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**A.I.D.S. CONFERENCE**

**NEWCASTLE**

**11TH-13TH FEBRUARY, 1986**

**THE BLOOD TRANSFUSION SERVICE**

by

**Regional Blood Transfusion Centre,  
Plymouth Grove,  
Manchester,  
M13 9LL.**

There is convincing evidence available from studies involving donor-recipient pairs that the HTLV III/LAV virus can be transmitted by the transfusion of infected blood and blood products (1, 2, 3). The first case of transfusion associated A.I.D.S. was reported in an infant transfused in 1982 (4) and up to August, 1985, 194 cases had been reported to the Centre for Diseases Control in Atlanta, U.S.A. (5). In the U.S.A. approximately three million transfusions are performed each year so that the incidence of disease following transfusion is low; however the transmission of the virus may be much higher and with the long incubation period for A.I.D.S. it may still present a significant problem for the future.

Although the comparable figure for the U.K. is <sup>five</sup> ~~four~~ cases, two of whom have received their transfusions abroad, the Blood Transfusion Service in the United Kingdom could not be complacent with respect to the transmission of the A.I.D.S. virus from the transfusion of red cells, and products manufactured at Regional Blood Transfusion Centres. Such products, of course, cannot be heat-treated in the manner found to be satisfactory for fractionated coagulation factor products and albumin.

An immediate priority for the Blood Transfusion Service was to discourage those donors who belonged to groups of people who were particularly susceptible to the development of A.I.D.S., viz: homosexual men who have many different partners, drug addicts - male and female, using injections and sexual contacts of persons suffering from A.I.D.S. At this time, of course, there was no screening test to detect persons who had been exposed to the causative virus for A.I.D.S.

A pamphlet was issued in September, 1983, by the Department of Health and Social Security and distributed to potential blood donors and, coincidentally,

a health education campaign was undertaken which stressed the dangers of the transmission of the virus causing A.I.D.S.

During 1984, the causative virus for A.I.D.S. was recognised as the retrovirus termed "human T-cell retrovirus, Type III (HTLV III)" or "lymphadenopathy associated virus (LAV)" (6, 7) and a significant correlation between the antibody developed to HTLV III/LAV with patients suffering from A.I.D.S. was found (8). Various commercial companies began the process of developing a suitable screening test for anti-HTLV III/LAV and the first test systems became available in March, 1985.

Further information became available about A.I.D.S. and in January, 1985, it was necessary to issue a second leaflet to blood donors with a widened range of persons at risk from A.I.D.S., viz: practising homosexual and bisexual men, drug abusers - both men and women, who inject drugs and sexual contacts of people in these groups. It was also stated that A.I.D.S. had also occurred in a number of haemophilic patients who are treated with blood products and that there was evidence that those people who had lived in Haiti or Central Africa, particularly Zaire and Chad may be at risk from A.I.D.S.

Persons in the above risk groups were requested not to donate blood, and positive action was taken by ensuring that each potential donor received a copy of the leaflet and they were asked prior to donation whether they had read the leaflet. By 1985, there was evidence that certain donors were concerned that they could develop A.I.D.S. by giving blood and in order to counter this potential for reduction in donor recruitment a statement to the effect that it was not possible for this to occur was included within the leaflet.

It would have been reasonable to expect that when the test for the detection of anti-HTLV III/LAV was available, problems for the Blood Transfusion Service throughout the world would be greatly reduced, since the elimination of potentially infectious donations could be achieved. In the event the advent of ELISA screening tests for anti-HTLV III/LAV on blood donations raised further problems both of an ethical and practical nature.

Several aspects of the use of the screening tests had to be considered.

(1) Specificity, sensitivity and reproducibility of the test

Development of the ELISA test for anti-HTLV III/LAV was carried out in the U.S.A. initially, and the first tests licensed by the Food and Drugs Administration had impressive sensitivity and specificity. Sensitivity was between 93 and 99 per cent and specificity greater than 99 per cent. It must be stressed that at this time the prevalence of anti-HTLV III in the donor population was unknown but theoretical estimates, based on a prevalence of 1 in 1000, indicated that 68-89 per cent of all repeatedly positive donations were likely to be false positives (9).

