HIV antibody assay that gave false negative results: multicentre collaborative study

Barry G Evans, John V Parry, Philip P Mortimer on behalf of the Multicentre Collaborative Study Group

Public Health
Laboratory Service,
London NW9 5EQ
Barry G Evans,
consultant
epidemiologist
John V Parry,
clinical scientist,
grade C
Philip P Mortimer,
consultant virologist

Correspondence to:
Dr Evans
bevans@phls.co.uk

BMJ 1997;315:772-4

Abstract

Objective: To identify false negative results arising from the use of a commercial kit to detect antibody to HIV-1 and HIV-2 between July 1995 and March 1996.

Design: The 56 laboratories in the United Kingdom that were using the assay were asked to retrieve and retest specimens with an alternative assay for HIV-1 and HIV-2. Details of false negative results were obtained and these serum samples further investigated.

Subjects: 24 181 patients tested with the assay who were reported as being negative for HIV antibody. An additional 497 patients were confirmed as HIV positive with the assay.

Results: Serum samples of 20 973 of the patients were retested, and four patients were found to have had false negative results with the kit; three further patients were found to have had false negative results in the course of other laboratory testing. The seven patients with false negative results with the kit were of diverse risk group and HIV-1 subtype. Four had evidence of recent HIV infection.

Conclusion: The commercial kit had a sensitivity of 99.2% (497/501), or less if the additional three patients with false negative results were taken into account.

Introduction

Early in 1995 Abbott Laboratories modified its assay kit for HIV antibody (IMx HIV 1/2 3rd Generation Plus) to increase the sensitivity for the recently described outlier HIV-1 variants, collectively designated subtype O. This assay (code 8B32) was sold in the United Kingdom from July 1995 after it had been evaluated on 785 serum samples containing HIV antibody (S Dooley et al, fifth joint meeting of the European Group for Rapid Viral Diagnosis and the European Society against Virus Diseases, Prague, September 1995) and several panels of sequential specimens from plasma donors undergoing HIV antibody seroconversion.

On 29 March 1996 Abbott wrote to all laboratories that had been supplied with the assay, stating: "Information obtained from users of this product has

identified a small set of high titre positive samples yielding some discrepant results with other assays." Customers were advised to "discontinue use of [the assay] or evaluate each specimen both undiluted and at a 1:4 dilution." This followed a report by a public health laboratory to Abbott on 5 February of a false negative result with the kit and, reputedly, similar findings in France and Spain. At the end of March 1996 it also emerged that two other public health laboratories had just found false negative results with the kit in the course of confirmatory testing for HIV infection. Considerable alarm was created among those who had been screened for HIV infection since the previous July, and an urgent retesting programme was therefore begun at the start of April 1996.

Methods

By 8 April all user laboratories in England and Wales had been sent a questionnaire, and those with stored specimens that had been tested by the assay and found to be negative had agreed either to test them by another assay or to send them to a reference laboratory for retesting. Similar action was taken in Scotland and Northern Ireland.

Telephone contact was maintained to monitor progress, and on 17 and 18 April a second questionnaire was sent by facsimile to determine how many specimens had been found to be positive for HIV antibody with the kit, whether retesting of stored specimens that had negative results with the assay had been completed, and for how many patients stored specimens could not be traced. Laboratories were asked to refer specimens that were previously negative with the kit but positive in another assay to the Virus Reference Division of the Central Public Health Laboratory in London.

Results

An initial analysis was published on 12 April 1996,¹ and this is updated here. Of the 66 laboratories in the United Kingdom cited by Abbott Laboratories as users of its assay, 56 used the kit as their sole screening assay. Twenty eight were NHS laboratories, 18 public health laboratories, and 10 private laboratories. Five laboratories had tested over 1000 patients with the kit; 17 had screened fewer than 100 patients.

Of over 24 000 patients originally found to be negative for HIV antibody with the kit, almost 21 000 had stored specimens retested with an alternative assay (table 1). Four of these patients' specimens were confirmed as positive for HIV antibody.

Clinicians treating these four patients were advised that an erroneous result had been issued. Table 2 shows details of these four patients and of three others whose serum samples had given false negative results

Table 1 Retesting of patients who had been tested for HIV antibody with Abbott assay between July 1995 and March 1996

	Original result with Abbott assay		Retested by another assay	Insufficient serum to retest	Serum not stored
	Negative	Positive			
England	21 114*	484	19 029	189	1412
Scotland	1 314	13	1 294	0	7
Wales	1 455	0	355	0	1100
N Ireland	298	0	395	3	0
Total	24 181*	497	20 973	192	2519

*Four subsequently found to be falsely negative.

in the Abbott assay (all originally tested between January and March 1996). The serum samples available for study from these seven patients were positive in all other screening enzyme immunoassays by which they were tested. One patient (case 1) had serological evidence of recent infection (positive for HIV IgM antibody, with only p24 and p160 bands on western blotting). Another patient (case 3) seemed to have had an acute illness related to HIV infection. Two other patients (cases 5 and 7) were known to have seroconverted within the previous three months. Apart from the specimen in case 1, which was not followed up further, all the specimens with false negative results and further specimens from each of these patients gave strong reactions to all major HIV-1 proteins on western blotting. Serotyping showed the presence of five different subtypes of HIV-1.²

The questionnaires established that 497 confirmed HIV antibody specimens had been identified after screening antibody with the kit between July 1995 and March 1996. This allows the sensitivity of the assay to be estimated as 99.2%—497/501 (497 true positive specimens plus 4 false negative specimens). It would be less if the other three false negative results are taken into consideration.

