DEPARTMENT OF HEALTH & SOCIAL SECURITY

PUBLIC HEALTH LABORATORY SERVICE AND DEPARTMENT OF HEALTH AND SOCIAL SECURITY
EVALUATION OF FIVE COMMERCIAL ANTI-HTLV III/LAV ASSAY KITS

HTLV III EIA
Virgo HTLV-III ELISA
Vironostika Anti-HTLV III
HTLV III BioEnzaBead
Wellcozyme anti-HTLV III

Abbott Laboratories Ltd
Electronucleonics Inc
Organon Teknika Ltd
Ortho Diagnostics (Litton)
Wellcome Diagnostics

PHLS Virus Reference Laboratory
P P Mortimer
Hilary J Moulsdale
J V Parry
Louise J Oldham
Marguerite S Pereira

PHLS Communicable Disease Centre
Janet Y Mortimer

DHSS Project Officers
D A Kennedy, Scientific and Technical Branch, Supply Division
P A Lister, Scientific Services, Medical Division

September, 1985

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SECTION I

Introduction

This report presents the results of evaluations, performed in June and July 1985 of five commercial anti-HTLV/LAV III assay kits, i.e.: HTLV III EIA-Abbott Laboratories Ltd; Virgo HTLV-III ELISA-Electronucleonics Inc (ENI); Vironostika Anti-HTLV III- Organon Teknika Ltd; HTLV III BioEnzaBead-Ortho Diagnostic Systems Ltd and Wellcozyme anti-HTLV III-Wellcome Diagnostics.

The kits were evaluated by testing a panel of sera that included specimens from blood donors, patients with HTLV III/LAV related disorders and individuals with other unusual serological features. The same sera were tested by two commercial radioimmunoassays, Compria and Gacria, and these results are presented for comparison. The sera have now been tested by Western blot analysis and results are summarised at Appendix D. Immunofluorescence tests have also been completed. The results are at Appendix E.

The protocol for the evaluations (Appendix A) was drafted by an ad hoc Expert Working Group set up by the Department of Health and Social Security. The PHLS/DHSS evaluations were funded by Supply Division, Department of Health and Social Security. Further evaluations are being undertaken using a protocol designed to investigate the value of the commercial anti-HTLV III/LAV kits for blood donor screening.

Acknowledgements

Sera were kindly provided by Dr M Contreras, Dr P E Hewitt, Dr I V D Weller, Dr G Farthing, Dr D A McSwiggan, Dr B Jameson, Dr A M Denman, Dr B Bradley and Dr G F Bottazzo.

Miss Bhavna Mandalia and Miss Jennifer Wilson helped in the preparation of the report.
SECTION 2

Evaluation Report

Method

Four of the commercial assays were based on the same principle; i.e., a solid phase coated with antigen to which successively the specimen, an enzyme conjugated anti-human globulin, and a colour generating substrate were applied (see diagram below). The fifth commercial assay was of the competitive type; i.e., specimen and anti-HTLV III/LAV IgG conjugated with an enzyme were applied together to an antigen coated solid phase. The Compria assay was based on the same principle but anti-HTLV III/LAV IgG was labelled with $^{125}$I rather than enzyme conjugated. Gacria was based on a third principle. For this assay, antibody to the gamma chain of human IgG was coated onto a solid phase. IgG was 'captured' from the specimen and the presence of anti-HTLV III/LAV was determined by adding first HTLV III antigen and then anti-HTLV III/LAV labelled with $^{125}$I.

Three methods for detecting anti-HTLV III/LAV

Type 1 Ag  +  Specimen  +  anti human Ig Enzyme  +  colour forming substrate

(eg, Abbott, Eni, Organon and Ortho)

Type 2 Ag  +  { Specimen

  anti-HTLV III/LAV Enzyme

  +  colour forming substrate

  (eg Wellcome and Compria)

Type 3 anti-gamma  +  Specimen IgG  +  Ag  +  anti-HTLV III/LAV $^{125}$I (Gacria)
Although most of the commercial assays shared the same methodology their presentations differed:

- Abbott: 6mm polystyrene beads, antigen coated.
- ENI: microtitre 96 well plates
- Organon: microtitre strips of 12 wells
- Wellcome: microtitre strips of 2×8 wells
- Ortho: 4mm polystyrene coated ferrous beads

Compria and Gacria used 6mm polystyrene beads.

Table 1 summarises the steps in each of the seven assays and gives an estimate of the time needed to test 90 specimens. Note that the time to complete an assay and the number of manipulations involved varies considerably.

Three groups of sera were selected on epidemiological and clinical grounds according to the evaluation protocol (see Appendix A): 220 successive blood donors' sera received on 13 March 1985 at a Blood Transfusion Centre; 83 sera from patients in high risk groups (ie homosexual attenders at Genito-Urinary Medicine clinics, haemophilia patients) many of whom had illnesses attributed to HTLV III/LAV; and 57 sera from individuals with conditions likely to give rise to false positive reactions. These three groups are referred to here as 'donors' (numbers 1-220); 'high risk group', HRG (numbers 221-300, 338-340) and 'potential false positives', FPP (numbers 301-337, 341-360).

Four sera known to be anti-HTLV III/LAV positive by the Compria test were diluted from 1 in 50 to 1 in 800 in a serum known to be negative (normal human serum) and tested in duplicate. These were the only sera in the evaluation of which the anti-HTLV III/LAV reactivity was known at the time the evaluation panel was assembled.
All specimens used in the evaluation were aliquoted into 15 portions according to the protocol and stored at -30°C. A new set of aliquots was used for each evaluation. Each evaluation was done in four phases:

phase 1 - all sera tested.
phase 2 - all sera reacting positively in phase 1; for convenience the entire high risk group were re-tested.
phase 3 - (after heating at 56°C for 30 min) all sera re-tested.
phase 4 - all sera reacting positively in phase 3 re-tested plus other specimens in the high risk group as above. In this phase only specimens 1-30 were re-tested by the Abbott assay.

Assay kits were used as specified by the manufacturers in preliminary training sessions. Each phase was completed in a single day or, where appropriate, over one night. Results obtained for all phases of each assay evaluation were put on files in 'Datamaster' on an 'Apricot' computer. The analyses of these files of data are summarised in the tables and figures that follow.

Results

The analysis of the results takes account of four aspects of the kits: specificity, reproducibility, sensitivity and convenience to the user. In considering these aspects it should be borne in mind that all specimens in the group of blood donors and the group of potentially false positive specimens are very likely to have been anti-HTLV III/LAV negative, and that the consensus of findings on the high risk group specimens suggests that 11 of these were negative.

i. Distribution of results

The distribution of values around the mean cut-off specified by the manufacturer before and after heat treatment of the specimens for each assay is illustrated in Table 2 and the figures. The ranges of
values from the minimum to the mean-cut off value, and from the cut-off to the maximum for each assay have both been divided into ten equal intervals. If the ELISA reader scored OD >2 these values have been counted into an extra category. For the Wellcome assay, the intervals are shown in the reverse order because anti-HTLV III assay negative, rather than positive sera, produce a colour signal and the intervals are shown in reverse order. This adjustment is unnecessary in the methodologically similar Compria assay because its results are expressed as percentage inhibition of radiolabel binding. Attention is drawn to the following:

i. The discrimination between reactive and non-reactive specimens, i.e. the number of intervals separating the main blocks of specimens.

ii. The position of the potential false positive specimens relative to the blood donors' specimens.

iii. The existence of some anti-HTLV III-negative specimens in the high risk group.

iv. The changes caused by heating specimens to 56°C for 30 mins.

v. The numbers of specimens for which the values were within two intervals each side of the mean cut-off ('borderline specimens', Table 2).

