ANTI-HCV TESTING OF BLOOD DONATIONS

COMPENDIUM OF RECOMMENDATIONS
MADE BY THE
U.K. ADVISORY COMMITTEE ON TRANSFUSION TRANSMITTED DISEASES

August 1991

INTRODUCTION

There has been considerable debate about the introduction of anti-HCV testing of blood donations which has occupied many months.

The U.K. Advisory Committee on Transfusion Transmitted Diseases has discussed, in detail, all aspects of this additional test to be applied to blood donations and the subsequent handling of donors thought to carry HCV infection.

The minutes and various accompanying papers of this Committee have been circulated to RTDs over a period covering approximately 18 months. However, I thought it would be useful to combine the recommendations into a single compendium.

The status of these recommendations are that they have been put forward by an Advisory Committee of the NBTS and SNBTS. They have been made after careful consideration of all the issues involved. They should not be interpreted as Government policy but, instead should be regarded as guidance for use by the staff involved in the RTCs.

Some issues have not yet been finalised and attention is drawn to these where appropriate.

H.H. GUNSON
Chairman
U.K. Advisory Committee on
Transfusion Transmitted Diseases

August 1991

1. THE STARTING DATE AND ITS DEFINITION

1.1 It was recommended that the testing of blood and plasma would commence on a specified date. There would be no retrospective tests carried out on donations collected prior to that date.

1.2 The date of 1st September 1991 has been agreed for commencement of tests.

2. TESTING OF BLOOD DONATIONS AT RTCs

2.1 An "Action Chart" (page 2) indicates the steps recommended for testing donations at RTCs and gives a definition of a positive reaction.

2.2 An earlier chart included the performance of an ALT test. ALT levels would not assist in defining HCV seropositives, may confuse the issue, and may pose difficulties in including this data in the information technology required. It was agreed that ALT testing would not be recommended as a routine. ALT and other tests of liver function will, almost certainly, form part of the clinical follow-up.

2.3 The procedure outlined in this "Action Chart" may be modified following consideration of the results obtained in the eight week study of HCV confirmatory testing commencing 1st September 1991 (see Section 4).

3. CONFIRMATORY TESTING

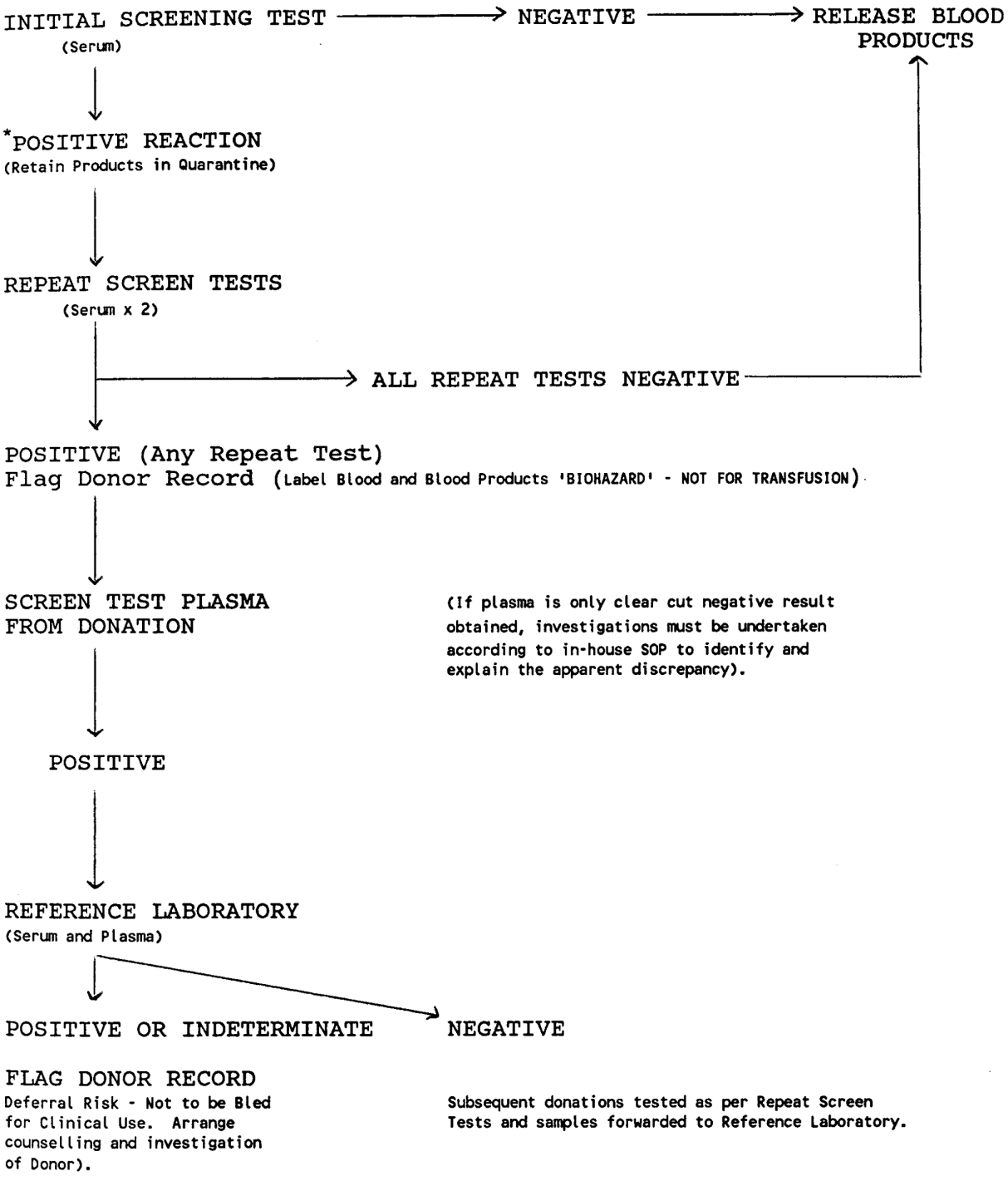
3.1 The preferred policy recommended was that samples of serum/plasma from a repeatably positive ELISA screening test should be sent to a reference laboratory for appropriate confirmatory tests.

3.2 Some members of the Committee considered that, providing appropriate expertise was available, confirmatory tests could be carried out at RTCs. This would not only reduce the workload of the reference laboratories but also the results would be available more quickly. It may also reduce costs.

3.3 It was pointed out, however, that although the 4 band RIBA II test appeared to be the most appropriate test at the present time, it may be replaced in the future by improved tests. Part of the work of reference laboratories is to evaluate novel tests and apply them to referred samples. Without reference work this could delay improvements in confirmatory testing.

3.4 It was recognised that, in particular, those samples which were RIBA II indeterminate would benefit from having a PCR performed and that the PCR result would be valuable in counselling donors.

ACTION CHART - ANTI-HCV TESTING



DEFINITION: Biohazard blood and products labelled 'BIOHAZARD' must be isolated and not used for transfusion. Products must be discarded or stored in Microbiological Laboratory for future investigation.

* Positive reaction is any test which meets manufacturers criteria or internal SOP where sensitivity is not less than manufacturers criteria.

DAC.HCVT (6/2/91)

3.5 The cost of performing a PCR test was the principal deterrent to their use in England and Wales and some members of the Committee considered that a donor who was confirmed seropositive, but PCR negative, would have to be referred for further clinical evaluation was sufficient reason for not using this test routinely. SNBTS were planning to carry out PCR tests on RIBA positive and indeterminate results.

3.6 A confirmatory test system could be operated without PCR tests, although in all likelihood this test would be needed by that part of the service performing the clinical follow-up of donors.

4. CONFIRMATORY TESTING DURING SEPTEMBER AND OCTOBER 1991

4.1 In view of the above difficulties Dr. P.P. Mortimer proposed that an eight week study of HCV confirmatory testing should be conducted during September and October 1991 with the results of the study reported by December 1991.

4.2 This proposal was considered at the RTD meeting and agreed. RTCs in the Northern Division would refer samples to PHLS in Manchester; those in the Eastern Division to University College Middlesex School of Medicine (UCMSM) and those in the Western Division to PHLS, Colindale.

