Previous studies have focused predominantly on selection alone rather than on selection with enrichment. As for other bacterial searches, it would be expected that some form of selective enrichment should perform the best. The stringency of search for *B cepacia* is, of course, the driving force for the need for selective or enrichment techniques. For the purposes of antimicrobial chemotherapy, the finding of lesser quantities of *B cepacia* compared to other pseudomonads may not alter a treatment regimen. In contrast, the belief that person to person transmission does occur with reasonable frequency has caused some to believe that absolute knowledge of carrier status is essential.

How should the carrier be defined and will all such carriers confer equal risk for transmission? If the simple identification of *B cepacia* at any site, regardless of quantity, defines the carrier, how many samples (and from which sites) should be acquired? If a carrier is identified, what criteria should be present before the patient ceases to be classified as a carrier? Our experience with the finding of transient oropharyngeal carriage despite the use of selective enrichment in one patient is a good example of a circumstance where the above dilemmas need to be resolved. Transient carriage of *B cepacia* after acquisition from summer camp exposure is known to occur. Although it is sensible to observe good personal and interpersonal hygiene so that respiratory pathogens in general, let alone *B cepacia*, are not

transmitted, the strict definition of a carrier and the social segregation that can arise, may not always be in the best interests of the patient. Studies which examine these issues may be better served by the use of selective enrichment media.

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False negative results in enzyme linked immunosorbent assays using synthetic HIV antigens

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Abstract

The sensitivity of 12 commonly used anti-HIV-1/HIV-2 enzyme immunoassays was evaluated. The assays, each of which utilises at least one synthetic HIV antigen, were tested against a panel of 1092 specimens previously designated anti-HIV positive. In a total of 13 104 tests there were eight false negative results attributable to assay insensitivity: three were on two serum samples collected close to sero-conversion and five on another serum specimen. These eight false negative results arose in seven different assays. Five other false results were attributable to

technical error. This false negativity rate indicates that all of the assays performed adequately and leads to an estimate of one false negative result in a thousand tests in routine diagnostic practice. Because of the antigenic heterogeneity of HIV strains, similar evaluations would be required in several regions before this satisfactory level of sensitivity in anti-HIV assays incorporating synthetic antigens could be said to be universal.

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Keywords: HIV screening, synthetic HIV antigens, false negative results.

Table 1 The 12 anti-HIV-1/2 ELAs studied

Assay No.	Assay name (Product No.)	HIV-1 antigen	HIV-2 antigen
1	Abbot recombinant HIV-1/HIV-2 3rd generation (3A10-24)	Recombinant (gag and env)	Recombinant (env)
2	Behring Enzygnost Anti-HIV-1/-HIV-2 (OWRP)	Peptide (gp41)	Peptide (gp36)
3	Biochrom HIV-1/HIV-2 Elisa Version 2 (D 7651)	Peptide (gp41 and gp120)	Peptide (gp36)
4	Biotest Anti-HIV-1/-2 Recombinant (807005)	Recombinant (p24 and gp41)	Recombinant (gp36)
5	Diagnostics Pasteur Genelabs MIXT (72267)	Recombinant (gp160)/peptide (env)	Peptide (env)
6	IAF Biochem Detect HIV (851403)	Peptide	Peptide
7	Innotest HIV-1/HIV-2 Ab (M422)	Recombinant (gag and env)	Peptide (env)
8	Ortho HIV-1/HIV-2 ELISA (932010)	Virion/recombinant	Peptide (env)
9	Sorin ETI-AB-HIV K (P3076)	Recombinant (p24 and gp41)	Peptide (gp36)
10	UBI HIV-1/2 EIA (6021)	Peptide (env)	Peptide (env)
11	Vironostika HIV MIXT (6005)	Virion	Peptide
12	Wellcozyme HIV 1 + 2 (VK 54)	Recombinant (gag and env)	Peptide (env)

Though false positivity in anti-HIV assays has been extensively studied, little information exists on rates of false negativity. Even in circumstances where the incidence of infection is high, it is rare for more than one anti-HIV assay, or a HIV p24 antigen as well as an anti-HIV assay, to be used and it is vital that the single anti-HIV assay chosen be adequately sensitive.