Table 3 shows the mean cut-off value, the maximum value (minimum in the Wellcome assay) and the mean value for three groups of specimens thought to be anti-HTLV III/LAV negative: the blood donor group, the potential false positive group and a sub-group of eleven specimens from the high risk group. Assuming that all these specimens are truly anti-HTLV III/LAV negative, no results should overlap the cut-off. Ideally, the mean values for the three groups should be similar within each assay.
ii. Discrepancies within assays

An equivocal range of results is defined for the Abbott, Compria and Gacria assays. The other assays classify all results as either positive or negative. All specimens giving positive or equivocal reactions were re-tested, as were the 11 specimens in the high risk group thought to be anti-HTLV III negative. The reproducibility of the assays is summarised in Table 4a (specimens before heat treatment, phase 1 and 2) and Table 4b (specimens after heat treatment, phases 3 and 4).

Only 30 heat-treated specimens (blood donors 1-30) were re-tested by the Abbott assay. The results were:

a. Initially positive on-repeat positive - 22
b. Initially negative on-repeat positive - 4
c. Initially equivocal, on-repeat positive - 4

iii. Discrepancies between assays

a. Before heat treatment of the specimens.

Blood donors: All the results on the 220 donors specimens were negative except for two results in the Abbott assay. These were:

Specimen 175: initially positive; (OD=0.129, cut-off 0.115) on repeat negative, (OD=0.027).

Specimen 184: initially equivocal; (OD=0.111) on repeat negative (OD=0.020).
High risk group  The following eleven specimens were negative by all assays:

224  AIDS contact
248  PCL
249  AIDS contact
253  PCL contact
254  
256  PCL, HBeAg carrier
270  haemophilia patient
271  
279  
282  
299  

Specimen no. 246 PCL, was initially negative in the Wellcome assay only (OD=0.554, cut off 0.514), on-repeat positive (OD=0.148).

Specimen no. 274 (haemophilia) was initially negative in the Ortho assay only (OD=0.367 cut-off 0.410) on repeat positive (OD=0.691). Though both results were close to cut-off, a repeat test in such circumstances was not called for in either manufacturers' instructions.

The remaining 70 high risk group specimens were positive in all the assays.

The discrepancies between the assays in testing the potentially false positive group of specimens are shown in Table 5.

b. After heat treatment of specimens.

The results by the Abbott assay were excluded from the analysis because of they were anomalous.
Blood donors  By the ENI assay 21 specimens were initially positive. On repeat testing (phase 4) only one of these specimens was positive. There were no positive results by the other five assays. No. 133 gave an equivocal result (27%) in Compria. On repeat the result was negative (6%).

High risk group  The results by the six assays agreed except for two specimens 271, 279, that were positive only by ENI, OD values respectively 0.106, 0.103, on-repeat negative 0.057, 0.030; and for one specimen, 278, that was negative only by the Gacria test, negative ratio 1.05, on-repeat positive ratio 25.1.

Potentially false positive group  These 57 specimens were all negative by the Wellcome, Compria, Gacria and Ortho assays. By the Organon assay no. 341, anti HLA DR 4 B5, was positive, (OD value 0.575, on repeat negative 0.424). By the ENI assay 25 specimens were positive.

iv. Titration of anti HTLV III positive sera
To assess the sensitivity of the assays four anti-HTLV III/LAV positive sera were serially diluted in anti-HTLV III/LAV negative serum and tested in duplicate. The duplicate values were averaged and the titre was expressed as the first dilution at which the average value for the duplicates fell below the cut-off. For assays defining an equivocal range the cut-off was the mid-point of that range. Where the titre value fell above the cut-off in the equivocal range it is marked 'E'. These titres are to be found in Table 6.

Note that the proximity of the normal human serum (NHS) value to the...
cut-off varies widely. The choice of a cut-off close to the NHS value raises the apparent sensitivity of an assay, but suggests that the ability to discriminate between real positive and false positive reactions may be impaired.

No other data allowing firm conclusions to be drawn about the relative sensitivity of the assays is available from the evaluations. The 72 high risk group sera thought to be anti-HTLV III/LAV positive gave positive reactions in all the assays with three exceptions: specimen 246 not heat treated (NHT) - Wellcome negative, positive on repeat; specimen 274 NHT Ortho negative, positive on repeat and specimen 278 heat treated Gacria-negative, positive on repeat. In the Organon and Wellcome assays almost all, 68 of the 72 sera, gave a strong reaction, i.e., were in intervals 19, 20 21 (see figure). The distribution of the results for the reactive sera shown in the figures suggests that there were few or no weakly anti-HTLV III/LAV positive sera in the evaluation. An investigation of the reactivity in the assays of weakly positive sera collected in an unbiased way would provide useful additional information about the effective sensitivity of the assays.

v. User assessment of the commercial anti HTLV III assays

The convenience of the assays may be judged by reference to Tables 1 and 7. Reliability in supplying and maintaining kits and equipment was noted. The provision of the Organon and Ortho kits was trouble free.
There were adjustments in the provision of the Abbott, ENI and Wellcome kits. Kits delivered by Abbott were withdrawn by the manufacturer and replaced by another set. Suspected non-functioning ENI kits had to be replaced by the manufacturer before the evaluation could be done. Two Wellcome plates (i.e. 2 x 96 wells) gave abnormally high OD readings. These were discarded and that part of the assay was re-done. The reader supplied by ENI broke down before the evaluation could begin. Otherwise the equipment supplied worked satisfactorily. The print-out of the reader supplied by Wellcome was very difficult to read.
SECTION 3
Tables and Figures

Tables (pages 14 - 21)
1. Anti-HTLV III/LAV Assays: Stages and Estimated Times to Test 90 specimens plus Controls
2. Number of specimens in Two Intervals Above and Below the Mean Cut-off.
3. Maximum and Mean Values of Groups of Sera Believed to be Anti-HTLV III/LAV Negative.
4a. Comparison of Initial and Repeat Results: No Heat Treatment of Specimens before Testing.
4b. Comparison of Initial and Repeat Results: Specimens Heat Treated before Testing.
8. (See Appendix D) Blood donor specimens reported to contain anti-HTLVIII by Western blot.
9. (see appendix D) Western blot analysis of high risk group specimens.

Figures (pages 21A and 21B, 22 - 34)
Figs 1 & 2 Abbott - Results before and after heat treatment
Figs 3 & 4 ENI - " " " " " "
Figs 5 & 6 Organon - " " " " " "
Figs 7 & 8 Ortho - " " " " " "
Figs 9 & 10 Wellcome - " " " " " "
Figs 11 & 12 Compria - " " " " " "
Figs 13 & 14 Gacria - " " " " " "

KENAAL
<table>
<thead>
<tr>
<th></th>
<th>Abbott</th>
<th>ENI</th>
<th>Organon</th>
<th>Ortho</th>
<th>Wellcome</th>
<th>Comprin</th>
<th>Gacria</th>
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<tbody>
<tr>
<td>Specimen pre-dilution</td>
<td>35*</td>
<td>40</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Loading the plate</td>
<td>35</td>
<td>20</td>
<td>20</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30 plus 150/37°</td>
</tr>
<tr>
<td>Specimen plus antigen</td>
<td>60/40°</td>
<td>30/37°</td>
<td>30/37°</td>
<td>90/38°</td>
<td>)</td>
<td>60/45°</td>
<td>16 hr/RT** 16hr/RT</td>
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<td>Incubation with conjugate</td>
<td>124/40°</td>
<td>30/37°</td>
<td>30/37°</td>
<td>30/37°</td>
<td>)</td>
<td>60/45°</td>
<td>150/37**</td>
</tr>
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<td>Colour development</td>
<td>30/RT</td>
<td>10/RT</td>
<td>30/RT</td>
<td>10/RT</td>
<td>20/RT</td>
<td>-</td>
<td>-</td>
</tr>
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<td>5</td>
<td>5</td>
<td>5</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Reagent preparation and washing time</td>
<td>30</td>
<td>10</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Number of stages</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Total time hr min</td>
<td>5.25</td>
<td>2.30</td>
<td>2.45</td>
<td>3.10</td>
<td>2.00</td>
<td>17.50</td>
<td>23.20</td>
</tr>
</tbody>
</table>