4.3 These proposals refer to England and Wales. The situation with respect to Scotland will be reviewed.

4.4 **The eight week RTC study of HCV confirmatory testing September - October 1991**

Introduction

There are at least three candidate anti-HCV screening assays available, Ortho, Abbott and UBI, and two confirmatory assays, RIBA II and Organon. Of the latter, RIBA II is currently being used by most transfusion services seeking to confirm anti-HCV screening results. Considerable published and unpublished data has accrued to suggest that most RIBA confirmed positive sera are reactive in all the three screening assays and that most RIBA confirmed positive sera are strongly reactive in screening assays.

Aim of the Study

The purpose of the eight week study (from 1st September 1991) is to examine whether, on screen reactive British donors, there is a close concurrence between RIBA II positivity and:

(i) combined repeatably positive reactions in screening assays

(ii) strong repeatably positive reactions in single screening assays

If concurrence is close it may be possible to interpose a second anti-HCV screening assay into Regional Transfusion Centres (RTCs) testing in between the initial screen and the referral for RIBA II or other confirmatory testing. This would result in significant savings in time and expense.

Procedure for the Study

(i) The study calls for all donor sera repeatably reactive in the initial screening assay in use at each RTC to be referred to one of the three confirmatory laboratories as previously agreed. The three confirmatory laboratories will contact individual RTCs to ensure that the arrangements for referral of samples are satisfactory and well understood.

(ii) Each referred sample will be tested with RIBA II and the result will be reported to the RTC within one week. This will allow the RTC to arrange for the disposal of the cellular and plasma components of the donation and take steps for referral of the donor if this is required. Plasma donations from RIBA II positives should be retained frozen for possible future use for standards or Q.C. panels.

(iii) Each referred sample will be tested with other ELISA screening tests including the test used by the referring RTC.

(iv) When the testing has been completed the results on individual donors will be reported to RTCs and the full study results collated and analysed centrally. A report of this analysis will be available by the end of December 1991.

(v) When the results have been discussed in the NBTS it should be possible to define a policy for the screening and referral of samples from blood donations.

Action to be taken in November and December

(i) During this period all repeatably reactive donor samples will have to be subjected to confirmatory tests.

(ii) RTCs should make their plans for the venue for these confirmatory tests and for the handling of the donations and donors.

Costs involved in the Study

(i) Based on the estimate that between 0.5 and 1% of donors are repeatably reactive in current HCV screening assays the expected number of specimens that will be referred will be between 1500 and 3000. (In fact, some RTCs are now consistently finding <0.5% samples are repeatably reactive).

(ii) All the tests performed by the confirmatory laboratories will be done at the cost of £62.50 per referred sample.

(iii) Cost of the analysis of results. Dr. Gunson is examining ways in which this can be accommodated.

5. DISPOSAL OF ANTI-HCV POSITIVE DONATIONS September-December 1991

5.1 During the course of the study being carried out by PHLS/UCMSM donations will have to be handled according to the results of a RIBA II test.

Following referral of a repeatably positive screening test there are three possible results.

5.11 **Screen Test Positive, RIBA II Positive**

All products from the donation should be discarded (although plasma may be required by PHLS, Colindale, for preparation of controls).

5.12 **Screen Test Positive, RIBA II Indeterminate**

All products from the donation discarded (although plasma may be required by PHLS, Colindale, for preparation of controls).

5.13 **Screen Test Positive, RIBA II Negative**

Cellular products discarded. Plasma used for fractionation only, (but please see Section 6).

Subject to local circumstances the donor may continue on the active panel for plasma for fractionation only.

5.14 Dependent upon the results of the study during September and October (with results due in December) the recommendations for disposal of donations may be amended.

6. PLASMA FOR FRACTIONATION

6.1 The Department of Health's Advisory Committee recommended that it was necessary to be consistent in the testing of plasma and whole blood, and, therefore, both should be tested for anti-HCV. However, the situation is not straightforward and this matter will have to be considered further.

6.2 There has, as yet, been no formal communication from the Medical Control Agency who are the Regulatory Authority for the product licences.

6.3 **The current situation is that BPL have agreed to accept plasma from donations specified in Section 5.13 providing this is operationally feasible.**

7. RECOMMENDED PROCEDURE FOR THE MANAGEMENT OF ANTI-HCV POSITIVE DONORS

The following is an ideal procedure for managing anti-HCV positive donors when counselling is to be undertaken at RTCs. It should be seen as a guideline to providing a reasonable standard of care, but it is acknowledged that for practical reasons the procedure may be adapted to local circumstances without jeopardising the standard of care, e.g. referral to a consultant physician by the donor's general practitioner after obtaining permission to do so.

7.1 Donors with repeatably positive screening test but negative confirmation

It is recommended that these donors should be kept on the panel, but placed on "medical hold" or some equivalent status which prevents transfusion of future donations. No policy has yet been formulated to allow re-entry of donors whose screening test subsequently becomes negative, pending the results of studies of test performance. Donors should not be informed of unconfirmed positive screening tests.

7.2 Donors with positive screening tests and positive confirmation, as defined by the specialist microbiological laboratory

7.21 As soon as positive confirmation results are received, the donor must be placed on **"permanently withdrawn"** status.

7.22 The standard letter is sent, informing the donor that the test is positive and requesting attendance for further samples (note: letter should be timed to reach donor during the first half of the week).

7.23 An appointment for initial counselling and assessment is offered at the earliest opportunity (note: allow at least 1 hour).

7.24 The initial counselling and assessment is carried out by a Medical Officer experienced in donor counselling.

7.25 The donor is informed that he or she must not give blood or carry an organ donor card. Permission to inform the donor's GP is requested.

7.26 The donor is given the written advice (provided by each Centre individually) on the implications of a positive test.

7.27 The donor should be given a contact telephone number for further advice.

7.28 The donor may be offered a second counselling interview within 1 week if it is considered necessary or at a time when the results of further testing, if performed, will be available.

7.29 Samples are taken for repeat HCV antibody tests (screening and confirmation), hepatitis B markers, liver function tests (preferably including ALT) and any other tests considered to be indicated by the Medical Officer.

7.3 **Donors confirmed anti-HCV positive on the second sample**

7.31 On receipt of the repeat results, provided they confirm the donor's seropositivity, the donor is informed and advised on the need for further investigation or follow-up.

7.32 The Medical Officer decides on the need for specialist referral or follow-up, based on the epidemiological features and results of liver function tests.

7.33 The donor's General Practitioner is informed by letter, with the donor's permission.

7.34 The question of look-back has still to be defined for England and Wales. In Scotland it is initiated in accordance with SNBTS policy.

7.4 **Donors with negative tests on the second sample**

7.41 Proceed according to flow chart to investigate the apparent discrepancy.

7.42 Review donor status in light of investigation, and proceed accordingly.

8. RECOMMENDATIONS FOR COUNSELLING OF HCV SEROPOSITIVE DONORS

8.1 Informing the donor

8.11 The Letter

The initial contact will usually be by a standard letter, which should be reassuring in tone and specifically mentions that the positive test is for hepatitis C and has nothing to do with AIDS. The donor will be invited to come back for further testing in order to clarify the significance of the findings. An early appointment should be offered.

8.12 The Interview - First Counselling Session

Requirements:

Donor record, including list of previous donations, screening and confirmatory test results with their interpretation, and any further test results on original serum if these are available, e.g. ALT, anti-HBc, anti-HBs.

8.13 Blood samples:

It is recommended that further specimens be taken in order to confirm the results on the donation and to check liver function tests.

8.14 Epidemiological data:

It is recommended that the route of infection should be identified and recorded at the first interview, as such data are likely to prove extremely valuable (eg drug abuse, previous transfusion, etc).

8.15 Breaking the news:

The initial news-breaking should be direct and simple, with the minimum of preliminary talk. The essential information is that one of the tests done on every donation has shown a positive reaction. Explain that this is a new test for a mild form of hepatitis, or jaundice, called hepatitis C. This can be passed on by blood transfusion, but we have not been able to test until now.

At this point it will generally be appropriate to allow the donor to ask questions, but it is recommended that the following information must be conveyed to the donor at the initial consultation, and preferably reinforced at a subsequent interview:

8.151 That chronic liver damage can occur, and that their liver function therefore should be assessed. If abnormalities are detected, long-term follow-up will be necessary.

