Public Health Laboratory Service

PHLS

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Dr Skinner
Mr Lister
Mr Kennedy
This is very useful and will I hope stimulate RTCs to get their act together. No doubt it will be debated in Manchester on 24 March.
[DHSS STD are funding this].
AS 15/3.

Our ref

Your ref

March 11th 1987

Dear Dr [illegible]

I enclose a draft report based on information gathered on a questionnaire completed by 23/24 of the RTCs which report their anti HIV results to the [illegible] in Manchester. I have circulated this draft to the RTC members with their code numbers and invited their comments and amendments which I plan to incorporate in the final version.

Yours sincerely

Janet [illegible]

I [illegible]

DHSC0000980_0001

DRAFT

March 10th 1987

RTC operation of anti-HIV screening, January 1987: a report to Regional Transfusion Directors with proposals for their consideration.

Introduction

Routine anti-HIV screening was introduced in the UK Blood Transfusion Service in October 1985 and has become an established part of donation screening. Although most Regional Transfusion Centres have used a single commercial test (Wellcozyme) throughout, there has seemed to be considerable diversity between RTCs in the details of test procedure. It was decided to investigate this diversity by asking each centre to report on the way the testing was being done.

Method

In January 1987 a questionnaire was sent to 24 RTCs asking for details of the way anti-HIV testing was currently performed in their centres. The questions included ones on the testing of quality control specimens, the ways in which specimens were dispensed and enzyme conjugate added, the operation of the plate washer, the criteria for retesting and referral of specimens, and any planned changes in the testing. The respondents were asked to include a photocopy of the read-out (and any further analysis) from a typical plate with their return. Each participating centre was given a one letter code, and their responses were entered on a microcomputer under that code. The original wording of these replies was retained as far as possible within the limitations of the data entry format.

Results

Twenty three of the 24 centres completed and returned their questionnaires. Their responses are given, under code, in the accompanying lists.

All but two of the centres (A and S) used the Wellcozyme test at the time of the survey. There was variation in the reported proportions of specimens retested, and evidence of a rise in the number of problems associated with the Wellcozyme test (List 1).

All the centres made use of the quality control specimens distributed by the PHLS Division of Microbiological Reagents and Quality Control (List 2). In several centres (H,L,O,R,T) one or more of these were used on every plate, and in all except 2 (C and P) they were in use at least once on any day on which testing took place. The DMRQC "panels of 6" were stated to be used at least once a week by every centre except E,Q and X.

The variations in the time taken in dispensing plates of samples, the timing of adding conjugate and the hardware used in the procedures are shown in List 3.

Four centres (H,I,M,A) reported variations from the kit instructions in washing technique, and these and the checks used on the operation of the washer are given in List 4.

Only four centres incubated the tests in a water bath. Most of the rest described some means of increasing the humidity in their incubator (List 5). Only one, Q, reported that it found such a precaution unnecessary.

It is not clear how many different makes of OD reader were in use, as it seems likely that what is basically the same machine can appear under different names. However at least 3 basic types are listed (Multiscan, Skatron and Dynatech) and Wellcome supply readers under their own label. Of these Multiscan and the readers supplied by Wellcome apparently offer a small matrix of qualitive results to

supplement the listing of OD readings.

The calculation of the cut-off value is subject to considerable local adaptation. The current Wellcozyme test called for the use of the mean of 3 cut-off control wells with an additional 10% defining the equivocal zone, but centres D,H,I and N used that mean plus 20% and V added 30% in defining the cut-off value for positive specimens. At some centres, eg M, the mean was used and the interval between it and 10% above is regarded as the "grey zone". Centre W has retained the use of a previous cut-off specimen.(List 6)

The selection of specimens for retesting varies both quantitatively and qualitatively (List 7). All centres define an equivocal zone adjacent to the cut-off and will retest any specimen falling within it, but the bound of this zone varies from "cut-off + 10%" to "cut-off + 30%". Many centres also retest specimens with OD values that seem to be outliers of the distribution of values for the negative specimens, either in the direction of the cut-off (weakly reactive specimens) or as "super negatives". However these specimens are difficult to select without computerised output, and several centres lack this. The selection of specimens for confirmatory testing is apparently influenced by this difficulty, as well as by policy. Some centres referred all specimens repeatably within any of the zones described above, while one, O, referred only those repeatably positive. Thirteen RTCs stated that they sent duplicates to the Middlesex Hospital of at least some of the specimens referred for confirmatory testing locally. A 14th used the Middlesex as its primary confirmatory centre (List 8).

The greatest diversity was apparent in the methods of analysing the results of anti-HIV testing (Lists 9 and 10). Nine centres

(T,H,O,P,Q,S,U,V) had no computer linkage, and relied on operator calculations and visual appraisal of the read-out to identify the positive and equivocal results. Five of these laboratories used the facility of their OD reader to re-read the plate and produce a small matrix showing the plate position of reactive specimens. The operator had to enter a value for the reader to use in distinguishing these. Optionally the range of reactive readings could be divided into 10 regions scoring 0 (strongly positive by Wellczyme) to 9 (close to the chosen distinguishing value). The remaining four centres (C,O,Q,U) relied solely on checking the printed list from the OD reader.

Fourteen centres had computer generated output. This varied both in its content and presentation (List 10). All except one, A, apparently produced a list of positive and equivocal results with the specimen identified either by its place on the plate or the donation number, or both. Figure 1 gives examples of some of the forms of OD and result output. The mean of the negative donation ODs was calculated in 10 centres, and their standard deviation in 9 of these (see list 10) . In centres B,G,M,R and W these values were used to find "super negatives". Other centres, e.g. K and N, used the cut-off mean multiplied by a chosen number to distinguish these. In one centre, B, the computer analysis of each plate was part of a much bigger system which also produced monthly summaries of the testing done, the repeats and the control specimens results.