* minutes

** with 125I anti-HTLV III/LAV
Table 2

2. NUMBER OF SPECIMENS IN THE TWO INTERVALS ABOVE AND BELOW THE MEAN CUTOFF
REFER ALSO TO FIGURES

<table>
<thead>
<tr>
<th>Specimen Group</th>
<th>Abbott</th>
<th>EMI</th>
<th>Organon</th>
<th>Ortho</th>
<th>Wellcome</th>
<th>Compria</th>
<th>Gacria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HT*</td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
</tr>
<tr>
<td>blood donor, n=220</td>
<td>2</td>
<td>211</td>
<td>0</td>
<td>49</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>high risk &quot;*&quot;, n=72</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>&quot; &quot; &quot;-&quot;, n=11</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>potential false positive, n=57</td>
<td>15</td>
<td>44</td>
<td>3</td>
<td>28</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>17</td>
<td>266</td>
<td>4</td>
<td>82</td>
<td>1</td>
<td>3</td>
<td>12</td>
</tr>
</tbody>
</table>

*HT= Heat Treatment
Table 3

MAXIMUM AND MEAN VALUES IN ASSAYS OF GROUPS OF SERA BELIEVED TO BE ANTI-HTLV III/LAV NEGATIVE

<table>
<thead>
<tr>
<th></th>
<th>Abbott HT</th>
<th>ENI HT</th>
<th>Organon HT</th>
<th>Ortho HT</th>
<th>Wellcome HT</th>
<th>Compra HT</th>
<th>Gartia HT</th>
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</thead>
<tbody>
<tr>
<td>Mean cut-off</td>
<td>0.12</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
<td>0.49</td>
<td>0.47</td>
<td>0.51</td>
</tr>
<tr>
<td>Blood donors</td>
<td>(max)</td>
<td>0.13</td>
<td>0.31</td>
<td>0.04</td>
<td>0.18</td>
<td>0.39</td>
<td>0.40</td>
</tr>
<tr>
<td>n=220</td>
<td>(mean)</td>
<td>0.04</td>
<td>0.16</td>
<td>0.01</td>
<td>0.06</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>High risk</td>
<td>(max)</td>
<td>0.06</td>
<td>0.48</td>
<td>0.04</td>
<td>0.11</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>group</td>
<td>(mean)</td>
<td>0.04</td>
<td>0.35</td>
<td>0.01</td>
<td>0.07</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>Potential</td>
<td>(max)</td>
<td>0.45</td>
<td>0.75</td>
<td>0.78</td>
<td>0.84</td>
<td>0.53</td>
<td>0.58</td>
</tr>
<tr>
<td>false</td>
<td>(mean)</td>
<td>0.09</td>
<td>0.36</td>
<td>0.05</td>
<td>0.13</td>
<td>0.11</td>
<td>0.17</td>
</tr>
<tr>
<td>positive</td>
<td>(mean)</td>
<td>0.04</td>
<td>0.16</td>
<td>0.01</td>
<td>0.07</td>
<td>0.08</td>
<td>0.12</td>
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*Minimum values shown for the Wellcome assay

HT=Heat treated
## Table 4a

### COMPARISON OF INITIAL AND REPEAT RESULTS: NO HEAT TREATMENT OF SPECIMENS BEFORE TESTING

<table>
<thead>
<tr>
<th>Initial Result</th>
<th>Repeat Result</th>
<th>Abbott</th>
<th>ENI</th>
<th>Organon</th>
<th>Ortho</th>
<th>Wellcome</th>
<th>Compria</th>
<th>Gacria</th>
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<tbody>
<tr>
<td>positive</td>
<td>positive</td>
<td>77</td>
<td>76</td>
<td>73</td>
<td>71</td>
<td>71</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>equivocal</td>
<td>4</td>
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<td>0</td>
<td>1</td>
<td>1</td>
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<td>0</td>
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<tr>
<td></td>
<td>negative</td>
<td>11</td>
<td>11</td>
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<td>equivocal</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>equivocal</td>
<td>positive</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>negative</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
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<tr>
<td></td>
<td>equivocal</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>0</td>
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| Number of specimens repeated | 100 | 87 | 84 | 83 | 83 | 83 | 84 |
Table 4b

COMPARISON OF INITIAL AND REPEAT RESULTS: SPECIMENS HEAT TREATED BEFORE TESTING

<table>
<thead>
<tr>
<th>Initial result</th>
<th>Repeat result</th>
<th>ENI</th>
<th>Organon</th>
<th>Ortho</th>
<th>Wellcome</th>
<th>Comprin</th>
<th>Gacria</th>
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<tr>
<td>positive</td>
<td>positive</td>
<td>91</td>
<td>71</td>
<td>71</td>
<td>72</td>
<td>72</td>
<td>71</td>
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<td>negative</td>
<td>positive</td>
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<td>positive</td>
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<td>0</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>negative</td>
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<td>11</td>
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<td>11</td>
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</table>

Equivocal negative

| Number of specimens repeated | 130 | 83  | 83   | 83   | 84   | 83   |

The Abbott data, which was anomalous, is omitted from this table. Only 15 specimens gave negative results after heat treatment.
Table 5

DISCREPANCIES BETWEEN ASSAYS ON TESTING POTENTIALLY FALSE-POSITIVE GROUP SPECIMENS

<table>
<thead>
<tr>
<th>No</th>
<th>Abbott</th>
<th>ENI</th>
<th>Organon</th>
<th>Ortho</th>
<th>Wellcome</th>
<th>Compria</th>
<th>Cacria</th>
</tr>
</thead>
<tbody>
<tr>
<td>319</td>
<td>E(−)*</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>321</td>
<td>E(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>235</td>
<td>+(+)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>337</td>
<td>E(−)</td>
<td>+(+)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>E(−)</td>
</tr>
<tr>
<td>341</td>
<td>+(+)</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>345</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>346</td>
<td>+(E)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>349</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>352</td>
<td>E(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>354</td>
<td>+(E)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>355</td>
<td>+(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>357</td>
<td>+(+)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>358</td>
<td>+(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>359</td>
<td>+(E)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>360</td>
<td>+(E)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

All 15 specimens were negative by Ortho, Wellcome and Compria. The remaining 42 potentially false positive specimens were negative in all assays.

* E = equivocal, repeat results shown in brackets.
## Table 6

TITRES OF FOUR ANTI-HTLV III/LAV POSITIVE SERA (PHASE 1)

<table>
<thead>
<tr>
<th></th>
<th>Abbott</th>
<th>ENI</th>
<th>Organon</th>
<th>Ortho</th>
<th>Wellcome</th>
<th>Compria</th>
<th>Gacria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
</tr>
<tr>
<td>10363</td>
<td>&gt;800E</td>
<td>-</td>
<td>200</td>
<td>200</td>
<td>&gt;800</td>
<td>&gt;800</td>
<td>400</td>
</tr>
<tr>
<td>10754</td>
<td>400</td>
<td>-</td>
<td>200</td>
<td>100</td>
<td>400</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>10761</td>
<td>400</td>
<td>-</td>
<td>100</td>
<td>&gt;800</td>
<td>400</td>
<td>200</td>
<td>&gt;800E</td>
</tr>
<tr>
<td>12424</td>
<td>400</td>
<td>-</td>
<td>200</td>
<td>100</td>
<td>400</td>
<td>&gt;800</td>
<td>&gt;800E</td>
</tr>
</tbody>
</table>

|                | mean   | NHS | 0.053  | 0.264* | 0.017 | 0.076 | 0.080 | 0.116 | 0.175 | 0.133 | 1.204 | 1.023 | -7 | 14 | 1.16 | 0.87 |
|                | cut-off |     | 0.119  | 0.124  | 0.10  | 0.10  | 0.398 | 0.413 | 0.427 | 0.376 | 0.599 | 0.478 | 37.5 | 37.5 | 3   | 3   |