8.152 That even where abnormal liver function is detected, the prognosis is good in the majority of cases, and treatment is available for those in whom more severe liver disease occurs.

8.153 That there is little known about the routes of spread of the virus in the population, and that sexual transmission does not readily occur. Detailed instructions about protecting others should be given, preferably in written form.

The following list of questions and answers is meant to illustrate sorts of questions the donors may ask and will give the basis for satisfactory answers. The questions are in no particular order.

Q: **What does a positive test mean?**

Since only donors with positive confirmatory tests will be counselled, it is reasonable to explain that we do extended testing with very specific tests, so that we are already fairly sure that the donor truly has antibodies to the virus known as hepatitis C.

This virus is very common in the population - about 1 in every 1000-1500 donors has a positive test. This may mean that they have been in contact with the virus at some time in the past. Emphasize that the tests detect antibodies, not the virus itself, and that the virus is not necessarily still present. If the PCR test for viral genome is available, a positive will mean that the donor must be regarded as infectious (but a negative does not necessarily rule out infectivity).

Q: **Does it mean I've got hepatitis?**

At the moment we have very little in the way of data from blood donors with anti-HCV, but from studies of patients who develop post-transfusion hepatitis C, we know that the vast majority have no symptoms whatever, the

infection just showing up as a rise in transaminases (sometimes referred to as "transaminitis"). In about half of those infected the liver function abnormality lasts 6 months or more. In a third of these a liver biopsy will reveal some evidence of inflammatory activity, and in approximately 10-15% this may ultimately result in chronic active hepatitis or cirrhosis. It is worth emphasizing that the natural history of the infection in transfused patients may be quite different. Thus, it is possible that the long-term consequences are much less serious for carriers in the general population than for patients infected by blood transfusion.

If the ALT has already been done the result will be very useful in finding the reply to this question. When it is not available, it is important to emphasize that a few simple tests will help to determine the significance of the test result for the donor.

Q: **Will I die of this?**

If the donor asks for a prognosis, it will be necessary to be slightly guarded without causing alarm. If the ALT is raised explain that there are many possible reasons for this, and that it will be possible to sort it out after one or two further blood tests have been done, but that sometimes a period of monitoring will be needed to be absolutely sure of the significance. It is felt by some hepatologists that very few cases of serious liver disease due to hepatitis C occur in the community, so for most people this is an incidental finding unlikely to cause serious disease or symptoms of any kind.

Progressive chronic hepatitis C has been treated successfully with Interferon, and though this treatment is at present experimental it holds out considerable promise for the future.

Q: **How did I get it?**

Though hepatitis C is very common in the community, we have little idea as yet of the routes of spread. We don't know if it can be spread by food or water, nor is much known about mother-to-baby spread, but sexual transmission can occur (albeit not as efficiently as other viruses, eg

hepatitis B). There seems to be a high incidence in intravenous drug misusers, suggesting that
parenteral spread is the most efficient. Thus, tattoos, ear-piercing, acupuncture, dental treatment, electrolysis and so on could be relevant.

Q: **Am I likely to infect other people?**

It is not yet known with certainty what proportion of antibody-positive donors will be true carriers, able to transmit to other people. Initial studies suggested that the majority of donors would not be infectious, but this was before a confirmatory test was developed. We should regard all donors with confirmed positive tests as potentially infectious.

Situations in which others are at risk are those in which blood or body fluids may be exchanged, eg blood transfusion, needle sharing, and probably sexual contact, though it may not be logical to take any additional precautions with a longstanding partner. A condom should be advised with new sexual partners, while the necessary precautions for longstanding partners should be talked through.

There is no evidence of risk associated with ordinary daily contacts within the same household, and some evidence that there is no risk of transmission. Ordinary rules of hygiene should be observed, and donors should be advised not to share toothbrushes or razors.

Q: **Can I ever give blood again?**

At the moment there is no prospect of readmitting seropositive donors, even if on follow-up they go seronegative. Further refinements in testing may lead to this being reconsidered.

Q: **What about my previous donations?**

The recipients of previous donations will be traced and their Consultants or GPs informed. We hope to obtain results of any tests carried out. However, it may cause distress to the donor to discuss this matter in any detail. A general comment suggesting that we are going to check to see that the

recipients are alright, that they get any treatment they may require, should be sufficient, **but should only be offered if the donor asks directly**.

Q: **Could I be sued if anyone was infected?**

We guarantee the confidentiality of the donor. We strongly advise that the donor's GP be informed, but we shall not divulge the information to any other party without the donor's consent.

Q: **Could I have got if from giving blood?**

No.

Q: **Should I tell anyone apart from my spouse? My employers, for instance?**

At present there are no official guidelines, and therefore no requirement exists to inform any other person.

It is recommended, however, that your General Practitioner should be informed.

Q: **Do I need to change my diet or take any other health precautions?**

Regardless of the results of ALT etc., donors should be advised that a period of medical supervision and repetition of the blood tests is advisable, either through their GP or at a suitable hospital clinic. The only specific advice justifiable is that those with liver dysfunction should avoid alcohol, and even those with normal liver function should take no more than modest amounts.

Q: **Will it affect my insurance policies?**

This result does not affect existing insurance policies, but in taking out any new policies the donor will be obliged to answer all questions truthfully. To do otherwise, or to appear to conceal relevant information, might make any policy invalid.

APPENDICES

I - Multicentre trial (Newcastle, Glasgow and North London RTCs) using the 1st generation Ortho and Abbott tests.

Summary of results of the trial.

II - Multicentre trial using the 2nd generation Ortho and Abbott tests and the UBI and Organon tests.

IIa - Phase I - Results from RTCs
IIb - Phase II - Results from UCMSM

III - Extended trial of 2nd generation Ortho and Abbott and UBI tests at 5 RTCs (Leeds, Liverpool, Newcastle, Bristol and Sheffield).

Preliminary report from J. Craske and W.K. Paver. (It has been agreed that RIBA tests will be performed on all samples submitted from RTCs and these will be reported at a later date).

IV - Extended trial of 2nd generation Abbott test at Glasgow RTC.

V - Abbott HCV EIA 2nd generation - batch variation. A report from Glasgow RTC.

PLEASE NOTE: The information given in these Appendices should not be published or referred to in any publication without the permission of the Regional Transfusion Directors and/or Directors of Specialist Laboratories concerned.

APPENDIX I

COMPARISON OF ANTI-HCV TESTS USING ABBOTT AND ORTHO 1ST GENERATION KITS

(A MULTI-CENTRE TRIAL)

SUMMARY OF RESULTS OF THE TRIAL

H.H. GUNSON
National Directorate

FEBRUARY 1991

1. INTRODUCTION

1.1 The study to compare the results of testing blood donor samples using anti-HCV tests provided by Abbott and Ortho has been performed at three Regional Transfusion Centres (RTCs) Glasgow (South West Scotland), Northern and North London.

1.2 Testing the same blood donor samples with both tests was Phase I of the trial; Phase II consists of additional tests on those samples which were found repeatably positive by both Abbott and Ortho tests which will be carried out in specialist laboratories.

1.3 Each RTC received sufficient tests for approximately 3,500 donor sample tests during the period September/October 1990.

1.4 The initial test kits supplied by Ortho were found at the North London RTC to give negative control OD results which invalidated the quality control of the plate. The Company were advised and the test kits were replaced by another batch. Substitution of the test kits was also carried out at the other two RTCs, although at Northern RTC the negative control ODs of the first batch of test kits were well within the quality control limits. A single kit lot number was used at Glasgow RTC, and at the Northern RTC. Three different lot numbers were used at N. London RTC.

1.5 Modification of the computer programme for the Abbott Commander System installed at the North London RTC had to be undertaken by the company to allow the tests to proceed satisfactorily.

1.6 At the Northern RTC, Abbott installed a Parallel Processing Centre (PPC) specifically for the trial. The % cut-off values using this equipment varied between 0.413 and 0.547 which was higher than the OD of between 0.3 and 0.4 predicted by Abbott. On the one occasion that the equipment, which had been in use at the RTC regularly for several months, was used to perform the tests, the cut-off value was 0.383. Also, on this date the OD values for the positive control sera were virtually identical compared with greater variability with the PPC installed for the trial. This PPC may have had a minor fault but without inclusion of quality control panels the effect of this could not be evaluated. Cut-off values at Glasgow and North London RTC were consistently below an OD of 0.4.