In recent years, anti-HIV assays based on purified viral lysate antigen have been superseded by assays employing recombinant DNA derived and oligopeptide antigens.¹² These synthetic antigens are safe to manufacture and assays based on them have generally detected anti-HIV earlier in the course of HIV infection than the lysate antigens that they have replaced.¹ They might, however, lack sufficient antigenic diversity to identify all individuals infected with HIV and in that sense be no more sensitive than assays based on native antigens. To test this hypothesis we took over 1000 reportedly anti-HIV positive serum specimens collected in London and screened them by current anti-HIV 1/2 assays incorporating synthetic antigens marketed by 12 kit manufacturers.

Methods

Manufacturers of commonly used anti-HIV kits based on synthetic antigens were invited to submit kits for the evaluation. Twelve anti-HIV-1/2 enzyme immunoassays (EIAs) were included (table 1).

A panel of 1092 serum specimens previously determined to be anti-HIV-1 positive was as-

sembled from specimens received for routine testing by the Westminster Hospital microbiology laboratory, London. Specimens were aliquoted in volumes of about 1 ml into the wells of high capacity 96-well microtitre plates (Beckman 071). The aliquots were stored frozen, in which state they were transported to the Virus Reference Division. They were kept there at -20°C until evaluated. For the nine weeks that it took to test the specimens once by the 12 EIAs the specimens were stored at $+4^{\circ}\text{C}$. No tests were repeated at this stage.

The anti-HIV status of specimens that gave a result in any of the assays discordant with that previously determined by the Westminster Hospital microbiology laboratory was further investigated by western blot (HIV Blot 2.2, Genelabs Diagnostics S.A., Geneva, Switzerland) and "in house" IgG and IgM anti-HIV capture EIAs.^{3,4} In addition, new aliquots of the original specimens were sought for further testing. Each assay was used according to its manufacturer's protocol.

Results

Of the 1092 specimens originally designated anti-HIV-1 positive, 1079 (98.8%) reacted in all 12 assays. The remaining 13 each gave a negative result in one or more assays. For eight of these a fresh aliquot of specimen was available from the Westminster Hospital microbiology laboratory for repeat testing, though only two of these aliquots were sufficient in volume to be retested in all of the assays.

The 13 specimens which gave rise to negative results were categorised, A to D (table 2),

Table 2 Details of patients and HIV results for the 13 serum samples that gave rise to unexpected negative findings in anti HIV-1/2 screening assays

Specimen	Western blot						Negative assays ^c	Clinical status	Exposure group	Comment
	First ^a (gag pol env)	Second ^b (gag pol env)								
A Specimens thought to have given rise to false negative results										
1. 7882	+	—	+	NA			10	No information	Homosexual	IgM anti HIV + + +; seroconversion
2. 9694	tr	tr	—	+	tr	—	3,10	Seroconversion illness	Not known	IgM anti HIV + + +; seroconversion
3. 8518	tr	—	tr	tr	—	+	1,4,7,8,9	“Known HIV positive”	Homosexual	Unreactive in 5 recombinant assays
B Specimens thought to have been incorrectly designated anti-HIV positive										
4. 2864	—	—	—	—	—	—	2,3,5,6,8,10,11	No information	Homosexual	Reactive only in recombinant assays
5. 8797	—	—	—	NA			1–3, 6–12	Asymptomatic	Homosexual	Two assays weakly reactive
C Specimens thought to have deteriorated in storage										
6. 3407	+	tr	+	+	+	+	2,3,6,7,11	Asymptomatic	Bisexual	Stronger reactions on fresh aliquot
7. 8123	+	+	tr	+	+	+	4,8,9	No information	Non-afican female	Stronger reaction on a fresh aliquot
8. 9306	+	+	+	+	+	+	1,3–5,7–10	Asymptomatic	Homosexual	Stronger reactions on fresh aliquot
9. 9869	+	+	+	NA			1,3–5,7–10	“Known HIV positive”	Homosexual	Non-reactive in a wide range of assays
D Negative results thought to have arisen due to technical error										
10. 7334	+	+	+	NA			11	No information	IVDU	
11. 9133	tr	+	+	NA			4	AIDS	Homosexual	
12. 9534	+	+	+	+	+		4	ARC	Homosexual	
13. 9904	+	+	+	+	+	+	1,10	“Known HIV positive”	Homosexual	