*exceeds cut-off
E=Equivocal
### TABLE 7: INDEPENDENT SCORING OF FIVE COMMERCIAL ANTI-HTLV III/LAV ASSAYS BY THREE OPERATORS

<table>
<thead>
<tr>
<th></th>
<th>Abbott</th>
<th>ENI</th>
<th>Organon</th>
<th>Ortho</th>
<th>Wellcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Training given by company representatives</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2. Clarity of written instructions</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3. Ease of use of equipment</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4. Presentation and ease of use of reagents</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>5. Ease of test procedure</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>6. TOTALS</td>
<td>41</td>
<td>32</td>
<td>48</td>
<td>29</td>
<td>42</td>
</tr>
</tbody>
</table>

Scores as follows:—  
- (bad)  
- (poor)  
- (adequate)  
- (good)  
- (excellent)  
- (very difficult)  
- (difficult)  
- (some difficulty)  
- (ease)  
- (very easy)
Legend

To show the distribution of positive and negative values in phases 1 and 3 for each of the seven anti-HTLV III/LAV assays, the ranges from minimum value to cut-off and from cut-off to maximum value have both been divided into ten equal intervals. The number of values in each interval for the three categories of specimen are displayed. If OD values >2 were recorded they have been counted into a 21st category. In the Wellcome assay for which OD values < cut-off are positive, the intervals are shown in reverse order.
Figure 1

ABBOTT NHT (PHASE 1):

MIN 0.013  MAX >2  CUT-OFF. 0.114
Figure 2

**ABBOTT HT (PHASE 3):**

- **DONOR**
- **HRG**
- **PFP**

MIN 0.014  MAX >2  CUT-OFF 0.114
Figure 3

ENI NHT (PHASE 1):

+200

+100

MIN -0.01 MAX >2 CUTOFF 0.1
Figure 4

ENI HT (PHASE 3):

DONOR
HRG
PFP

MIN -0.01 MAX 2 CUTOFF 0.1
Figure 5

ORGANOH NHT (PHASE 1):

MIN 0.065  MAX >2  CUT-OFF 0.489
Figure 6

ORGANON HT (PHASE 3):

DONOR

HRG

PFP

MIN 0.072  MAX >2  CUTOFF 0.467
Figure 7

LITTON-ORTHONMT (PHASE 1):

MIN 0.078  MAX 1.629  CUTOFF 0.406
Figure 8

LITTON/ORTHO HT (PHASE 3):

DONOR
HRG
PFP

MIN 0.077 MAX 1.352 CUTOFF .379
Figure 9.

**WELLCOME NHT (PHASE 1):**

- DONOR
- HRG
- PFP

MIN 0.102 MAX 1.650 CUTOFF 0.514
Figure 11

COMPRIA NHT (PHASE 1):

- DONOR
- HRG
- PFP

MIN -29  MAX 98  CUTOFF 37.5
Figure 13

GACRIA NHT (PHASE 1):

DONOR

HRG

PFP

MIN 0.1 MAX 60.36 CUTOFF 3
SECTION 4

Comments From Manufacturers

Manufacturers were asked to comment on the results of the evaluation of their product. The comments are reproduced below together with the evaluators' responses.

| i. Abbott Laboratories Ltd | 35 |
| ii. Electronucleonics Inc | 38 |
| iii. Organon Teknika Ltd | 45 |
| iv. Ortho Diagnostic System Ltd | 46 |
| v. Wellcome Diagnostics | 47 |
Mr. D. A. Kennedy,
14 Russell Square,
LONDON.

26th July 1985.

Dear Mr. Kennedy,

Thank you for the copy of the recent Colingdale evaluation concerning HTLV III. Our comments are as follows:-

Heat Treatment of Specimens

HTLV III E.I.A. from Abbott has been designed as a blood bank screening technique. It is generally considered unlikely that blood transfusion centres will wish to heat inactivate and so this is not a part of our standard operating procedure.

Indeed, if heat treatment has to be taken into account this generally means a trade off versus sensitivity which is to be avoided in blood transfusion screening where test sensitivity is extremely important.

As heat treatment is not a part of our standard operating procedure we do not advise it, though it is worth noting that we do have customers who heat inactivate and observe no interference with results. We can only speculate that variation in the method of inactivation leads to this discrepancy.

Discrepancies Between Assays

Two samples from the donor group were initially positive and became negative on re-testing. This gives a non-repeatable positive rate of 0.9%. Our own experience on a much larger sample shows the non-repeatable positive rate to be in the region of 0.4%. As the sample tested is very small it may be helpful to reference data from the package inserts of the various companies where this data is published, in addition to the results of this study.

In our view the number of samples tested is too small to make any meaningful inference.
Potential False Positive Group

Thirteen of the fifteen equivocables or positives in this group were clustered in the first interval above the cut-off. A similar picture is seen with samples which have been heat treated. It is our view that the most probable explanation is that the samples have deteriorated in some way during processing and storage. HTLV III E.I.A. from Abbott has been designed to be used on fresh samples. The number of such samples found in the blood transfusion environment will also be very low.

User Assessment

Abbott HTLV III E.I.A has been designed for blood transfusion screening of serum or plasma. Because of the high numbers involved the test has been automated with the Pro Quantum. The Pro Quantum automates the washing and reagent addition and greatly simplifies the procedure. We were therefore very disappointed that the Pro Quantum was not used during the evaluation, despite being offered and having been tested by Porten Down and found safe to use.

Similarly, the automated reading system, the Quantumatic was not used.

Again, we do not feel that the test has been used in the environment it was designed for, which is blood transfusion screening.

Sensitivity

Abbott HTLV III E.I.A has been designed to pick out even low level positives as are found in field testing. We notice that the assay demonstrated the required sensitivity and that all of the positive samples were detected on the first testing.

Yours sincerely,

Stephen Porter
GENERAL MANAGER

cc J.D. Hatt
Gary Coslett
Evaluators' Response to Abbott Laboratories Ltd

1. Heat Treatment of Specimens - Comments noted: Abbott Laboratories Ltd agreed the evaluation protocol.

2. Discrepancies Between Assays - Comments noted: Abbott Laboratories Ltd agreed the evaluation protocol.

3. Potential False Positive Group - Comments noted.

4. User Assessment - Comments noted: The washing and reading procedures were agreed with Abbott Laboratories Ltd and the equipment used, eg the "Pentawash", was supplied for the evaluation by Abbott Laboratories Ltd.

5. Sensitivity - Comments noted.
August 6, 1985

D. A. Kennedy,
Scientific and Technical Branch
DEPARTMENT OF HEALTH AND SOCIAL SECURITY
14 Russell Square
London WC1B 5EP England

Dear Mr. Kennedy:

Our technical personnel have had a chance to review the draft report prepared by the PHLS Reference Laboratory following the evaluation of Electro-Nucleonics VIRGO HTLV III Elisa Antibody Test. Their comments are attached. Some of the comments may reflect a difficulty in interpreting this draft of the report. If any of the response appears to result from a misunderstanding of the data, please let us know. Obviously given the short time available, not all points have been raised. However, we would like to know why the "H-9" plate provided to identify false positives was not used.

As indicated to you, we are interested in participating in the next stage of the evaluation. In view of the fact that we have now had much more experience with the product ourselves, we are confident that you will be more than satisfied with our product. An analysis of the data obtained from testing 350,000 units with the ENI product was presented at a Workshop at the National Institute of Health on July 31. An abstract of this paper is attached for your review. In the next study, we would want to have both our "H-9" specificity plate used and, if possible, our IFA product.