Concern was expressed on two counts by several major blood centres in the U.S.A.; the withdrawal of the positive anti-HTLV III/LAV reactive donations would actually lead to blood shortages and how, under such circumstances, could donors be properly advised with respect to their future life-style. Also the effect on donor recruitment could be serious; if potential donors were aware of a significant false positive rate in the tests some may be reluctant to come forward to donate their blood.

It was important, therefore, that highly specific confirmatory tests were available within a relatively short time after routine screening of blood donations was undertaken so that false positive reactive tests on donors could be identified and the donor recalled for a further donation. Critics of this policy who argued that, at least, positive reacting donations could be eliminated did not recognise the practical problems involved in the avoidance of further blood collections from donors without advising them of the reasons for not requiring their blood donations in the future.

(2) The possibility of false negative anti-HTLV III/LAV tests

Despite the publicity by the Blood Transfusion Service to discourage persons in high risk groups not to present as donors, it was feared that some persons in these groups would present as blood donors as a convenient and anonymous way to find out whether they were anti-HTLV III/LAV positive. Whilst the blood of such persons may well have been eliminated by performing the screening tests there was the danger that a false negative result would be obtained from an infectious donor. This would have made the safety of blood donations less secure if persons in high risk groups, who had refrained from donating blood whilst the test was not available, now presented as blood donors.

It was important, therefore, to ensure that alternative test venues were made available for persons, other than blood donors, who wished to know their anti-HTLV III/LAV status.

(3) Information given to blood donors

Many tests are carried out on blood donations; these include blood grouping, antibody investigations, tests for hepatitis B surface antigen and syphilis. These tests have been recognised by the profession as necessary and donors were not informed prior to donation of the nature of the tests; although they were aware that tests were carried out on their blood.

However, the significance for the donor of a positive anti-HTLV III/LAV test was such that it was agreed that it was essential that the donor should be informed that the anti-HTLV III/LAV test was to be performed on their donation and that their agreement should be sought prior to their donation.

This has been achieved in the U.K. by the issue of a third leaflet in September, 1985. In addition to the previous requests to donors in high-risk groups to refrain from donating blood, donors were informed that their blood would be tested for the antibody to A.I.D.S. and that they would be asked at sessions to agree to this test being performed; this is obtained by asking the donor to sign a statement indicating his/her consent.

It was recognised that concern that the test was to be performed may discourage some persons from donating and the effect on recruitment would have to be observed closely.

During the period between March and October, 1985, a succession of events took place which were considered to be essential pre-requisites for the introduction of the screening tests in the Blood-Transfusion Services of the U.K. Thus:

- (1) An evaluation of the available test systems for anti-HTLV III screening was undertaken by the Public Health Service Central Laboratory. From this, three tests emerged as the most suitable for screening blood donations in the U.K. Transfusion Centres.
- (2) Of these three, two test systems, Organon-Teknika and Burroughs-Wellcome were subjected to an evaluation within the Blood Transfusion Service at the Regional Transfusion Centres in Manchester and Edgware. This study revealed several interesting findings and is still to be finalised and detailed results cannot yet be presented. However, it was found that both tests were suitable for use in Regional Transfusion Centres.
- (3) Arrangements were made with the Public Health Laboratory Service for the performance of confirmatory tests on repeatable positive reactors found by anti-HTLV III/LAV screening of blood donations.
- (4) It was agreed that the initial counselling of blood donors whose confirmatory test was positive would be carried out by

senior medical staff in Regional Transfusion Centres and these persons attended training courses at St. Mary's Hospital, Paddington.

- (5) Alternative testing venues were established by Regional Health Authorities so that tests were available for members of the public other than blood donors.
- (6) A training programme for the scientific and technical staff in Regional Transfusion Centres was commenced, so that on the nominated day of 14th October, 1985, all Centres began routine testing of all blood donations.