In many centres graphs of each plate's OD values were apparently routinely used both as a check on the performance of that test plate, and to help in the selection of "outlying" negative specimens which did not fall in one of the numerically defined repeat zones. The range

and scale of these graphs showed variation, and a selection are reproduced in Figure 2. One, from R, used 0.5 of a standard deviation of the negative ODs as its scale unit, and did not show values beyond +/- 5 SDs from the mean. Other graphs showed the distribution over either the whole possible range of OD values or the range of values from that plate. Some marked the control specimens by a distinct symbol, while others used the same symbol as for test specimens or did not show control specimens on the graph. One centre (I) had the control results displayed on a separate graph below that of the test specimens.

The questionnaire did not include an enquiry about plate validation, but on 10 of the returns the method of validation was obvious. In 6 cases this was part of the computer print-out, in 4 it was performed by the operator. There were computer listed OD values for the control specimens in 10 cases. In several centres the output, whether computer or operator generated, included a field to be signed by the individuals responsible for checking the results.

The comments made and changes foreseen in testing are given in List 11.

Discussion

Between the introduction of screening in October 1985 and the end of January 1987 73 anti-HIV positive blood donations were found in the British Isles. This gives a rate of about 1 positive in every 50000 donations, which is considerably lower than the rate in other parts of Western Europe. The difference in incidence could reflect a lower rate of sero-positivity in the British population or more effective exclusion from donation of those at high risk. However the possibility remains that the difference in rate is attributable to positive

reactions being overlooked or to false negative reactions occuring in the tests. The responses to this enquiry should be examined with these possibilities in mind.

Overlooked positive reactions

As Figures 1 and 2 show there is a wide range of output from the BTS anti-HIV testing, and the positive results are much easier to distinguish on some, where they are clearly listed, than on others, where the operator has to pick them out from the OD reader print-out. The number of specimens examined will affect the demands on the concentration of operators checking output for positives, but probably even the smallest centres should have some machine assistance with this job. The use of an OD reader with the "matrix" option would at least high-light those results which demand closer attention. Computer linkage provides the oportunity for listing all screen positive specimens and should prevent positive results being missed.

False negative reactions

The avoidance of false negative results is more difficult to achieve. Some of the considerations which arise are general in nature, but others relate to the Wellcozyme test and its dominant position in anti-HIV testing in the Transfusion Service. In general it has been accepted that there are HIV positive specimens that are strictly speaking test negative but nevertheless show some reactivity. This was demonstrated at South London RTC last April when a specimen was found which was repeatably between 10 and 30% above cut-off and was positive on confirmatory testing (B Cant, personal communication). Because of the existence of such specimens all centres define an equivocal zone or "grey area" and reactions falling in this zone are identified with

or without the aid of a computer. Any specimen which is repeatably in this category should be referred for confirmatory testing. The width of the equivocal zones defined and the terminolgy used vary between RTCs and it would be useful if both could be standardized throughout the Transfusion Service, for each assay in use.

Another class of results recognised by most centres is those "reactive but beyond the defined equivocal zone". All specimens repeatably showing some reactivity deserve further attention, whatever the screening assay used. However, it is particularly important that the Wellcozyme test should be kept under review in this way. As this kit is not widely used by other European or the North American transfusion centres it has been suggested that the low rate of positivity found in the British Isles may be attributable to a lack of sensitivity in this test. Routine retesting of such weakly reactive specimens by a methodologically different test is needed to investigate this suggestion. This can be done either within the RTC or by a confirmatory laboratory. It is only possible to recognise these donations with reference to the distribution of the OD values of the other negative specimens on the plate. It is difficult to find them without the help of a computer to give the characteristics of this distribution against which the results can be checked for outliers. This may be done through the production of a graph, or for the Wellcozyme test where the negative results are approximately normally distributed, in terms of the mean and standard deviation. With the Wellcozyme test "super negatives" should also always be looked for as such specimens have not, in effect, been tested for anti-HIV; any donation repeatably in that category should be tested by a methodologically different test. The consistent recognition of "super

negatives", too, is difficult without the aid of a computer to either draw a graph or compute the mean and standard deviation of the negative donation results of each plate .

Theoretically the possibility of an undetectable false negative arises in the Wellcozyme test if "stickiness" competes with anti-HIV positivity to produce an OD value in the normal negative range. A record of the number of repeatably "super negative" results found in each RTC is needed to estimate the undoubtedly very low probability of such an event in donor screening.

Proposals for your consideration

1) That anti-HIV OD readings should be computer linked in all RTCs and that the computer system should:-

a) identify and list all specimens with positive or equivocal ODs.

b) check that the plate meets test validity criteria.

c) draw a graph showing all the results, with the control specimens distinguishable from test ones.

d) (Wellcozyme only) calculate the mean and standard deviation of the negative donations on each plate and use these values to identify "weakly reactive" and "super negative" results.

2) That the output of every plate should be signed by the individuals responsible for reading and checking it.

3) That the validity of each plate should be checked and recorded on its output.

4) That attempts should be made to standardise throughout the Transfusion Service:-

a) the terminology (cut-off, equivocal, weakly reactive, and super negative)

b) the criteria for retesting specimens

c) the criteria for referral for confirmatory testing.

5) That counts be kept and gathered centrally of the numbers of specimens referred under each criterion.

JANET MORTIMER
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