Sincerely,

Victoria M. Leitz, Ph.D.
Director International Sales and Marketing

VML/jm
REPORT ON THE EVALUATION OF
ANTI-HTLV III/LAV ASSAYS
Prepared for DHSS by PHLS Virus Reference Laboratory
15 July 1985

Comments by Electro-Nucleonics; Code Letter B

Table I

The colour development time for the ENI assay is 10 minutes at
room temperature; not 30 minutes.

Heat Treatment

Heat treatment is specifically proscribed in the Electro-
Nucleonics Package Insert. The reason for this is the increased
incidence of false positive reactions, as was observed in this
study.

However, we appreciate the concern regarding potential infectivity
of samples and the desire for heat treatment. In order to be
able to clearly identify false positives, we provided the
evaluating laboratory with the procedure recommended by
Electro-Nucleonics to distinguish these samples. This
procedure involves the use of an "H9" plate which contains
as antigen a preparation of non-infected H9 cells. The use of
this has been clearly demonstrated to identify non-specifically
reacting false positives. We would ask why this was not used
in the study.

Potentially false positive group

The report indicates that 25 of 57 potentially false positive
samples were positive by the Electro-Nucleonics test. The
table does not appear to reflect this. We would appreciate
clarification of this issue. The table clearly illustrates
a large number of false positive results by Test A on the
group of potentially false positive group of specimens. This
is not noted in the test. It indicates a significant degree of
reactivity of antinuclear or anticellular antibodies with the
test plates. Again, these types of false positives can be
easily distinguished by use of the H9 Specificity Test.

Titration of Anti-HTLV III positive sera

We do not agree that titration is a true measure of sensitivity
when the protocol described is used. Dilution of a high titer
antibody will be detected at the highest dilution on the test
with the highest surface area, ie. Test A. This does not measure
true sensitivity to low levels of antibody present in actual test
samples. The sensitivity claims in the manufacturer's product
inserts are a better indication of sensitivity to known positive
samples.
Table 7

We would like to receive a more detailed critique of the various aspects of the assays summarized in Table 7. We would especially welcome knowing which items contributed to the difficulty in running the test and in the use of the reagents.

Victoria M. Leitz, Ph.D.
Director International Sales
and Marketing

NML/jm
WORKSHOP

EXPERIENCE WITH HTLV-III ANTIBODY TESTING - UPDATE ON: SCREENING, LABORATORY AND EPIDEMIOLOGIC CORRELATIONS

Sponsored by:

Center for Drugs and Biologics, FDA
National Institutes of Health, and
Centers for Disease Control

July 31, 1985

NIH-Building 10, Masur Auditorium
Bethesda, Maryland
Electro-Nucleonics HTLV-III Testing: Sensitivity (HILV-III ELISA), Specificity (H9 ELISA), Confirmation (IFA)

VIRGO HILV-III ELISA screening test data collected on over 350,000 units showed an initial reactive rate of 0.68%. The repeat ELISA reactive rate was 0.27% for an overall specificity of 99.7%. These results reflect an average over a four month period. In the first two weeks of testing, the repeat reactive rate was 0.55%. With the deferral of HTLV-III reactive donors, the repeat reactive rate for the two weeks in July has fallen to 0.09%, for a specificity of 99.9%. The sensitivity of the test, as determined by the ability to detect antibody in 267 of 268 individuals with diagnosed AIDS is 99.6%. Recently completed clinical trials on an improved HILV-III ELISA which utilizes a divisible strip microassay plate and a floating cutoff yields the same level of sensitivity (99.6%) and higher specificity in both initial and repeat screening tests. With the use of VIRGO H9 Specificity Test, differentiation of true positive from false positive results is possible. Use of the H9 specificity test distinguishes almost 90% of the minimally reactive ELISA samples as nonspecific to HTLV-III. All of the AIDS samples reactive to HILV-III ELISA were specific positives when using the H9 test (100%). Additionally, to aid in donor/patient counseling an indirect fluorescent antibody test has been developed at ENI, with sensitivity and specificity comparable to the HILV-III ELISA. It is rapid (1 hr) and considerably less laborious than the Western Blot technique for confirmation of the presence of HTLV-III antibody. With this test, ENI has documented an association between repeatedly reactive samples just about the cutoff on the HILV-III ELISA and the presence of antinuclear or anti-HLA antibodies.
Evaluator's Response to Electronucleonics Inc

1. Use of the "H-9" plate

The confirmatory system using H9 plates was presented to us as an experimental procedure. It asked for initially positive assays to be repeated in duplicate, with a pair of control (i.e., H9 plate) assays. The final result was then to be calculated as

\[
\text{mean of duplicated repeats} - 0.1 \\
\text{mean of control results}
\]

Only specimens for which this value was >3 were to be considered as confirmed positives. It was not possible to follow this procedure in full, but the value

\[
\text{repeat result} - 0.1 \\
\text{control result}
\]

has been calculated for all initially positive specimens, with the results shown below.

Difficulties

When the control result = zero, the calculation was undefined. When the control result was less than zero, the result was nonsense. Both these cases would be less likely to arise when there were duplicate control readings, but they need to be allowed for in the manufacturer's instructions.

(NB: Negative OD readings are given by some ELISA readers including the one used (by agreement) in this work. The reader supplied by ENI was not in working order and could not be used.)
Results with "H-9" plates

Before heat treatment

All the positives in the HRG were confirmed as positive. Three (337, 341 & 345) of the four in the PFP group which were positive in both Phase I and Phase II were erroneously confirmed as positive, though with values (7.6, 5.5, 4.7) which were lower than for any of the "true" positive specimens. The fourth, 349, was negative (0.3).

After heat treatment

Of the 21 BD specimens positive in phase 3, 20 were negative when repeated (phase 4) and the 21st was negative after the calculation had been done.

The calculation did not alter the repeat results for the HRG specimens. These were in agreement with the findings by the other assays.

Twenty-five of the PFP specimens were positive in phase 3. Seven of these were negative on repeat (phase 4), and a further 13 were negative after the calculation had been done. The other five (307, 337, 341, 345 and 349) remained erroneously positive.

2. Table 1 Comment noted: Table 1 has been amended accordingly.

3. Heat Treatment Comment noted: ENI agreed the evaluation protocol.

See comments above on our experience with the "H-9" plates.

4. Potentially False Positive Group - Comment noted. See above the information on the use of HTLV III "H-9" plates.

5. Titration of Anti-HTLV III Positive Sera - ENI agreed the evaluation protocol which included testing of serum dilutions. The company's views on other ways of measuring sensitivity are noted, and attention is drawn to the evaluators' comment about this on page 11.

6. Table 7 - The company is invited to send a representative to visit the evaluators so that these detailed issues may be discussed.

Mr. D. A. Kennedy,
Scientific & Technical Branch,
Dept. of Health & Social Security,
14 Russell Square,
LONDON WC1B 5EP.

Dear Mr. Kennedy,

Evaluation of anti-HTLV III

Thank you for your correspondence dated 19th July, 1985 enclosing a copy of the data on our product Vironostika anti-HTLV III. We are happy with the results and the raw data supplied and look forward to the B.T.S. evaluation and the full report being made available.

Yours sincerely,

S. J. Jacques
Marketing Manager

Evaluators' Response - Comments noted.
Dear Mr. Kennedy,

Thank you for sending me a copy of the PHLS evaluation report on Anti HTLV-III kits. I was pleased to see that the Ortho/Litton kit gave what appear to be good results with the sera tested.