^a Western blot result on specimen initially archived; ^b western blot results on fresh aliquot of same specimen; ^c see table 1 for key to identify the EIAs; tr, trace reaction observed; NA, fresh aliquot not available; ARC, AIDS related complex; IVDU, intravenous drug user.

according to the four possible reasons for the failure of assays to give a positive reaction (though in individual cases it cannot be certain why an apparently false negative reaction arose). The categories were:

A—Specimens that revealed true assay insensitivity. Two patients were categorised as seroconverters on the basis of their western blot profile and the presence of strong IgM anti-HIV reactivity. A third was believed to be anti-HIV positive on the basis of western blot reactivity which just met minimum criteria for positivity although some doubt remains about whether this patient was truly infected with HIV. His specimen was unreactive in five assays based mostly on “recombinant” antigens. In the test on specimens from these three patients there were eight (0.061%) false negative results.

B—Specimens thought to have been incorrectly designated anti-HIV positive. Samples from two patients were thus categorised on the basis of western blot analysis. No clinical information was available on one patient; the other was asymptomatic.

C—Specimens thought to have deteriorated in storage. Four specimens were categorised as such on the basis of a substantial increase in the reactivity of western blot and other tests on a fresh aliquot of the same specimen. In some instances the second aliquot was of sufficient volume to be retested by those assays that had been negative and a positive reaction was obtained.

D—Specimens in which negative results were thought to be because of technical error. Five tests, on four specimens, that were initially negative were positive on repeat testing of the original specimen. This suggests that the specimen or one of the reagents had not been added to the test well, although the possibility that the solid phase was not coated with antigen during manufacture could not be ruled out.

Discussion

In this study we investigated the ability of 12 commercial anti-HIV screening assays that use synthetic HIV antigens to detect anti-HIV in 1092 specimens previously found to be anti-HIV positive during routine laboratory diagnosis. There was a high degree of concordance in detecting positive specimens (almost 99% of the positive specimens were detected by all 12 assays) and there was no difference in ability to detect anti-HIV between antigens derived from the expression of recombinant HIV DNA and oligopeptide antigens.

At first, there was at least one apparently false negative result for 13 (1.2%) of the specimens previously reported as anti-HIV positive. However, subsequent testing clarified these findings and indicated that only a few of the false reactions were attributable with certainty to assay insensitivity. Other factors that were or might have been responsible for the appearance of false negativity were: (1) incorrect original designation of the specimen as positive or continuing uncertain serostatus; (2) deterioration of the specimen in storage; and (3) technical error.

If (1) and (2) are excluded as irrelevant to the issue of whether false negative results can occur in routine testing, then the false negativity rate in this study was about 1 per 1000 tests.

A number of lessons can be drawn from this study. Firstly, there is no evidence that assays based on either type of synthetic antigen, recombinant DNA coded polypeptide or synthetic oligopeptide, are consistently less sensitive though there is a suggestion, as previously shown,⁵ that some assays incorporating oligopeptide antigens might be marginally less sensitive at seroconversion. On the other hand, one serum in this study apparently positive on western blot analysis idiosyncratically failed to react in five of the seven assays that incorporated antigens derived from recombinant DNA; one of the other two “recombinant” assays blended oligopeptide with recombinant antigens. Secondly, our study suggests that technical errors may be responsible for as many false negative results as intrinsic assay failures. Thirdly, it should be noted that specimens collected during seroconversion may give rise to negative but, nevertheless, often elevated optical density values. Thus, a “high” negative result in a patient known to be at increased risk should prompt further investigation and, if possible, a request for a follow up specimen. A specimen taken at an interval of a week or more from a subject undergoing seroconversion will usually display positive reactivity. Fourthly, long term storage of specimens at a nominal temperature no lower than -20°C and the need to hold serum samples for several weeks at 4°C during EIA testing, as in this study, may lead to loss of anti-HIV reactivity: fewer false negative results might be expected with fresh specimens such as are routinely tested in clinical and transfusion laboratories. When specimens are stored, regular checks must be made to ensure that freezer temperatures have not risen above -20°C .