1.7 All three RTCs reported that the tests were easy to perform and that the manufacturer's instructions were "user friendly". All tests were performed according to these instructions.

1.8 Northern RTC commented that the lack of a suitable computer package for the statistical analysis of the Abbott tests resulted in many manual calculations having to be carried out.

They also commented that the ADAMS assay data analysis and management systems used by Ortho had some operational problems because of its lack of flexibility in coping with the method of distributing samples onto the plates used at the RTC. Some blank wells read as positives and corrections had to be made. However, the plate reactive sample matrix report was useful for selecting initial screen positives and the control summary report and the daily reactive summary report were valuable. The ability to use wells in a required number makes the system versatile and cost-effective.

2. SUMMARY OF RESULTS USING ABBOTT AND ORTHO TESTS

2.1 These results are presented in Table 1 and Figure 1 and in percentage terms in Figure 2.

2.2 It can be seen that the number of initial screen positives obtained with the Ortho test is higher than with Abbott tests, but the repeatable positive rate was similar with both tests at Newcastle and North London RTCs. The repeatable positive rate for Abbott tests at Glasgow RTC was higher than that for Ortho (Figure 2).

2.3 The difference in the rate for initial screen positives is not statistically significant. However, if this trend occurred in the routine screening of 2.5 million donations it would result in a considerably greater cost, not only for the purchase of an increased number of Ortho test kits, but also in staff time involved in withdrawing into quarantine the initial screen positives and repeating the tests on a subsequent occasion.

3. COMPARISON OF REPEATABLE POSITIVE RESULTS USING ABBOTT AND ORTHO TESTS

3.1 The comparisons are shown in Table 2 and in Figure 3 in percentage terms.

3.2 It can be seen from these comparisons that the two test kits identify two population of donor samples which overlap. Samples positive with both test kits are only one-half to one-third of the total repeatable positive samples.

3.3 It is hoped that the resolution of a truly positive anti-HCV result can be obtained during the second phase of the study.

4. REFERRAL OF REPEATABLY POSITIVE SAMPLES

4.1 A total of 69 samples (Glasgow 25, Northern 25 and North London 19) were referred to each of the three specialist laboratories.

4.2 At each specialist laboratory 6 of the repeatably positive samples were PCR positive. One additional positive was found at the University of Edinburgh. Of the 6 PCR positives, 5 were positive with the RIBA II test and 1 gave an indeterminate result.

An additional 3 samples gave indeterminate results with RIBA II.

4.3 All 6 PCR positive samples were reactive using both Abbott and Ortho tests.

5. COMMENTS

5.1 The screening tests used seem to identify a significant number of repeatably positive results which were not found to be antibody positive with the RIBA II test.

5.2 All RIBA II positive samples were shown to contain viral RNA.

5.3 A more specific screening test is desirable. There is an early indication that the second generation tests will have improved specificity and a more limited study on some of the same series of donor samples is currently being undertaken.

5.4 With the Ortho screening test used in this study for the 2.2 million donations per year in England and Wales would result in approximately 10,400 repeatably positive results of which approximately 1250 would be RIBA II positive, PCR positive.

The corresponding data using the Abbott test is 9000 with 1250 RIBA II, PCR positives.

Centre	No. Samples Tested	ABBOTT ISP*	(%)	RR**	(%)	ORTHO ISP	(%)	RR	(%)
GLASGOW	3516	18	(0.51)	18	(0.51)	23	(0.65)	14	(0.40)
NORTHERN	3539	13	(0.37)	12	(0.34)	25	(0.70)	21	(0.59)
N. LONDON	3578	14	(0.39)	13	(0.36)	16	(0.44)	15	(0.42)
Total	10633	45	(0.42)	43	(0.40)	64	(0.60)	50	(0.47)

* ISP - Initial Screen Positive
** RP - Repeatable Positive

TABLE 1

Initial screen and repeatable positives using Abbott and Ortho anti-HCV tests at three RTCs

		REPEATABLE POSITIVES			
Centre	No. Samples Tested	No. Abbott Positive only	No. Abbott and Ortho Positive	No. Ortho Positive only	Total
GLASGOW	3516	9	9	7	25
NORTHERN	3539	4	8	13	25
N. LONDON	3578	4	9	6	19
Total	10633	17	26	27	69

TABLE 2

Analysis of repeatable positive results using ABBOTT and ORTHO anti-HCV tests in three RTCs.