Of the 21 Centres where anti-HTLV III/LAV screening of blood donations was being performed, initially 16 elected to use the Burroughs-Wellcome test and 5 the Organon-Teknika test. Before presenting a summary of the results for the U.K. from the first three months of testing, it is useful to review briefly the experiences obtained using the Organon test at my own Centre during this period. I should like, at this stage, to express my gratitude to Principal Scientific Officer at the Manchester Regional Transfusion Centre for providing me with statistical data which I will present.

The manufacturer provides control antisera which are used to calculate the cut-off value for each micro-titre plate of 90 test sera. Whilst the great majority of donor sera give O.D. values significantly below the cut-off value, with an occasional result clearly above, others may give O.D. values below the cut-off but clearly higher from the values of



the majority of negative results. A "Low Positive" control supplied by the Central Laboratory of the Public Health Laboratory Service gave O.D. values close to the manufacturer's cut-off value. On some plates this was above and on others below the cut-off.

Since it was known that this serum contained anti-HTLV III, it seemed prudent, therefore, to regard certain results where the O.D. was less than that of the cut-off value as equivocal, since on repeat testing a proportion of these were found to be positive, i.e. giving an O.D. value above that of the cut-off. Table 1 shows an analysis of the equivocal results obtained in the performance of 33,608 tests, divided into 10 per cent steps to an O.D. value of 50 per cent below that of the cut-off. It can be seen that the number of results increases the further the O.D. values are from the cut-off, but even at the 50 per cent level, on repeat tests 7 per cent of the results were found to be positive. The percentage of tests which were either positive or equivocal on repeat testing tends to rise the nearer the initial result was to the cut-off value.

If one accepted that confirmatory tests should be performed on the 159 of the 396 units of blood which were found either positive or equivocal on repeat tests, this would mean that almost 0.5 per cent of the total blood collected would have to be quarantined. This is in addition to those units found to be positive on the initial screen and subsequently repeatably positive. Not only does this create a considerable workload, but with short-dated products such as platelet concentrates wastage is caused. at the Public Health Service Laboratory, Manchester, carried out confirmatory tests on over

300 of these equivocal results and found them to be uniformly negative. It was concluded, therefore, that these were not true positives and a value of less than 20 per cent of the cut-off was chosen to define an equivocal result, since this covered the majority of the results obtained with the P.H.L.S. "LOW POSITIVE" control serum.

There was a batch variation in the finding of positive and equivocal results as shown in Table 2 where the number of tests found to be positive on initial screening tests varied from 0.04% to 0.57% in the six batches used. A smaller variation in the equivocal results was observed.

Experience with the Burroughs-Wellcome test has been more limited at the Manchester Regional Transfusion Centre and comments on possible differences between batches cannot be made. However, using a value of 10 per cent above the cut-off value, to define an equivocal result, a proportion of repeat tests have proved to be positive. The findings described with respect to the Organon test, therefore, are not confined to it alone, and a degree of variation has to be expected with the current ELISA tests for anti-HTLV III/LAV.

An analysis of the anti-HTLV III/LAV tests carried out on blood donations throughout the U.K. to the end of December, 1985, is shown in Table 3. These have been compiled with the co-operation of the Regional Transfusion Directors who have sent their results to the Manchester R.T.C. for collation. It can be seen that many more tests have been carried out using the Burroughs-Wellcome kit, and with this test it is

interesting to note that on initial screening the total number of positive and equivocal results have decreased during the period October to December. One can only speculate on the reason for this; it may be that with time, experience in performing the test has increased, leading to better definition of results or it may be related to batch number although this could not be proven since there are a large number of batches involved. Also, during this period changes in the cut-off value were made by the manufacturer.

With the Organon test, the percentage of positive and equivocal results has not changed markedly during the period.

Despite the changes in the percentage of initial positive or equivocal results with the Burroughs-Wellcome test, the percentage of repeatable positives/equivocal results has remained similar in the three months. The number of repeatable positive/equivocal results with the Organon test is more variable and consistently higher than with the Wellcome test. This may be explained, at least in part, by the fact that the Organon test will give repeatable positives with anti-lymphocytic antibodies which are not detected by the Wellcome test.

