currently use the following protocol for anti-HIV screening/confirmation.

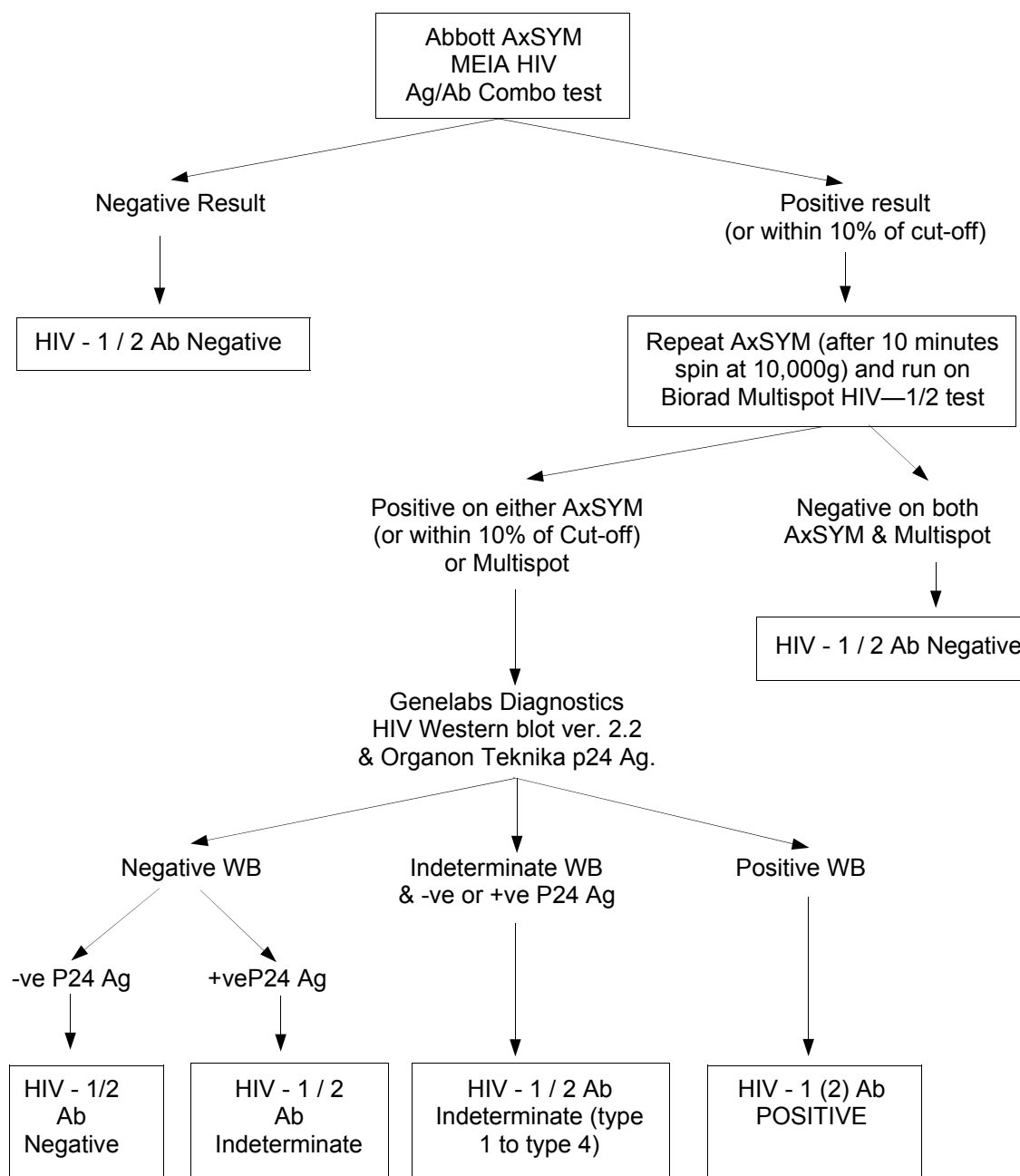


Fig 2. Current HIV Antibody screening protocol

consent from the patient.

