AIDS SCIENTIFIC AND TECHNICAL WORKING GROUP: NOTES OF A MEETING ON JULY 18TH 1986 AT NIBSC.

There was considerable overlap between the presentations of the individual speakers; as a result the notes are, whenever possible, arranged by subject matter rather than by speaker.

1. Introduction.

Representatives from the Central Public Health Laboratory, Colindale, were present at the meeting for the first time (John Parry, William Jessen and Tony Taylor). Representatives from the Medicines Inspectorate will be present at future meetings.

2. Tests for HIV antibody at NIBSC.

a) Samples are initially tested by an ELISA method (now almost exclusively by the Wellcome method particularly for immunoglobulin preparations for which the indirect methods are insuitable; the Du Pont kit may be used on occasion). ELISA positive (+ve) and equivocal samples are tested by immunoblot (IB). ELISA negative (-ve) samples are not routinely tested by IB but of those tested so far none have proved to be +ve.

b) Immunoglobulin products tested at NIBSC: January to July 1986.

		ELISA		IB
Manufacturer	No. of samples	+	±	+
A	31	1	13	0
B	31	2	0	2
C	9	1	2	1
D	9	1	2	1
E	3	1	0	1
F	1	1	0	1
G	1	0	0	0
H	1	0	0	0

Three batches of IB +ve immunoglobulin have been detected at NIBSC since the last meeting of the working group.

i) PFC IV Ig: this was ELISA -ve when tested at PFC but was sufficiently near the cut-off point to cause concern. The source plasma (>4000 donations) was not screened for HIV antibody. A single HIV antibody +ve donation was later reported to have been included in the pool.

ii) Commercial Im Ig: the manufacturers of this product contracted out their HIV antibody tests. The sample was repeatedly -ve when tested by the contractors (method not known). The manufacturers subsequently confirmed it was +ve by 'in-house' testing.

iii) Commercial IVIg (modified): this was -ve when tested by the manufacturer.

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Other immunoglobulin preparations of interest were:

iv) Two batches of BPL Ig (batches GG288 and GD111B) gave single bands at about 65K in IBs of both HIV infected H9 cells and uninfected H9 cells. The protein that the antibody is directed against has not been identified; it is not albumin but may be an antigen present in the cytoplasmic membrane of H9 cell lines. Various T and B cells are being examined to determine whether the antibody binds to these.

v) Two batches of CMV specific IvIg from PFC gave atypical ELISA results (-ve but clearly 'more +ve' than normal batches). The batches are IB -ve at NIBSC. The immunoglobulin was prepared from BPL source plasma.

c) Factor VIII (F8) and Factor IX (F9): in the period January to July 1986 no HIV antibody +ve F8 or F9 preparation was found. Of stored batches, retrospectively tested, one batch is strongly +ve by ELISA and will be checked by IB.

The phenomenon of elevated absorbance values given by F8 in the Wellcome ELISA test was discussed. TJS reported that at BPL any fibrinogen containing samples were examined by an indirect ELISA rather than the Wellcome test. Similar elevated absorbance values were reported by PFC and NIBSC. Unlike BPL, NIBSC do not find that F8 masks the presence of HIV specific antibody when the product is spiked. However due to the considerable differences in the purity and formulation of F8 preparations from different manufacturers, it is possible that the 'masking' may not occur for all products. The considerable difference in the IgG content of F8 from various manufacturers was considered a factor that would influence the likelihood of detecting +ve batches.

d) Assessment of antibody detection methods.

i) The need to standardise the immunoblot method and its interpretation more carefully was discussed. Various reports have been published of the need to detect certain bands in immunoblots before they can be considered to be +ve. At NIBSC, +ve samples have always given at least 2 bands, usually the p24 core protein and its precursor p55; NIBSC use a cell lysate antigen which contains more p55 than purified virus preparations. Strong +ve samples may be diluted by up to 1/10,000 and still give detectable bands in IB; there is an indication that the antibody to glycoproteins gp41, gp120, gp160 dilute out more quickly than antibody to the core proteins. It was reported that at NIBSC, IB +ve samples have been detected that do not have antibody to gp41 (contradicts other published claims). CDC now accept the presence of a single band to indicate a +ve IB. The problems in the use of streptavidin/biotin systems to enhance the sensitivity of IBs were discussed; in addition to the specific binding to biotin streptavidin can bind to many proteins giving artefactual bands.

ii) ELISA methods: NIBSC have found the Wellcome, Du Pont and Abbott tests to be of about equal sensitivity; the Organon test is less sensitive. The Abbott and Organon tests gave a higher rate of false +ves particularly for stored sera.

At Colindale the new Ortho test kit and the rapid Pasteur kit have been partially evaluated. The Abbott confirmatory test has yet to be examined but the supporting literature is reported to be impressive.

iii) Other methods.

Both NIBSC and Colindale have evaluated the Karpas immunoperoxidase slide test for HIV antibody. Both found the interpretation of results to be subjective. NIBSC did not use the original Karpas cells for the test.

A Japanese gelatine agglutination test kit has been checked at NIBSC; equivocal results were obtained.

iv) Interpretation of results from different methods.