FIG. 1: REPEAT REACTIVES v ALL TESTS

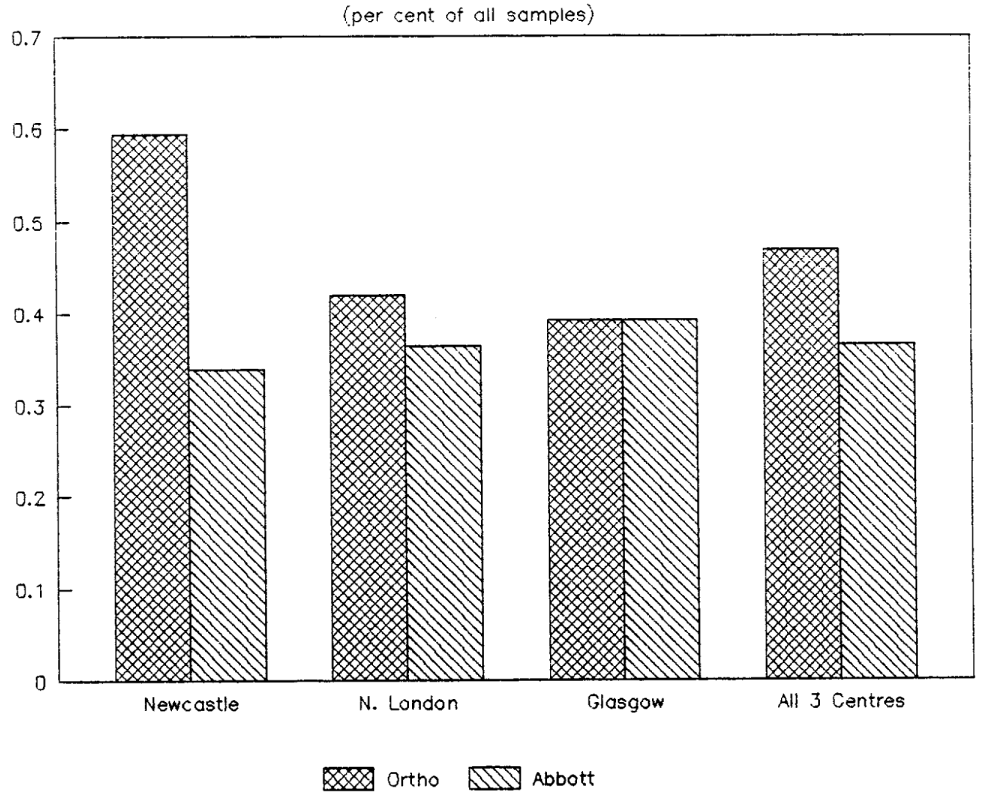


FIG. 2: REPEAT v INITIAL REACTIVES

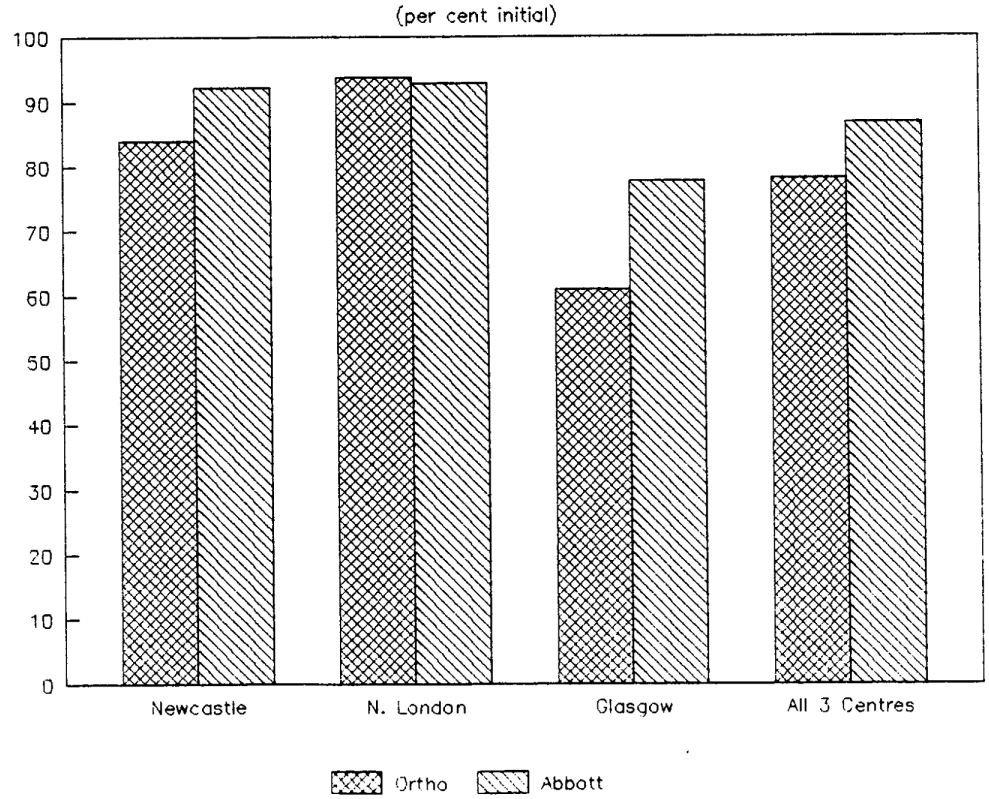
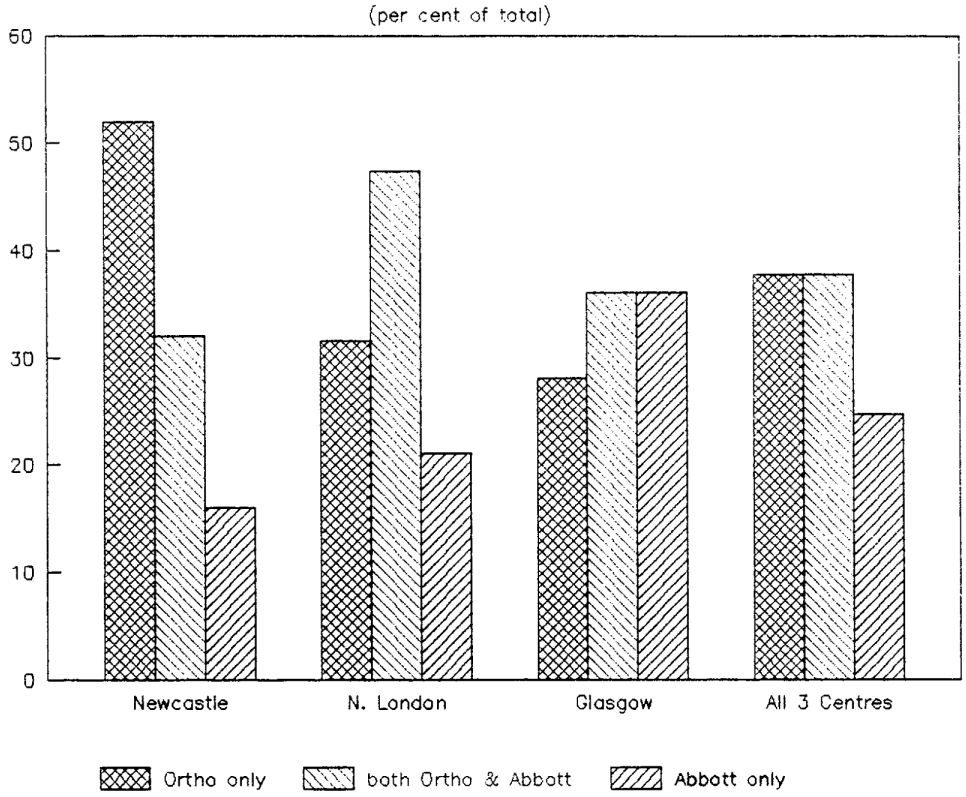


FIG. 3: ANALYSIS OF REPEAT REACTIVES



APPENDIX IIa

IN CONFIDENCE

COMPARISON OF ANTI-HCV TESTS

USING - 2nd GENERATION ABBOTT
2nd GENERATION ORTHO
UBI and ORGANON TEST KITS

SUMMARY OF PHASE I OF THE MULTI-CENTRE TRIAL

H.H. GUNSON
NATIONAL DIRECTOR

3.7.91.

1. INTRODUCTION

1.1 As many samples as possible from the multi-centre trial of the 1st generation Ortho and Abbott anti-HCV tests have been tested, according to the manufacturer's instructions, with Ortho, Abbott 2nd generation, UBI and Organon anti-HCV tests.

1.2 The tests using Abbott kits were performed in all three RTCs (Glasgow, Northern and North London), but the tests using Ortho-2, UBI and Organon were performed at the North London RTC.

1.3 All samples which have been found repeatably reactive have been sent to a specialist laboratory for RIBA-2 and PCR testing.

1.4 The confirmatory test results are not yet available and the contents of this report are a summary of Phase I of the trial, i.e. the results of the screening tests at the three RTCs.

2. RESULTS

2.1 The initial reactive results are shown in Table 1. It can be seen that results for Abbott 1 and Abbott 2 and Ortho 1 and Ortho 2 at Glasgow are very similar. There is some variation in the initial reactives for the Ortho tests at Northern and North London, but this is not consistent. UBI has the highest rate of initial reactives.

2.2 Repeatably reactive results are shown in Table 2. The results for Abbott and Ortho are consistent between the three RTCs. UBI has the highest repeatably reactive rate, but shows a 100% variation between Northern and North London samples.

2.3 The 2nd generation tests from Ortho and Abbott identify different but overlapping populations compared with the 1st generation tests.

2.4 Comparison of the overlap of repeatably reactive results using the Abbott-2, Ortho-2, UBI and Organon tests are shown in Table 3. For this purpose the results from each test have been combined for all three RTCs.

The lack of specificity of the UBI test is clearly apparent from the results shown in this table.

2.5 **In the first trial comparing 1st generation Ortho and Abbott tests there were six positive results confirmed with RIBA-2 and PCR tests. These have all been detected as a repeatable positive result with Ortho-2, Abbott-2 and UBI. The Organon test failed to react with two of the previously confirmed positives.**

There was one serum from Glasgow which gave an indeterminate result with RIBA-2 and was PCR negative in the first trial. This serum was repeatably reactive with Ortho 2, UBI, but was unreactive with Abbott 2 and Organon. In contrast, a RIBA-2 indeterminate PCR+ at North London in the first trial was repeatably positive with Ortho 2, Abbott 2 and UBI, but failed to react with Organon.

3. CONCLUSIONS

3.1 Ortho 2, Abbott 2 and UBI tests have given repeatably reactive results with all the confirmed HCV seropositives from the first trial.

3.2 UBI test has a higher initial and repeatably reactive rate than Ortho-2 and Abbott-2.

3.3 Organon test is insensitive and failed to react with two confirmed HCV seropositives.

3.4 The multiple incubations with the UBI test are a disadvantage for routine use with large numbers of samples.

3.5 Ortho changed the cut-off value during the trial and this had the effect of reducing the number of repeatable reactives.