I would like to make two comments about the report, the first being about Table 1; the times taken for the various steps in the test. The time noted for reagent preparation and washing is 25 minutes. This would appear to be somewhat excessive and both an experienced user of our kit and myself feel that this time should be reduced by 5 to 10 minutes. Once reagents are prepared there is sufficient material to perform more than one batch of tests which also indicates that this 25 minute period is an over-estimation. The average time per test would therefore be reduced to approximately 3 hours.

The second observation I would like to make concerns the results obtained with sample No. 274 (haemophilia). Our kit was the only one to give a negative result with this sample, the O.D. being 0.367. On repeat testing however, the result was positive and the new O.D. was 0.691. As the O.D. almost doubled between the two tests it would appear that there was probably a pipetting error the first time the sample was tested. Having spoken to one of the people at Colindale who performed the tests, they agree this is a possibility.

I would therefore ask that this sample be either repeated to confirm that our kit truly gives a positive result with it, or that the result be excluded from the analysis altogether. Should you wish to discuss these comments further please do not hesitate to contact me.

Yours sincerely,

PETER SAVAGE
Product Manager

Evaluators' Response

1. Table 1 - Comments noted.
2. Sample No. 274 (haemophilia) - Comments noted. The possibility of operator error is acknowledged - see Evaluators' commentary at page 50.
EVALUATION OF ANTI-HTLV III KITS AT THE PHLS VIRUS REFERENCE LABORATORY

Thank you for the copy of the draft PHLS report which was received here on Monday 22nd July. In general we found the report both interesting and informative, although a summary of results would have been useful. Specifically our comments are as follows:

1 Page 5, the table entitled 'Anti HTLV 3/LAV assays: stages and estimated times to test 90 specimens plus controls as specified by the supplier'.

The data for product E (the Wellcome test) against the heading 'colour development' should read '20/RT' and not '20/45°' as stated. We would like to see this corrected.

2 Page 25, penultimate paragraph.

The report comments that our instructions do not require repeat tests when results are very close to the cut off value. This is an aspect of our test which we are investigating further by means of our own scientific evaluation trials. It is likely that we will modify our protocol before the product is made widely available.

3 Page 26, the last paragraph entitled 'potentially false positive group'.

The report states that the 57 specimens were all negative by the Compria, Gacria and D assays but omits to mention that they were also negative by assay E (the Wellcome test). We would like to see this statement added.
Page 30, the paragraph entitled, 'user assessment of commercial anti HTLV III assays'.

The report states that there were 'minor adjustments in the provision of the Abbott and Wellcome kits'. We are surprised at this comment as we were under the impression that the manufacturers of the kits evaluated would remain anonymous. In every other respect the Wellcome test has been referred to as 'E'.

Another comment refers to the same problem. The inference from the report is that replacement or additional kits had to be supplied as a result of a failure in some respect with those kits originally provided. We would like it noted that in the case of the Wellcome test additional kits were supplied at our request following the sudden and unexplained reporting of over development of colour in two of the plates used. This over development of colour, however, did not affect the reading of results and the evaluating team chose not to repeat the tests using the replacement kits. Despite an albeit brief conversation with the PHLS staff on the problem at the time of the incident no satisfactory explanation has been found. Work carried out here since the trial does indicate that the reported problem can be recreated through the addition of too much (x 2) TMB substrate.

Our last comment on this section relates to the statement that 'the print out of the reader supplied by E (Wellcome) was very difficult to read'. Our concern here relates to the fact that at no time during the pre evaluation training or during the evaluation itself was this problem reported. Had we known, this minor problem would have been quickly corrected.

Page 31, Table 7.

Our comment here is the general one that we would have found the data much more useful had it been a little more detailed. For example the timings stated on page 5 are very informative in the context of giving more detail on items 4 and 5 in Table 7. It would have been nice to have had similar information on items 1 to 3.

If any of the above comments are not clear please do not hesitate to contact me. We look forward to seeing the final version of the report.

Yours sincerely

P D F SYMONS
Production Manager
Evaluators' Response to Wellcome Diagnostics

1. Table 1 - comments noted: table has been amended accordingly.
2. Need for Repeat Tests - comments noted.
3. Potentially False Positive Group - comments noted.
4. User Assessment - comments noted: The evaluators did repeat the tests using new plates and reagents.
5. Table 7 - comments noted.
SECTION 5

Commentary by the Evaluators

A brief discussion of the results will be found in Mortimer, P P Parry J V and Mortimer J Y 1985, Which anti-HTLV III/LAV assays for screening and confirmatory testing?, Lancet, (in the press), but it is hoped that readers will study the preceding tables and figures and make their own assessment of the results. Potential users will have divergent requirements of commercial anti-HTLV III/LAV assay kits and it is preferable that each makes his own interpretation of the data. In particular, the needs of diagnostic and blood transfusion laboratories are not the same. Diagnostic laboratories will especially want to avoid false positive results. Transfusion laboratories, on the other hand, will accept some false positive results as an extra assurance against false negative ones, provided that they enjoy the support of laboratories where confirmatory test are done and provided that false positive reactions do not occur too frequently.

With these considerations in mind we make the following recommendations to those using commercial anti-HTLV III/LAV kits:

1. That a high level of suspicion be maintained regarding the possibility of false negative results, and a better understanding of the circumstances in which these may arise be sought. They may be particularly common early in infection and late in HTLV III/LAV related disease. The commonest cause of false negative reactions, however, will be operator errors and every care should be taken to eliminate these.
2. When screening blood donors false positive results will be commoner than true positive results, especially when Type 1 assays are used. In the United Kingdom the services of the eight laboratories (the Public Health Laboratories at Birmingham, Bristol, Leeds, Manchester, Newcastle and Oxford; Virus Reference Laboratory, CPBL, Colindale, and Virology Section Middlesex Hospital/University College Hospital Medical School) designated to provide confirmatory tests should be used so that true results can be distinguished from false ones. This will be done by applying a range of independent anti-HTLV III/LAV tests.

3. False positive results often arise in testing heat treated specimens.

4. Most false positive reactions, and most false negative reactions that are intrinsic to the test as opposed to being due to operator error will be close to the manufacturer's "cut-off" value. All positive reactions and any negative reactions within the first one-fifth of the range of optical density values from cut-off to the end of the negative range (ie, within two intervals - see table 2) should be retested. If the result is unchanged the specimen should be referred to one of the eight confirmatory laboratories. It may also be advisable to refer some unrepeatably positive specimens to a confirmatory laboratory.

5. Finally, though particular commercial kits have been recommended on the basis of this data (DHSS letter 1 August 1985 - see Appendix B), none of the commercial assays is either perfect or wholly unsuitable. Users should consider their local needs and the costs of the kits as well as the results of these evaluations in deciding which commercial assay to choose. Our results for each kit were obtained on one day, and on one batch using relatively few specimens. Continuing assessment of commercial anti-HTLV III/LAV assay kits is needed, and it is
desirable that there should be some diversity countrywide in the range of kits in use. Moreover it should be borne in mind that the performance of the kits can only be monitored if the services of the designated confirmatory laboratories are used and these laboratories are supplied with the necessary information on screening tests and results.
SECTION 6

APPENDICES

A. Evaluation Protocol

B. Sera Used in DHSS/PHLS Evaluation of Anti-HTLV III/LAV Assays

C. DHSS Letter of 1 August 1985, Evaluation of Screening Test Kits for HTLV III/LAV Antibody

D. Western blot analysis of the evaluation panel of specimen

E. Immunofluorescence tests on the evaluation panel of specimens
APPENDIX A

PROTOCOL FOR THE DHSS EVALUATION OF ANTI-HTLV III KITS AT THE PHLS VIRUS REFERENCE LABORATORY.