Thirteen positive results have been confirmed, giving an overall incidence in the U.K. of 0.002 per cent or 1 in 45,731, although there are regional differences. Three of the 13 confirmed positives were detected by the Organon test (i.e. 23%) which correlates reasonably well with the proportion of the total donations tested with this system (i.e. 32%). At the initial interview, at least 10 of the 13 donors with confirmed positive tests fall into recognised high risk groups and one

was a donor from Sub-Saharan Africa. The frequency of positive results is much lower than those reported from the U.S.A.; Schorr et al (10) reported that in screening 1.028 million American Red Cross blood donors for anti-HTLV III, the initial positive reactivity was 1.0 per cent, repeat positives 0.17 per cent and the projected prevalence of confirmed positives by Western blot was 0.038% (1 in 2600). Thirty-six of the first 41 donors interviewed were in high-risk groups. It may be that the prevalence of HTLV III infectivity is higher in the U.S.A. than the U.K., and in a recent report it is estimated that there may be 1.7 million adults in the U.S.A. who have been exposed to the virus, including 64,000 not belonging to an identified risk group (11), but perhaps, also, our publicity designed to discourage donors in high risk groups has been particularly effective.

I am sure that now anti-HTLV III/LAV testing is included as one of our routine screening tests for blood donations, everyone is relieved in that we recognise that we are doing the utmost to secure a supply of blood and products from R.T.C.'s with the maximum safety with respect to the transmission of the A.I.D.S virus. The introduction of testing has not, as far as one can tell, had a significant effect on the number of donors coming forward to give blood; and donors can be reassured that if a positive result is found on their blood donation they will be treated in a confidential and sympathetic manner and given as much help as possible from the Senior Medical Staff in the Blood Transfusion Service.

MANCHESTER R.T.C.ANALYSIS OF EQUIVOCAL RESULTS - ORGANON TEST

NO. OF TESTS = 33608  
 NO. OF EQUIVOCAL RESULTS = 396 (1.18%)

% CUT-OFF O.D. VALUE	NUMBER	%	REPEAT TESTS		
			EQ. %	POS. %	NEG. %
50	198	(50)	45 (23)	14 (7)	139 (70)
51-60	66	(17)	24 (36)	8 (12)	34 (52)
61-70	53	(13)	15 (28)	4 (8)	34 (64)
71-80	42	(11)	16 (38)	6 (14)	20 (48)
81-90	23	(6)	8 (34)	5 (22)	10 (43)
91-100	14	(4)	6 (43)	0	8 (57)

TABLE 1

MANCHESTER R.T.C.POSITIVE AND EQUIVOCAL RESULTS ON INITIAL SCREEN  
(ORGANON TEST)

BATCH NO.	NUMBER TESTED	NUMBER POSITIVE	%	NUMBER EQUIVOCAL	* %
239	7509	3	(.04)	3	(.04)
260	7201	15	(.21)	10	(.14)
272	5728	22	(.38)	6	(.10)
268	7246	14	(.19)	7	(.10)
306	5924	34	(.57)	11	(.19)
298	563	2	(.36)	1	(.18)
TOTAL	34171	90	(.26)	38	(.11)

1 CONFIRMED POSITIVE

\* Defined as within 20% of the cut-off value

TABLE 2

ANALYSIS OF ANTI-HTLV III RESULTS FOR U.K.

TEST KIT	OCTOBER	NOVEMBER	DECEMBER
WELLCOME			
TOTAL NO. OF TESTS	140351	162279	147031
NO. POS./EQ. RESULTS	1878 (1.3%)	925 (.57%)	355 (.24%)
NO. REPEATABLE POS./ EQ. RESULTS	20 (.014%)	20 (.012%)	18 (.01%)
ORGANON			
TOTAL NO. OF TESTS	50207	48816	45830
NO. POS/EQ. RESULTS	221 (.44%)	336 (.69%)	204 (.44%)
NO. REPEATABLE POS./ EQ. RESULTS.	54 (.1%)	81 (.16%)	26 (.06%)
TOTAL NO. OF TESTS	190558	211095	192861
NO. OF CONFIRMED POS.	3	3	7

SUMMARY: 13 CONFIRMED POSITIVES IN 594514 TESTS, i.e. 0.002% (1 in 45731)

TABLE 3

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