The relative sensitivity of ELISA methods and modifications of the immunoblot technique vary. An unpublished observation from Perth, Australia, was reported. A blood donor was found to be HIV antibody positive by an ELISA method. At a subsequent donation the individual was -ve by a different ELISA method but positive by IB. There are other cases of ELISA -ve, IB +ve sera. Conversely cases of IB -ve, ELISA +ve individuals have been reported from whom HIV has been isolated. A recent case of a blood donor who was HIV antibody -ve, but who transmitted HIV to the recipients of his blood has been reported. Colindale reported observations on sera that were ELISA -ve, IB +ve but IgM +ve as measured by an 'in-house' RIA. The role of IgM detection during the 'window' before a significant IgG response is observed was briefly discussed. NIBSC have attempted an IB using detection of IgM rather than IgG but with little success; the lack of IgM in the samples tested may be partly to blame.

v) WHO studies organised by NIBSC.

In an initial study 26 participating centres are testing a panel of 7 sera distributed by NIBSC for HIV antibody by an 'in-house' IB method and a commercial ELISA kit. In a second study being organised, standard HIV antigen (type 1 and possibly type 2 in the future) will be distributed with standard sera and immunoglobulin preparations for comparative immunoblots.

3. <u>HIV antibody screening within the NBTS and PHLS (Alyson Smithies)</u>.

a) Summary of tests by BTS.

October 1985 to May 1986.

UK & Eire 1,706,580 tests; 39 +ve (3 female)
UK only 1,696,348 tests; 39 +ve (3 female)

6959 samples were initiallty equivocal of which 642 gave repeatedly equivocal results.

Since February 1986 old and new donors have been analysed separately. Of 141,293 new donors 7 were confirmed HIV antibody +ve. This is a rate of 1 in 20,000 (0.005%) compared with 1 in 43,000 (0.002%) for all donors.

Methods used: at present 91.3% of tests are done by the Wellcome ELISA method. This is a significant change since April 1986; up to this time 40% of tests were by the Organon ELISA kit.

b) Evaluation of current screening methods.

The almost exclusive use of a single screening method is a possible cause for concern. Various comparative studies have been set up to monitor the sensitivity of the Wellcome assay. In addition Dr. Tedder's laboratory is acting as a final confirmatory centre for the NBTS and PHLS. ELISA +ve and equivocal samples (all samples within 20% of the cut-off point) are checked. In addition 'super-negative' samples (see below) and high risk samples (HBsAg +ve but HIV antibody -ve) are also checked. The confirmatory tests are 'in-house' competitive radioimmunoassay and antigen (?) capture assays, the Du Pont ELISA kit and immunoblotting. The inclusion of this extended sample range should help to determine whether the Wellcome test under-reports +ve samples.

c) Interpreation of ELISA results.

The working group was reminded of the need to make use of the distribtuion of absorbance values of HIV antibody negative samples when interpreting the results of ELISA tests rather than just relying on the manufacturers calculated cut-off point. A computer programme allowing such an examination is in use at the Edinburgh RTC.

d) 'Super-negative' samples.

The existence of serum samples giving abnormally high absorbance values in competitive immunoassays (mainly the Wellcome ELISA test but to lesser extent competitive RIA) was reported by Colindale. These samples have the ability to mask known HIV antibody positive samples in pools. A suggested explanation for this phenomenon was the non-specific cross-linking of the competing antibody to the antigen.

4. Provision of HIV antibody +ve sera for laboratory use.

The difficulty in obtaining adequate quantities of material for use in the establishment and standardisation of laboratory methods was discussed. It was reported that the PHLS should receive samples for these purposes. Two antibody +ve donors from Manchester are donating plasma/serum for this purpose.

5. Laboratory Reagents.

A circular is to be issued shortly by the DHSS advising that laboratories purchase and use reagents and reference preparations that have been screened for HIV antibody at source. An acceptable alternative is the heat treatment of the reagents.

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6. Infectious hazards of immunoglobulin prepared from pool containing donation from an individual having a soft-tissue sarcoma.

Dr. Lane sought views and advice on a batch of anti-D fraction II which could be at risk due to possible viruses causing soft-tissue sarcoma. It was generally agreed that: there is no evidence for a retroviral aetiology of soft-tissue sarcoma; 'normal' plasma pools probably routinely contain donations from individuals with undiagnosed malignancies; and the routine fractionation procedure probably partitions or inactivates any potential retroviruses. However no firm advice was offered other than to test the material for HIV antibody, for reverse transcriptase activity, to attempt virus isolations and to seek further advice from the CSM (B). The source plasma of this material is untested for HIV antibody. Although it should be possible to trace most of the donors and assess their antibody status 100% follow-up is very unlikely.

7. Anti-HIV testing of source plasma : CSM recommendations.

Although it was indicated that the CSM(B) recommendations were aimed at the commercial manufacturers it was generally felt that BPL and PFC would have no option but to follow the recommendations.

8. Additional virus implicated in AIDS.

Dr. Schild reported unconfirmed rumours that a large parainfluenza type virus has been repeatedly isolated from individuals suffering from neurological complications and Kaposis sarcoma associated with AIDS (US military).

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