INITIALLY REACTIVE RESULTS (IR)

TEST	GLASGOW		NORTHERN		N. LONDON	
	No. Tested	IR(%)	No. Tested	IR(%)	No. Tested	IR(%)
1. Abbott 1	3516	18(0.51)	3539	13(0.37)	3578	14(0.39)
2. Ortho 1	3516	23(0.65)	3539	25(0.70)	3578	16(0.44)
3. Abbott 2	3516	18(0.51)	3473	16(0.46)	3562	14(0.39)
4. Ortho 2	3516	21(0.60)	3467	13(0.37)	3562	28(0.78)
5. UBI	3516	26(0.74)	3467	35(1.0)	3562	66(1.85)
6. Organon	3516	5(0.14)	3467	5(0.14)	3562	24(0.67)

TABLE 1

REPEATABLY REACTIVE RESULTS (RR)

TEST	GLASGOW		NORTHERN		N. LONDON	
	No. Tested	RR(%)	No. Tested	RR(%)	No. Tested	RR(%)
1. Abbott 1	3516	18(0.51)	3539	12(0.34)	3578	13(0.36)
2. Ortho 1	3516	14(0.40)	3539	21(0.59)	3578	15(0.42)
3. Abbott 2	3516	15(0.42)	3473	12(0.35)	3562	9(0.25)
4. Ortho 2	3516	12(0.34)	3467	13(0.38)	3562	14(0.39)
5. UBI	3516	23(0.65)	3467	19(0.55)	3562	37(1.04)
6. Organon	3516	4(0.11)	3467	2(0.06)	3562	10(0.28)

TABLE 2

TESTS	NO. REPEATABLY REACTIVE SAMPLES
ORTHO 2, ABBOTT 2, UBI, ORGANON	6
ORTHO 2, ABBOTT 2, UBI only	5
ORTHO 2, ABBOTT 2, ORGANON only	2
ORTHO 2, ABBOTT 2 only	11
ORTHO 2, UBI only	3
ORTHO 2 only	13
UBI only	59
ORGANON only	8
ABBOTT 2 only	7

TABLE 3

DIVISION OF VIROLOGY
UNIVERSITY COLLEGE AND MIDDLESEX
SCHOOL OF MEDICINE

Supplemental testing for anti-HCV on referred blood donor samples from the NBTS three-centre trial.

Report on results.
12 August 1991

Professor R S Tedder
Mr C Perrons

REPORT OF CONFIRMATORY TESTS

A second screen using 2nd generation ELISAs incorporating structural proteins was carried out on 10,545 stored samples from the initial three Blood Trnasfusion Centres trial. Repeatedly reactive samples were sent to UCMSM for confirmation, those already picked up on the first screen were not included. The number received were:

North London BTC (NLBTC)	60
Glasgow and West of Scotland (GWS)	33
Northern Regional BTS (NBTC)	28
Total	121

On receipt of these samples they were immediately stored at -20°C. The following tests were carried out, once only, at UCMSM:

Ortho RIBA 2
In-house ELISA (P22/NS5)
Nested PCR using NCR "mini" primers

The volumes of samples from NLBTC and GWS were inadequate and rendered full supplemental testing difficult.

Ortho RIBA 2 (Tables)

All 121 samples were tested and scored by eye. 120 were unreactive, but this included 10 samples that showed a faint band on either the c22-3 or c33c protein. One sample (349618/Glasgow) was reactive to the structural protein c22-3 and would be classified as indeterminate by the manufacturer's criteria.

In-house anti-HCV ELISA (Figure)

All 121 samples were subjected to 1 test using UCMSM in-house ELISA. Of these 12 were reactive, including five of the 11 samples which showed some reaction on the RIBA 2 strips.

PCR for HCV RNA

The samples were not considered the most appropriate for PCR because:

1. The conditions the sample had undergone at the BTC were unknown.
2. The samples had been thawed twice at UCMSM for the above serology.
3. The volume of some of the samples received were very small. (The normal amount used in UCMSM PCR is 500µl).

In view of this and to validate as far as possible the use of sub-optimal samples, the three previously PCR-reactive samples from NLBTC were assayed for HCV RNA. Since they remained PCR-positive the remaining samples were then put forward for PCR testing.

30 samples were picked for PCR by the following criteria:

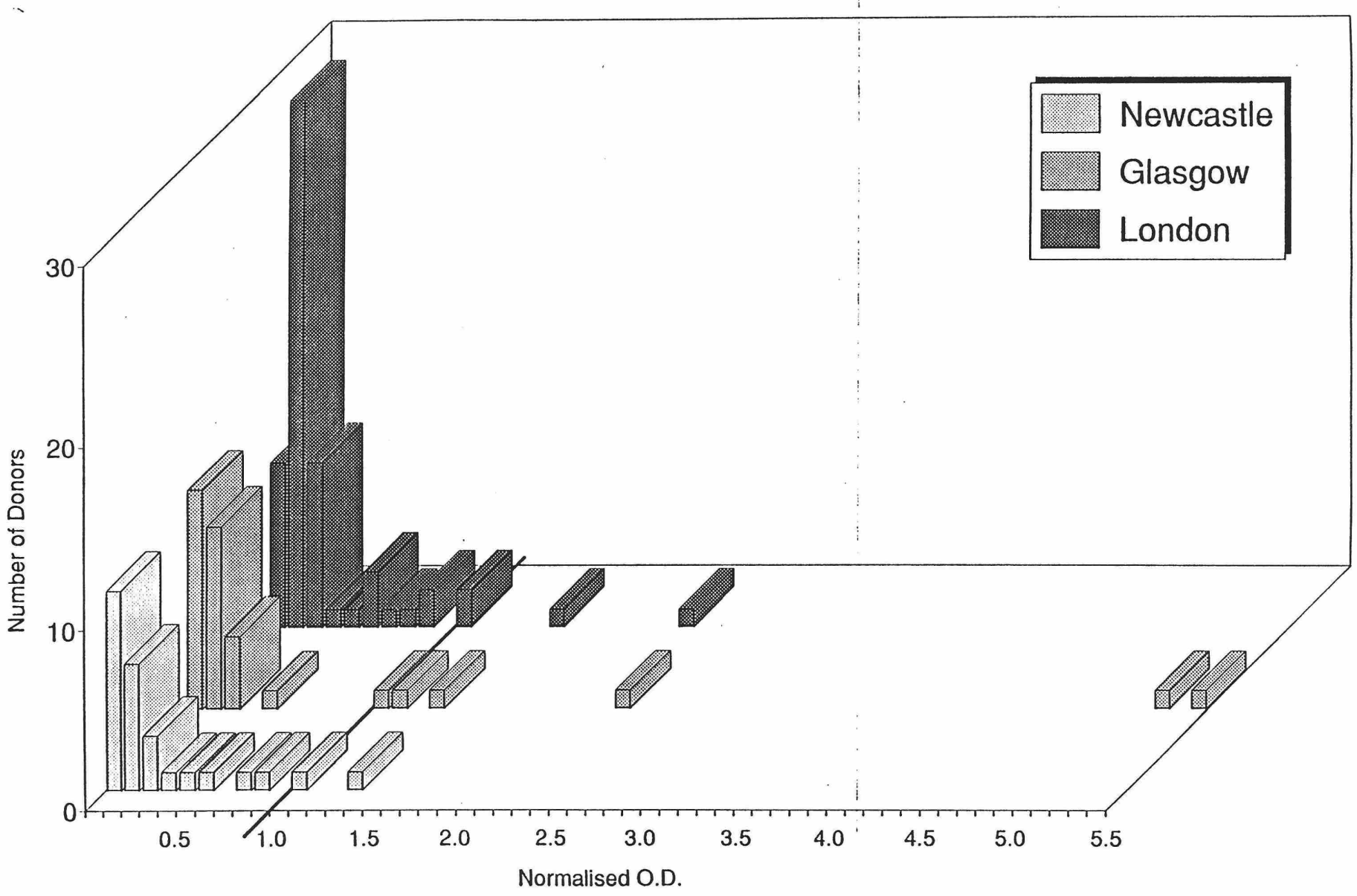
1. Any reactivity on RIBA 2, including faint bands.
2. Reactive on Ortho ELISA and Abbott ELISA.
3. Reactive on in-house ELISA and Ortho or Abbott ELISA.
4. Two samples reactive only in the in-house ELISA were also included.

Of these 30 samples there was insufficient left of number 343610/Glasgow to test and sample 349618/Glasgow had only 50µl left, while many others had less than the required 500µl. The 29 samples tested were all PCR negative. The result on 349618 is at variance with the reported result from Edinburgh University.

Summary

Only one serum (349618, GWS) from the additional 121 referred specimens was considered to show evidence of HCV infection by RIBA. Neither that sample, nor any of the other 28 samples, were found to contain HCV RNA.