11. The HIV Unit and PaLMS provide a 24-hour telephone consultation service on HIV testing.
12. Repeat testing on a new specimen should be performed on each patient diagnosed as “HIV-positive” for the first time.

Tests in the Algorithm/Protocol

1. Combo Test

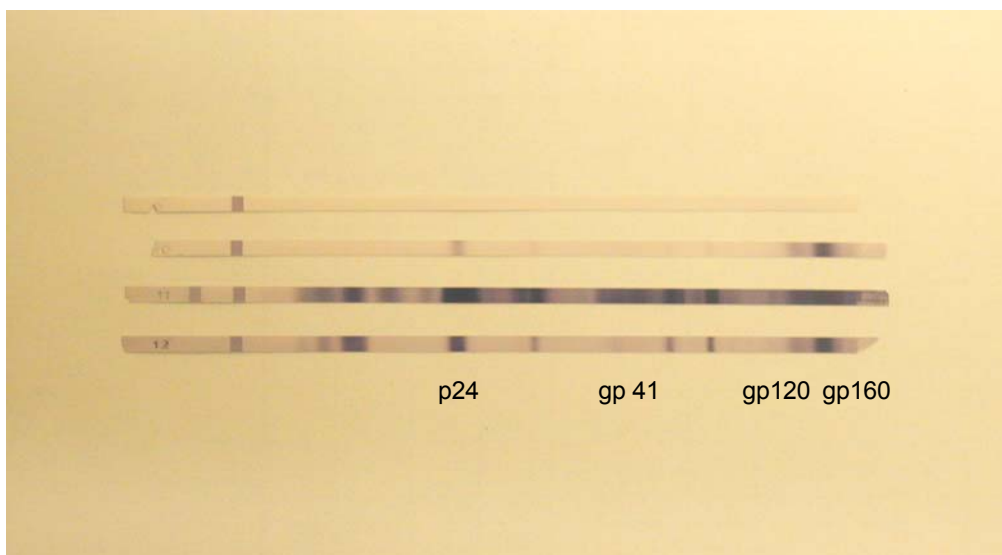
Our current main test is the Combo test on the AxSYM Analyser. This test detects antibodies to HIV-1 and HIV-2, and HIV core p24 antigen.

2. HIV Antigen

Previously we measured HIV p24 antigen by a specialised separate ELISA test. Since the new Combo assay detects HIV antigen at the same sensitivity as the ELISA test, we do not expect to see requests in the future for separate measurement of specific HIV p24 antigen. If a physician is considering such a request please contact the laboratory (Ext 67546).

3. Western Blot

The Western Blot is still considered the “gold standard” for confirmation of the specificity of a “positive” screening test- but it has to be stated it is not “perfect”. It is performed as required on screening positive serum. The test requires overnight incubation to test for the development of positive bands of interaction between specific HIV antigens of known molecular weight pre-loaded on the test strips, and antibodies in the patient’s serum. Any precipitation bands that develop are compared with known standards (Fig 3). If they meet the agreed standards the final result is reported as “HIV-positive”.³



*Fig 3. HIV Ab Western blots, including positioning of important Antigens.
From top: HIV Negative control, HIV-1 weak positive control,
HIV-1 / 2 strong positive control, HIV –1 positive patient*

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Comments on Algorithm/Protocol

- NSAHS is a low prevalence area for HIV and final report is “Negative” in over 99% of requests. Since we run 2 assay runs each day Mon-Fri turnover time is short for over 95% of samples.
- The requesting physician must note the comment on the Report and our earlier comments on “Negative” test results concerning the period between “exposure” and specimen date.
- The confirmatory tests comprise a repeat of the screening test plus a test for antibody by a different type of test plus a Western Blot Assay.
- When a patient shows a positive screening test then the patient shall not be given an official final result until Western Blot has been finalised.