The specimens of 2519 patients had not been stored (table 1). As many as possible of these patients were recalled, bled, and retested by a different assay.

Discussion

Within three weeks of the defect in the kit being acknowledged by the manufacturer the retesting of over 20 000 specimens had been completed and four negative results retracted. Many more patients were made anxious because few knew which assay had been used at screening. Laboratories and clinics that had not used the test were contacted by concerned patients, and many patients had to be recalled because their serum specimen had not been stored.

The absence of false negative reactions in early trials, the failure of the retesting programme to identify false negative results with the Abbott kit among patients screened between July and December 1995, and the occurrence of all seven false negative results between January and March 1996 suggest that the defect was present only in the batches of the assay distributed in the United Kingdom after January 1996. If so, the working sensitivity of the kit between January and March 1996 was considerably lower than the 99.2% estimate. A previous study of 12 kits assaying antibody to HIV-1 and HIV-2 in specimens in the United Kingdom found that their sensitivities were 99.9% or greater.³

Most of the NHS and public health laboratories had stored all of their patients' serum samples, but less than 30% of the samples tested in private laboratories had been stored. Regrettably, not all of the patients who had been tested by private laboratories could be traced, so their serostatus remains unknown.

We failed to identify any specific feature common to all patients whose specimens gave false negative results with the Abbott kit—for example, low titre of HIV antibody or reactivity to HIV IgM antibody. The samples were of several subtypes, and an unexpectedly high proportion (four of seven) had evidence of recent

Table 2 Laboratory data on seven patients with false negative results in Abbott assay

Case No	Sex	HIV-1 and HIV-2 electroimmunoassay*			Titre (×10 ³)†	V3 peptide serotype
		Biotest	Innotest	GACELISA		
Identified during retesting						
1	Male	2.3‡	13.4§	10.8	26	Untypable
2	Male	8.9	7.3	10.9	400	C
3	Female	9.9	8.2	12.6	1600	D
4	Male	10.6	8.6	11.5	400	A
Other cases						
5	Male	9.9	8.1	7.9	100	E
6	Male	9.0	6.3	10.0	1600	B
7	Male	9.7	10.4	6.4	6	B

*Values are ratios of optical density of test sample to optical density of cut off point, values >1 being positive.

†Modification of the Serodia gelatin particle agglutination test for HIV-1.

‡Murex Wellcozyme HIV 1 + 2 (VK55) assay used.

§Clonesystems Detect-HIV assay used.

infection. Abbott Laboratories have since attributed the defect to the complement activity of some fresh undiluted serum samples. Its modified assay, launched in the United Kingdom in September 1996, incorporates edetic acid (EDTA) in the diluent to inhibit this activity.

This incident has stimulated debate about means of preventing future false negative results in screening assays for HIV antibody. Firstly, before release or modification of such kits, they should be evaluated using many known positive specimens both by the manufacturer and by an independent laboratory. To be confident statistically that the sensitivity of a kit significantly exceeds even the estimated sensitivity of the defective kit described here (99.2%) would, however, entail examining several thousand positive serum samples.

Secondly, specimens from cases in which the clinical suspicion of HIV infection is raised should be tested by two screening assays, not just one. Unfortunately, such a suspicion is not always communicated to the laboratory.

Thirdly, laboratories screening specimens for HIV antibody must retain samples.

Finally, even single HIV assay failures should be reported to the Adverse Incident Centre of the Medical Devices Agency in England (tel: 0171 972 8223 or 8273; fax: 0171 972 8113) or to the Adverse Incident Centres in the health departments in Northern

Key messages

- Dual screening should be considered for patients known to be at increased risk of HIV infection
- Although assay kits for HIV antibody generally show high sensitivity, false negative results may still arise during routine use and go undetected
- Tested serum samples should be stored so that they may, if necessary, be checked.
- Any kit failures should be reported to the Medical Devices Agency or Adverse Incident Centres, as well as to the manufacturer
- All laboratories should be informed of test kit failures, whether or not they have used the suspect kit

Ireland, Scotland, or Wales, as well as to the manufacturer. Then, if a pattern of false reactivity emerges, rapid action can be taken.

This incident shook public confidence in HIV testing, which is expected to be virtually 100% sensitive. Vigilance is needed to avoid false negative results.