In-house anti-HCV Elisa on repeatedly reactive samples



		ORTHO RIBA 2					In-House EIA				PCR	
ecimen	5-1-1	c100-3	c33c	c22-3	Interpretation	Test	Cutoff	Normal		Vol/ul	Result
57215	-	-	-	-	Non-Reactive	0.04	0.491	0.08			
59716	-	-	-	-	Non-Reactive	0.035	0.491	0.07			
93842	-	-	-	-	Non-Reactive	0.087	0.491	0.18			
93881	-	-	-	-	Non-Reactive	0.234	0.491	0.48			
93887	-	-	-	-	Non-Reactive	0.262	0.491	0.53			
93889	-	-	-	-	Non-Reactive	0.175	0.491	0.36			
21724	-	-	-	-	Non-Reactive	0.075	0.491	0.15			
39257	-	-	-	-	Non-Reactive	0.043	0.491	0.09		150	neg
43266	-	-	-	-	Non-Reactive	0.069	0.491	0.14			
13924	-	-	-	-	Non-Reactive	0.039	0.491	0.08		200	neg
59782	-	-	-	-	Non-Reactive	0.494	0.491	1.01			
83944	-	-	-	-	Non-Reactive	0.069	0.491	0.14			
83952	-	-	-	-	Non-Reactive	0.065	0.491	0.13			
225782	-	-	-	-	Non-Reactive	0.085	0.491	0.17			
716463	-	-	-	-	Non-Reactive	0.052	0.491	0.11			
788882	-	-	-	+/-	Non-Reactive	0.352	0.491	0.72		150	neg
203770	-	-	-	-	Non-Reactive	0.114	0.491	0.23		200	neg
718516	-	-	-	-	Non-Reactive	0.142	0.491	0.29			
759920	-	-	-	-	Non-Reactive	0.078	0.491	0.16			
239393	-	-	-	-	Non-Reactive	0.059	0.491	0.12		150	neg
716531	-	-	-	-	Non-Reactive	0.286	0.491	0.58			
785051	-	-	-	-	Non-Reactive	0.391	0.491	0.80		150	neg
718608	-	-	-	+/-	Non-Reactive	1.093	0.491	2.23		150	neg
793836	-	-	-	-	Non-Reactive	0.053	0.491	0.11			
783947	-	-	-	-	Non-Reactive	0.102	0.491	0.21			
793955	-	-	-	-	Non-Reactive	0.062	0.491	0.13			
759927	-	-	-	-	Non-Reactive	0.439	0.491	0.89			
243366	-	-	-	-	Non-Reactive	0.326	0.491	0.66			
243369	-	-	-	-	Non-Reactive	0.52	0.491	1.06			
243731	-	-	-	-	Non-Reactive	0.749	0.491	1.53		150	neg
253595	-	-	-	-	Non-Reactive	0.112	0.491	0.23			
253600	-	-	-	-	Non-Reactive	0.053	0.491	0.11			
716382	-	-	-	-	Non-Reactive	0.102	0.491	0.21			
757198	-	-	-	-	Non-Reactive	0.062	0.452	0.14			
766690	-	-	-	-	Non-Reactive	0.065	0.452	0.14			

			ORTHO RIBA 2					In-House EIA				PCR	
ecimen		5-1-1	c100-3	c33c	c22-3	Interpretation		Test	Cutoff	Normal		Vol/µl	Result
25752		-	-	-	-	Non-Reactive		0.104	0.452	0.23			
16418		-	-	-	-	Non-Reactive		0.087	0.452	0.19			
57241		-	-	-	-	Non-Reactive		0.04	0.452	0.09			
93965		-	-	-	-	Non-Reactive		0.04	0.452	0.09			
25793		-	-	-	-	Non-Reactive		0.066	0.452	0.15			
57266		-	-	-	-	Non-Reactive		0.041	0.452	0.09			
12202		-	-	-	-	Non-Reactive		0.057	0.452	0.13			
43332		-	-	-	-	Non-Reactive		0.072	0.452	0.16			
16511		-	-	-	-	Non-Reactive		0.066	0.452	0.15			
18507		-	-	-	-	Non-Reactive		0.05	0.452	0.11			
57367		-	-	-	-	Non-Reactive		0.11	0.452	0.24			
85046		-	-	-	-	Non-Reactive		0.047	0.452	0.10			
90914		-	-	-	-	Non-Reactive		0.049	0.452	0.11			
47112		-	-	-	-	Non-Reactive		0.092	0.452	0.20			
25875		-	-	-	-	Non-Reactive		0.055	0.452	0.12			
85074		-	-	-	-	Non-Reactive		0.076	0.452	0.17			
85078		-	-	-	-	Non-Reactive		0.039	0.452	0.09			
90504		-	-	-	-	Non-Reactive		0.057	0.452	0.13			
90982		-	-	-	-	Non-Reactive		0.111	0.45	0.25			
90989		-	-	-	-	Non-Reactive		0.043	0.45	0.10			
12490		-	-	-	-	Non-Reactive		0.058	0.45	0.13			
66981		-	-	-	-	Non-Reactive		0.026	0.45	0.06			
94354		-	-	-	-	Non-Reactive		0.082	0.45	0.18			
94453		-	-	-	-	Non-Reactive		0.234	0.45	0.52			
97920		-	-	-	-	Non-Reactive		0.05	0.45	0.11			

		ORTHO RIBA 2					In-House EIA			PCR	
ecimen	5-1-1	c100-3	c33c	c22-3	Interpretation	Test	Cutoff	Normal	Vol/µl	Result
37474	-	-	-	-	Non-Reactive	1.039	0.45	2.31	150	neg
37545	-	-	+/-	-	Non-Reactive	0.054	0.45	0.12	100	neg
37595	-	-	+/-	-	Non-Reactive	0.52	0.45	1.16	50	neg
31892	-	-	-	-	Non-Reactive	0.051	0.45	0.11		
31901	-	-	-	-	Non-Reactive	0.063	0.45	0.14		
43351	-	-	-	-	Non-Reactive	0.033	0.45	0.07		
43538	-	-	-	-	Non-Reactive	0.062	0.45	0.14		
43610	-	-	-	+/-	Non-Reactive	2.35	0.45	5.22		
43455	-	-	-	-	Non-Reactive	0.091	0.45	0.20		
43462	-	-	-	-	Non-Reactive	0.037	0.45	0.08		
32168	-	-	-	+/-	Non-Reactive	0.027	0.45	0.06		
32187	-	-	-	-	Non-Reactive	0.046	0.45	0.10	150	neg
38003	-	-	-	-	Non-Reactive	0.037	0.45	0.08		
11918	-	-	-	-	Non-Reactive	0.063	0.45	0.14		
43681	-	-	-	-	Non-Reactive	0.045	0.45	0.10		
32260	-	-	-	-	Non-Reactive	0.1	0.45	0.22		
32291	-	-	-	-	Non-Reactive	0.021	0.45	0.05		
38171	-	-	-	-	Non-Reactive	0.05	0.45	0.11		
43811	-	-	-	-	Non-Reactive	0.456	0.45	1.01	150	neg
43825	-	-	-	-	Non-Reactive	0.048	0.45	0.11		
43853	-	-	-	-	Non-Reactive	0.036	0.45	0.08		
43890	-	-	-	-	Non-Reactive	0.048	0.45	0.11		
43935	-	-	-	+/-	Non-Reactive	0.011	0.45	0.02	100	neg
32482	-	-	-	-	Non-Reactive	0.011	0.45	0.02	50	neg
32540	-	-	-	-	Non-Reactive	0.03	0.45	0.07	100	neg
43967	-	-	-	-	Non-Reactive	0.009	0.45	0.02	50	neg
44009	-	-	-	-	Non-Reactive	0.04	0.45	0.09		
44038	-	-	-	-	Non-Reactive	0.104	0.45	0.23		
49618	-	-	-	4+	Indeterminate	2.455	0.45	5.46	50	neg
38502	-	-	-	-	Non-Reactive	0.106	0.45	0.24		
38552	-	-	-	-	Non-Reactive	0.181	0.45	0.40		
32675	-	-	-	-	Non-Reactive	0.013	0.45	0.03		
32334	-	-	-	-	Non-Reactive	0.599	0.45	1.33	100	neg

Northern Region Blood Transfusion Centre (Newcastle-on-Tyne)