1. Procurement of Products for Evaluation, Duration of Evaluation and Training of Evaluators

The objective is to purchase for evaluation a package which contains sufficient anti-HTLV III tests, together with ancillary reagents and consumables that are recommended by the manufacturer. These will be used in conjunction with the equipment, such as spectrophotometers and plate washers, that are recommended by the manufacturer. It is the intention to lease this equipment for the duration of the evaluation. It is estimated that evaluation of each kit will be completed within eight weeks from delivery of the package. Before evaluation starts, the manufacturer will be invited to train the evaluators in the use of the kits and equipment and to satisfy himself that the evaluators are properly trained.

2. General Conduct of the Evaluation

All products will be used in exactly the manner laid down in the manufacturer's literature or as described during the training period.

3. Content of the Evaluation

The objective is to assess the performance of the kits, used in exactly the manner recommended by the manufacturer, when tested against a panel of sera from patients with known AIDS and AIDS-related illness, high titre anti-HTLV III positive sera in dilution, sera from patients with other pathological conditions unrelated to HTLV III, and the same panel of sera after heat treatment at 56°C. The panel is more fully described below. An
assessment will be made of the usefulness of the training provided, clarity of operating instructions, packaging and labelling, safety, and ease of use and reliability of equipment. A record of the 'back up' provided by the manufacturer will be kept.

3.1 The panel of sera will be made up as follows:

a. At least 200 sera from volunteers having met the clinical criteria of suitability to donate blood for transfusion.

b. 80 sera from clinically 'HIGH RISK' patients, including those attending sexually-transmitted disease clinics with the clinical symptoms of AID or AIDS-related illness and haemophiliacs who have been given Factors VIII concentrate. It is expected that many will be anti-HTLV III POSITIVE. During the evaluation, these sera will be tested twice by the kit being investigated. The second test will be done within one week of the first and before the expiry date of made-up reagents.

c. 4 sera from cases which meet the clinical criteria of persistent generalised lymphadenopathy (evaluators' comment: the four sera used were from haemophilia patients who had regularly received commercial factor VIII concentrate and have high titre of anti-HTLV III when assayed by the VRL radio-immunoassay and immunofluorescence methods). These sera will be prediluted with anti-HTLV NEGATIVE sera (VRL methods) in doubling steps between 1/50 and 1/800. Aliquots will then be frozen (see Storage of Sera, below). After thawing, each aliquot will be assayed by the VRL methods as a check of stability during storage. Each aliquot will be tested in duplicate by the kit under evaluation.
d. At least 10 sera from cases of virologically and clinically established viral adenopathies, such as rubella and infectious mononucleosis, and at least 10 sera from cases of rheumatoid factor-positive rheumatoid arthritis, lymphoma, etc (see Addendum below).

3.2 Storage of Sera
Aliquots of sera will be distributed into the wells 4 x 5 well plastic trays. Each tray will be coded and sealed with a thick adhesive plastic cover. The trays will be placed in lidded plastic boxes and kept in a dedicated freezer at —30°C. Thawing will be carried out at room temperature.

3.3 Heat-treatment of Sera and Subsequent Testing
After thawing trays containing aliquots of all the sera in the panel, including the prediluted sera, will be floated in a water bath kept at 56°C for 30 minutes. Thereafter, assays will be performed on them by the kit under evaluation.

3.4 Other aspects of the evaluation
The following features of the kits will also be assessed:

- the packaging and labelling of the materials
- the clarity of the operating instructions and the training provided by the manufacturer
- the ease of use and reliability of the products supplied for evaluation. Comments will be based on entries in a log book in which all significant events, such as change of reagents, change of operator, maintenance of equipment and requests to the manufacturer for assistance, will be recorded.
d. the safety of the kit: this will be assessed with reference to information provided by the manufacturer on labels, in package inserts and during training.

4. **Discrepant Results**

A discrepant result will arise where the results produced by the kit under evaluation and by one or both of the methods performed by the Virus Reference Laboratory, fail to agree. If this occurs, the tests will be repeated on the same aliquots of the serum. The results will be recorded and if there is still a discrepancy, the serum in question will be stored at -30°C to await further investigations. (Evaluators' note: the procedure used for dealing with discrepant results did not follow this instruction, which proved impractical. The tests by virus reference laboratory methods were not the first to be done and, in any case, are not regarded as "reference methods").

5. **Evaluation Report**

Manufacturers will be given the opportunity to comment on the results of the evaluation of their product before the report is made available to the Health Service. Manufacturer's comments, where relevant, will be entered into the report.

6. **Addendum**

The group of sera in paragraph 3.1(d) (viral adenopathies, rheumatoid factor positive, lymphoma etc) is to be enlarged by the addition of specimens from SLE patients, sera positive for cold agglutinins and sera reactive with the HLA-DR antigens.

May 1985

KENAAL
APPENDIX B

Sera Used in DHSS/PHLS Evaluation of anti HTLV III/LAV Assay

Blood donor group

1 - 220 inclusive

<table>
<thead>
<tr>
<th>Blood donor group</th>
<th>Successive blood donations received on March 13 1983 at a blood transfusion centre</th>
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</thead>
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<tr>
<td>High risk group (HRG)</td>
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</tr>
<tr>
<td>221</td>
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<td>PGL, HBsAg carrier</td>
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<tr>
<td>223</td>
<td>AIDS contact</td>
</tr>
<tr>
<td>224</td>
<td>AIDS contact</td>
</tr>
<tr>
<td>225</td>
<td>PGL</td>
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<td>PGL</td>
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<tr>
<td>235</td>
<td>PGL contact</td>
</tr>
<tr>
<td>236</td>
<td>AIDS contact</td>
</tr>
<tr>
<td>237</td>
<td>AIDS contact</td>
</tr>
<tr>
<td>238</td>
<td>PGL</td>
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<td>PGL</td>
</tr>
<tr>
<td>240</td>
<td>PGL</td>
</tr>
<tr>
<td>241</td>
<td>Lymphadenopathy, oral candida, recent herpes</td>
</tr>
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<td>PGL, HBsAg carrier</td>
</tr>
<tr>
<td>243</td>
<td>AIDS contact</td>
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<tr>
<td>244</td>
<td>PGL</td>
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<td>AIDS</td>
</tr>
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<td>PGL</td>
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<td>247</td>
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<td>PGL</td>
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<td>PGL HBeAg carrier</td>
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</tr>
<tr>
<td>270 - 300 inclusive</td>
<td>Haemophilia</td>
</tr>
<tr>
<td>338</td>
<td>AIDS</td>
</tr>
<tr>
<td>339</td>
<td>AIDS</td>
</tr>
<tr>
<td>340</td>
<td>AIDS</td>
</tr>
</tbody>
</table>
Potential false positive group (PFP)

- Rheumatoid factor positive
- anti rubella IgM positive
- anti HAV IgM positive
- anti HBc IgM positive
- Paul Bunnell positive
- anti human parvovirus (B19) IgM positive
- promyelocytic leukaemia
- Hodgkin's disease
- Ac lymphoblastic leukaemia
- multiple myeloma
- chronic lymphatic leukaemia

AIDS cases, see high risk group

- anti HLA DR4, B5
- " DR1, 2, A2
- " DR4, 7
- " DR3, 7
pan-reactive anti-lymphocyte antibody positive human sera identified by the UK transplant service

Single species auto antibody positive human sera

Sera used in dilution series (1/50-1/800)

i. V 10363/85
ii. V-10754/85
iii. V 10761/85
iv. V 12424/85
EVALUATION OF SCREENING TEST KITS FOR HTLV III ANTIBODY

In his press release dated 27 June the Minister for Health announced that a test would be introduced to screen all blood given by blood donors for antibodies to the virus which causes AIDS. In his letter of 30 July, Mr Hart advised Regional General Managers of the need to provide alternative testing arrangements, outside the National Blood Transfusion Service, for people who fear they may have been exposed to the virus.