In spite of the false negative results and technical errors that were identified in this study, 99% of the positive specimens were anti-HIV reactive by all of the 12 assays. Although their performances may have fallen short of perfection, we conclude that all the assays are sufficiently sensitive for use as screening tests in the population from which these specimens were drawn. However, as our study population was entirely London based and predominantly composed of homosexual men, our results do not rule out the possibility of a higher false negative rate in other locations and risk groups. For these, the restricted number of epitopes represented by HIV antigens synthesised in European and North American laboratories might not always react with antibodies elicited by heterogeneous strains of HIV.⁶

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Anticomplementary activity in serum samples from patients with acute parvovirus B19 infection

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Abstract

Of 65 serum samples submitted for diagnostic purposes which proved to be anticomplementary by complement fixation test, 49 were parvovirus B19 IgM positive. Forty four of the 49 serum samples were from patients with arthropathy. Acute parvovirus B19 infection should be suspected when a patient has symptoms of disease of the joints and the serum is anticomplementary.

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Keywords: Parvovirus B19, immune complexes, arthropathy.

The complement fixation test (CFT) utilises the failure of complement induced lysis of sensitised red blood cells to detect immune complexes formed by the addition of a laboratory "test" antigen to a patient's serum containing specific antibody. CFT remains a widely used method for diagnosing viral and bacterial infections, but preformed immune complexes in a patient's serum may cause an "anti-complementary" effect in serum to which no "test" antigen has been added and render the test indeterminate.

We wish to report that this anticomplementary effect is frequently encountered in acute parvovirus B19 infection. Infection with parvovirus B19 has a spectrum of clinical manifestations and it is likely that the full range of parvovirus B19 disease is still to be resolved.¹ The reticular rash and acute polyarthralgia associated with parvovirus B19 infection are thought to be precipitated by immune complex deposition.² Adult polyarthropathy resulting from parvovirus B19 is easily confused with other symmetrical polyarthropathies.

Methods

Over 15 months, Ashford Public Health Laboratory received 88 serum samples which proved to be anticomplementary during routine CFT examination. To date 43 of these unselected specimens have been examined retro-

spectively for evidence of parvovirus B19 infection. Twenty two anticomplementary serum samples from Norwich Public Health Laboratory (selected from patients with symptoms suggestive of parvovirus B19 infection) were also tested. Sequential serum samples from the same patient were often not anticomplementary after a period of two to three weeks.

Results

Twenty seven of the 43 Ashford serum samples and all 22 Norwich serum samples were parvovirus B19 IgM positive by IgM capture radioimmunoassay.³ All of the samples were parvovirus B19 DNA negative by dot blot hybridisation assay.⁴ One of the 23 positive Ashford and two of the 22 positive Norwich serum samples were also positive for rheumatoid factor. The two rheumatoid factor positive Norwich serum samples were also weakly positive for rubella IgM.

Discussion

The higher proportion of positive results for parvovirus B19 IgM in the Norwich serum samples is probably explained by the fact that they were preselected from patients with symptoms suggestive of parvovirus B19 infection. All had joint involvement and their mean age was 36 years.⁵ Of the 27 of 43 parvovirus B19 IgM positive Ashford serum samples, 22 were from patients with joint involvement (mean age 39 years) but only one of 16 of the parvovirus B19 IgM negative samples were from patients with joint symptoms.

The presence of circulating immune complexes is a feature of parvovirus infections in humans and animals as exemplified by the severe immune complex glomerulonephritis of Aleutian disease in mink.⁶ It is of interest that one of the parvovirus B19 IgM positive patients from Ashford also had glomerulonephritis confirmed by biopsy.

Clinical and laboratory staff should suspect acute parvovirus B19 infection when serum from an adult patient with joint symptoms is

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