K Osborne and J McMenamin coordinated the data collection and helped in drafting the report. The retesting exercise relied on the excellent cooperation of many epidemiologists and laboratory scientists throughout England, Wales, Scotland, and Northern Ireland. J Booth, I Chrystie, P Morgan-Capner, E M Ndawula, J Paul, G Underhill, and M Zuckerman kindly provided specimens and results for further analysis. B Davis, D

Goldberg, V King, and C Roberts also gave valuable personal time to the exercise. C-P Pau generously supplied reagents for HIV serotyping.

Funding: No additional funding.

Conflict of interest: None.

- 1 Anonymous. Retesting of sera for antibodies to HIV-1/HIV-2. *Commun Dis Rep CDR Wkly* 1996;6:125.
- 2 Pau C-P, Kai M, Holloman-Candal DL, Luo C-C, Kalish ML, Schochetman G, et al. Antigenic variation and serotyping of HIV type 1 from four World Health Organisation-sponsored HIV vaccine sites. *AIDS Res Hum Retroviruses* 1994;10:1369-77.
- 3 McAlpine L, Parry JV, Shanson D, Mortimer PP. False negative results in ELISA using synthetic HIV antigens. *J Clin Pathol* 1995;48:490-3.

(Accepted 14 March 1997)

Epidemiology and clinical management of meningococcal disease in west Gloucestershire: retrospective, population based study

Philip AL Wylie, David Stevens, William Drake III, James Stuart, Keith Cartwright

See editorial by
Cartwright

Paediatric
Department,
Gloucestershire
Royal Hospital,
Gloucester
GL1 3NN
Philip AL Wylie,
senior registrar
William Drake III,
senior registrar
David Stevens,
consultant
paediatrician

Public Health
Laboratory,
Gloucestershire
Royal Hospital
James Stuart,
regional
epidemiologist
Keith Cartwright,
consultant
microbiologist

Correspondence to:
Dr Cartwright
kcartwright@phls.
co.uk

BMJ 1997;315:774-9

Abstract

Objective: To study changes in the epidemiology and management of meningococcal disease in one health district during a period of high local incidence of disease.

Design: Prospective case ascertainment and data collection over 14 years, with retrospective analysis of cases.

Setting: West Gloucestershire (population 320 000).

Subjects: Residents developing meningococcal disease between 1 January 1982 and 31 December 1995.

Results: 252 cases of invasive meningococcal disease were identified, of which 102 (40%) were officially notified and 191 (76%) were confirmed by culture from a deep site. The observed disease incidence of 5.6/100 000/year was about 2.7 times the national incidence (as measured by either statutory notifications or reference laboratory reports). The period 1983-90 was characterised by a prolonged localised outbreak due to serogroup B serotype 15 sulphonamide resistant (B15R) strains. General practitioners gave benzylpenicillin before hospital admission to 18% of patients who presented with meningococcal disease in the first half of the study period and to 40% who presented in the second half. The overall case fatality rate was 6.7% (17/252). Four deaths were directly or indirectly related to lumbar puncture. Of 120 patients whose lumbar puncture yielded meningococci, nine (8%) showed no abnormality on initial examination.

Conclusions: Neither laboratory records nor formal notifications alone can give an accurate estimate of the incidence of meningococcal disease. Because of the dangers of lumbar puncture, the frequency of misleading negative initial findings, and the advent of new diagnostic techniques, the need for samples of cerebrospinal fluid should be critically questioned in each case of suspected meningococcal disease.

Introduction

The two principal sources of data on the incidence of meningococcal disease in England and Wales are notifications of meningococcal meningitis and septicaemia to the Office of National Statistics (formerly the Office of Population Censuses and Surveys) and isolates and samples from clinical cases submitted to the England and Wales Meningococcal Reference Unit at Manchester Public Health Laboratory. After a peak in 1974, the annual incidence of meningococcal disease declined steadily in England and Wales until the mid-1980s, and then rose again.¹ Between 1990 and 1994 the annual incidence of meningococcal disease remained at a higher level of two to three cases per 100 000 population.²

A prolonged outbreak of meningococcal disease caused mainly by B15R (serogroup B serotype 15 sulphonamide resistant) strains began in west Gloucestershire in 1982.³ Gloucester Health Authority and Gloucester Public Health Laboratory coordinated the public health response. An enhanced surveillance scheme was established to ascertain all suspected and confirmed cases. Considerable local efforts were made to inform the public and healthcare providers about meningococcal disease and, from 1986 onwards, to encourage general practitioners to give parenteral benzylpenicillin to all patients suspected of having the disease before hospital admission. This same advice was promulgated to all doctors in the United Kingdom by the chief medical officer in 1988.⁴

We reviewed changes in the epidemiology and clinical management of meningococcal disease in west Gloucestershire during the 14 years to 31 December 1995.

Subjects and methods

Clinical and laboratory definitions

All patients with meningococcal disease were permanent or temporary residents of west Gloucestershire at