The first stage of the evaluation of commercially available test kits has now been completed on behalf of DHSS by the Public Health Laboratory Service. The outcome of that evaluation has been considered by a panel of experts and a summary of their recommendations is attached. The National Blood Transfusion Service is now undertaking its own 2nd stage evaluation covering aspects peculiar to the use of kits in the blood donation screening context.

A more detailed account of the PHLS evaluation will be available on request later this month. Any enquiries should be addressed to David Kennedy, Room 325, 14 Russell Square, London WC1B 5EP: Tel: 01 636 6811; or Peter Lister, Room 1004 Hannibal House, as above, Tel: 703 6380 ext 3328.

The Department has funded the PHLS to set up laboratory facilities to confirm the results of any blood donations found positive in the National Blood Transfusion Service, and to test the samples taken in Departments of Genito-Urinary Medicine or elsewhere. However, recipients of this letter are asked to ensure that copies are passed to all those who might be involved in the supply and use of test kits.

Yours sincerely

M A HARRIS
Health Services Division
EVALUATION OF KITS FOR THE DETECTION OF THE ANTIBODY TO HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE III (ANTI-HTLV III)

On behalf of the Department, the Virus Reference Laboratory of the Public Health Laboratory Service has evaluated five commercially available kits for the detection of antibody to HTLV III, a marker of infection with the causative agent of the acquired immune deficiency syndrome (AIDS). The kits evaluated were those of Abbott Laboratories Ltd, Electronucleonics Inc, Organon Teknika Ltd, Ortho Diagnostic Systems Ltd and Wellcome Diagnostics. The evaluation protocol, which was agreed with an expert working group and the manufacturers, was designed to investigate the performance of the kits with sera from unselected blood donors, sera from groups of patients with AIDS or AIDS-related diseases, and sera from groups of patients in which false positive results were a possibility. The performance of the kits using sera that had been heat-treated to inactivate the virus was investigated, and they were also assessed for their ease of use. The evaluators were trained by the manufacturers' representatives and the kits were used in the way specified by the manufacturers in conjunction with equipment supplied by them.

The results of the evaluation were considered by the expert working group and manufacturers were asked to comment. The following recommendations are made by the Department:

Kits most suitable for use in Diagnostic Laboratories

Organon Teknika Ltd - Vironostika anti-HTLV III (Indirect ELISA)
Ortho Diagnostic Systems Ltd - HTLV III BioEnzaéhead (Indirect ELISA)
Wellcome Diagnostics - Welcozyme anti-HTLV III (Competitive ELISA)

These kits provided a clear distinction between positive and negative results, a low rate of false positives and gave reliable results with heat-treated sera.

The other kits were less satisfactory. In particular, they produced an unacceptable number of apparently false positive results, and generally gave unreliable results with heat-treated sera. Abbott Laboratories has since emphasised that heat-treatment of sera before testing was not part of the company's standard operating procedure. Manufacturers made a number of comments which will be included in the full report.

Evaluation in Blood Transfusion Centres

The second stage of the evaluation is designed to investigate the performance of kits in large scale screening of blood donations. The expert working group considered that the results of the PHLS evaluation indicated that the following products were particularly suitable for use in Blood Transfusion Centres.
Organon Teknika Ltd - Vironostika anti-HTLV III
Wellcome Diagnostics - Wellcozyme anti-HTLV III

These kits were especially easy to use. Wellcome's product, which had five procedural steps (the lowest number), could provide results in 2 hours. Organon's kit, which had 8 procedural steps, could provide results in 2 hours and 50 minutes. These two kits are the first to be assessed on behalf of the DHSS in the second stage of the evaluation.

Further Information

A full report of the first stage evaluation will be available later in August, and the results of the second stage evaluation will be reported at a later date.

Further information can be obtained from:

Mr D A Kennedy
DHSS
Scientific and Technical Branch
Room 325
14 Russell Square
London WC1B 5EP

Mr P Lister
DHSS, Medical Division
Room 1004
Hannibal House
Elephant and Castle
London SE1 6TE
Western blot analysis of the evaluation panel of specimens

The specimens used in this investigation, except for the four diluted sera, were submitted randomised and under code to Biotech Research Laboratories, Inc. Rockville, Md, U.S.A. for Western blot analysis. These examinations were done on unheated specimens only. The results were reported as reactive/non-reactive against two protein bands of HTLV III, p 24 and gp 41. For three specimens a reaction against p 55 was noted. Specimens that were reactive against either p 24 or gp 41 were considered by Biotech Laboratories to contain anti-HTLV III.

Results were as follows:

Blood donors: 214 specimens were non-reactive. Five contained anti-HTLV III (see Table 8). One reacted against p 55 only. All 220 were anti-HTLV III negative in phase 1 by the seven solid phase assays.

<table>
<thead>
<tr>
<th>by Western blot</th>
<th>p 24</th>
<th>gp 41</th>
<th>p 55</th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>+</td>
<td>-</td>
<td>+ 1</td>
</tr>
<tr>
<td>174</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>189</td>
<td>+</td>
<td>-</td>
<td>+ 2</td>
</tr>
</tbody>
</table>

High risk group: On Western blot analysis 73 specimens contained anti-HTLV III including the 72 positive by the solid phase assays for anti-HTLVIII:67 of these reacted against both p 24 and gp 41, 3 against gp 41 only and 3 against p 24 only. One of the latter (no 254) was anti-HTLV III negative in all seven solid phase assays. The remaining ten high risk group specimens (which were negative in the seven solid phase assays) were non-reactive by Western blot analysis (see Table 9).
Table 9: Western blot analysis of high risk group specimens

<table>
<thead>
<tr>
<th>Result by</th>
<th>Western blot</th>
<th>+</th>
<th>+</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>all 7 solid phase assays</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

No of specimens | 72 | 1 | 0 | 10

Potentially false positive group

All 57 specimens in this group were non-reactive.

Comment

Five blood donor specimens were anti-HTLVIII positive by Western blot and these were probably false positive results. Similarly one apparently false positive result arose in the high risk group. In none of these 6 cases did the specimens react against both p 24 and gp 41. Single band reactions occurred in only 5 (72) specimens out of 72 in the high risk group thought to be truely anti-HTLV III positive. There were apparently no false negative results by Western blot analysis.

These findings suggest that it would be unwise to rely on the use of Western blot analysis alone to confirm positive results obtained in anti-HTLV III/LAV assays.
APPENDIX E

Immunofluorescence tests on the evaluation panel of specimens

The plates containing one set of heat treated specimens were recoded into a random series and tested blind by Dr M S Pereira for immunofluorescent antibody to HTLV III (see Mortimer P.P., Jesson W.J., Vandervelde E.M. and Pereira N.S. 1985, British Medical Journal, 11 pp.1176-78). Her results were:--

i) Blood donors: all 220 anti-HTLV III negative.

ii) High risk group: 14 out of 83 anti-HTLV III negative, viz.
    numbers 224, 228, 248, 249, 253, 254, 256, 270, 271, 279,
    282, 299, 339, 340. The remaining 69 were anti-HTLV III positive.

iii) Potential false positive group: 55 out of 57 were anti-HTLV III
    negative. Numbers 309 and 337 were anti-HTLV III positive.

NB Immunofluorescence tests on the dilutions of the four anti-HTLV III
    positive sera tested in the other evaluations will be done and reported
    later.

Comment

Three specimens, 228, 339, 340, that were anti-HTLV III positive by all phase 1
assays in the evaluations were negative by immunofluorescence and two, 309,
337, that were anti-HTLV III negative by all phase 1 assays (except 337 by ENI)
were positive by immunofluorescence. This amounts to 3 probable false negative
and 2 probable false positive results. The degree of accuracy is comparable
with that achieved by the more accurate of the solid phase assays and by
Western blot analysis. It indicates that, in experienced hands,
immunofluorescence antibody testing would be a useful confirmatory anti-HTLV
III procedure if used in conjunction with other confirmatory tests.

KENAAL

67.