Indeterminate HIV Results

Our biggest problem with Final Reports is the term “**Indeterminate**”. What does it mean? Both the requesting doctor and our computer system dictate that we issue a final report. The serum shows a persistent screening-positive result so we cannot report it as “Negative”. It does not, however, fulfil the criteria to be reported “Positive”, so the term “Indeterminate” was adopted. In a way it is similar to the verdicts given in Scottish courts: “Guilty”, “Not Guilty” and “Not Proven”. Indeterminate specimens can be classified into different “Types” depending on the patterns of bands.

The key is to realise that at this time (ie the date of the specimen), the laboratory is unable to provide a firm diagnosis. For our laboratory and population, most are cases of false-positive reactions eg a cross-reaction between antibody to another infectious agent and the HIV test. Follow-up-generally over a longer period, eg months, produces the final conclusion. The laboratory can give some assistance with discussion but a key factor will be clinical assessment of the patient and this will often involve the HIV Unit and appropriate counselling.

Some of the cases labelled “Indeterminate” are, in fact, examples of early HIV infection in which the patient has not yet developed the full range of anti-HIV antibodies that would generate a “Positive” result. Such patients progress to a Positive result with monitoring and follow-up testing. The time-line for the follow-up tests for patients labelled as “Indeterminate” should be discussed with the laboratory.

HIV Viral Load Testing

Our laboratory routinely measures the HIV Viral Load in known HIV-positive patients as part of the assessment and monitoring of the dynamics between the host immune system and HIV infection. The HIV Viral Load result is used in making the decision whether to start the patient on multi-drug anti-HIV therapy –HAART (Highly Active Anti Retrovirus Therapy).⁴

After the initiation of HAART it is essential that the viral load be measured to assess the response to treatment. A successful response is seen as a significant fall in viral load, with the aim of reducing it to “undetectable”.

The test is performed on the Roche COBAS Amplicor automated system. HIV RNA is extracted from plasma, amplified by PCR, and the level calculated by comparison with an internal standard of known concentration. The result is reported as “Copies of HIV viral RNA/mL plasma” and the lower limit of detection of the Ultra-sensitive assay in use is <50 copies/mL.

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Conclusion

In the 18 years since the first report, HIV infection/AIDS has become installed as a fact of life in our society, and testing for HIV has become a necessity. The quality of the tests has improved remarkably, but the system for "HIV testing" still requires essential interaction between clinicians and the laboratory.

References

1. Rich RR, Fleisher TA, Shearer WT, Kotzin BL, Schroeder HW. Clinical immunology Principles and Practice (Harcourt, London) 2001.
2. Stewart, G. (Ed). Managing HIV (Medical Journal of Australia, Sydney) 1997. Note: A new edition of this publication will be released June 2003.
3. Genelabs Diagnostics HIV Blot 2.2 assay kit insert Sep 1999
4. Sande MA, Volberding PA. The Medical Management of AIDS (WB Saunders, Pennsylvania) 1999.

Pathology F A Q

Following the changes to the guidelines for administration Anti-D there have been many questions asking if there is a table that outlines the appropriate doses.

The answer is yes, see below. In the future of InfoLink there will be a more in depth look at the rationale behind the current guidelines.

NHMRC RECOMMENDATIONS FOR USE OF RH D IMMUNOGLOBULIN

	Reason for administration	Dose / manufacturer
ANTENATAL	Potentially sensitising event up to 12 weeks gestation	CSL Rh D immunoglobulin 250 IU IMI
	Potentially sensitising event S>12 weeks gestation	CSL Rh D immunoglobulin 625 IU IMI
	Antenatal prophylaxis - primigravidae	CSL Rh D immunoglobulin 625 IU IMI (at 28 and 34-36 weeks)
	Antenatal prophylaxis - multiparous	Nil
POSTPARTUM	Rh D positive baby	WinRho 600 IU IMI
	Rh D negative baby	Nil

Send suggestions for FAQs to InfoLink Editor 9926 6395 or Email: mkhardy@doh.health.nsw.gov.au

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