		ORTHO RIBA 2					In-House EIA				PCR	
ecimen	5-1-1	c100-3	c33c	c22-3	Interpretation	Test	Cutoff	Normal		Vol/µl	Result
67193	-	-	-	-	Non-Reactive	0.358	0.45	0.80			
91781	-	-	-	-	Non-Reactive	0.04	0.45	0.09		500	neg
79962	-	-	-	-	Non-Reactive	0.018	0.45	0.04		250	neg
56853	-	-	-	+/-	Non-Reactive	0.457	0.45	1.02		500	neg
42625	-	-	-	-	Non-Reactive	0.037	0.45	0.08		250	neg
32351	-	-	-	-	Non-Reactive	0.584	0.45	1.30		500	neg
44413	-	-	-	-	Non-Reactive	0.243	0.45	0.54			
42851	-	-	-	-	Non-Reactive	0.068	0.45	0.15		500	neg
44428	-	-	-	-	Non-Reactive	0.06	0.45	0.13		500	neg
42448	-	-	-	-	Non-Reactive	0.065	0.45	0.14			
67456	-	-	-	-	Non-Reactive	0.024	0.45	0.05			
67419	-	-	-	-	Non-Reactive	0.18	0.45	0.40		500	neg
42762	-	-	-	-	Non-Reactive	0.09	0.45	0.20			
56702	-	-	-	-	Non-Reactive	0.081	0.45	0.18			
91773	-	-	-	-	Non-Reactive	0.019	0.45	0.04			
42342	-	-	-	-	Non-Reactive	0.023	0.45	0.05			
56790	-	-	-	-	Non-Reactive	0.038	0.45	0.08			
80147	-	-	-	-	Non-Reactive	0.021	0.45	0.05			
32341	-	-	-	-	Non-Reactive	0.331	0.45	0.74			
67255	-	-	-	-	Non-Reactive	0.087	0.45	0.19			
67310	-	-	-	-	Non-Reactive	0.111	0.45	0.25			
42412	-	-	-	-	Non-Reactive	0.103	0.45	0.23			
04519	-	-	-	-	Non-Reactive	0.026	0.45	0.06			
59992	-	-	-	-	Non-Reactive	0.017	0.45	0.04			
80249	-	-	-	-	Non-Reactive	0.074	0.45	0.16			
43052	-	-	-	-	Non-Reactive	0.015	0.45	0.03			
32260	-	-	-	+/-	Non-Reactive	0.155	0.45	0.34		500	neg
15519	-	-	-	+/-	Non-Reactive	0.048	0.45	0.11		500	neg

FURTHER EVALUATION OF ANTI-HCV BLOOD DONOR SCREENING IN 5 TRANSFUSION CENTRES

INTRODUCTION

The decision by the Department of Health to introduce donor screening of hepatitis C antibody in the U.K. Blood Transfusion Service in the autumn of 1991 prompted a review of the methods available for preliminary donor screening. Methods for confirming the specificity of plasma donations found to be reactive in the screening tests were also reviewed by a Working Party consisting of members of the NBTS Advisory Committee and virologists who are responsible for confirmatory testing for HIV and hepatitis B for the Blood Transfusion Service..

An initial study of donor screening for anti-HCV carried out in Scotland and the South of England showed that first generation anti-HCV tests had an 0.5% reaction rate and that further confirmation (or supplementary tests) with a recombinant immunoblot test (RIBA) supplemented by tests for HCV specific RNA in donor serum or plasma by the polymerase chain reaction (PCR) was a feasible approach.

It is evident that the cost of testing every serum sample repeatedly reactive in the screening anti-HCV test by RIBA (£30 per sample) and some by PCR (£100 per sample) would make confirmatory testing unacceptably costly for the transfusion centres. The introduction of second generation assays for anti-HCV by 3 manufacturers (Ortho II, Abbott II and UBI) which included an increased assay of recombinant antigens in these tests and a second generation RIBA - RIBA II, which contains 4 recombinant proteins, prompted this study.

METHODS

Five Regional Transfusion Centres (RTC), Newcastle, Leeds, Liverpool, Sheffield and Bristol, were asked to screen serum obtained from blood donations over a 6 week period by one of the 3 second generation immunoassays. Serum samples found to be repeatedly reactive in the screening immunoassays were referred to Manchester Public Health Laboratory (PHL) for confirmatory testing. All referred samples were tested at Manchester (PHL) by the same enzyme immunoassay (EIA) as that used by the referring transfusion centre and also by other 2 enzyme immunoassays in a feasibility study to determine whether testing referred donor serum by the other two available immunoassays would reduce the proportion of sera with apparently 'specific' reactions, and therefore reduce the number of sera requiring RIBA II and PCR testing. The 3 enzyme immunoassay tests for anti-HCV which were used were the second generation assays produced by Ortho and Abbott Diagnostics and an assay produced by United Biomedical Inc and marketed in the UK by AKZO.

PROCEDURE AT MANCHESTER PHL

1) Each serum sample was tested again by the same immunoassay used in the screening test at the Transfusion Centre.

2) All referred samples were also tested by each of the other 2 immunoassays available.

3) An initial group of 28 sera were tested by the RIBA II test irrespective of whether they were reactive or not in the screening immunoassay or reactive in the other immunoassays performed at Manchester PHL.

4) Based on the results of these initial tests, an algorithm was devised where only sera reactive with a test/cut-off ratio of greater than 2 in a single immunoassay or reactive in 2 or more immunoassays were selected for RIBA II testing.

5) The criteria used for assessing the results of RIBA test were exactly the same as the manufacturers criteria. Results were classified as NOT CONFIRMED; INDETERMINATE or CONFIRMED.

6) Selected sera were chosen for PCR testing on the basis of the RIBA results at a meeting of virologists at the Middlesex Hospital. These will be tested at the Department of Virology, Middlesex/UCH Medical School, London (Richard Tedder) and the Virus Reference Laboratory, CPHL, London (Philip Mortimer).

RESULTS

453 repeatedly reactive samples were received from the 5 Transfusion Centres (RTC's). Shortly after commencement of the trial Ortho Diagnostics recommended an alteration in the calculation of the cut-off in their assay. Only 3 of the referred samples originally tested by the Ortho assay failed to meet the criteria laid down for referral when this new cut-off was applied. Much further on into the trial AKZO recommended an alteration in the calculation of the cut-off for the UBI assay. This affected the results rather more and 35 of the specimens referred by the RTC's using this assay failed to meet the criteria for referral once the new cut-off was applied. All the data in this report have been derived using the new cut-off levels for both the Ortho and UBI assays. It was clear from the pattern of referral by the RTC's that performance improved as the centres gained experience with the

immunoassays and the proportion of samples with a reactivity close to the cut-off level declined (Table 1).

TABLE 1 - RESULTS FROM REGIONAL TRANSFUSION CENTRES.

RTC	Assay used	No tested	Initially reactive	Repeatedly reactive
Leeds	Ortho	24582	234 (0.95%)	179 (0.73%)
Liverpool	Ortho	10669	135 (1.26%)	87 (0.82%)
Newcastle	Abbott	26958	109 (0.40%)	96 (0.36%)
Sheffield	UBI	11870	130 (1.10%)[1] 84 (0.71%)[2]	97 (0.81%)[1] 65 (0.55%)[2]
Bristol	UBI	3569	31 (0.87%)[1] 18 (0.50%)[2]	26 (0.73%)[1] 16 (0.45%)[2]
total		77648	639 (0.82%)[1] 580 (0.75%)[2]	485 (0.62%)[1] 443 (0.57%)[2]

Note 1 - using original cut-off values
Note 2 - using new cut-off values

Following initial screening by the 3 enzyme immunoassays, samples which were reactive in 1 or more immunoassays but which were non-reactive in the immunoassay used by the referring RTC's were retested by that immunoassay; samples which were non-reactive in all 3 immunoassays were not retested. Samples showing discrepant results between the 3 immunoassays were also retested. A significant number of samples showed a different result on retesting, particularly those samples with a reactivity close to the cut-off level and it was clear that the reproducibility of the assays at levels around the cut-off was poor, even with the adjusted cut-off values. Six samples which had initially been non-reactive at Manchester PHL by the initial immunoassay

were shown to be clearly reactive on repetition. It was noticed that with the small volumes used in these assays (typically in the order of 20 ul) great care was needed in dispensing sera, especially if there was any fibrin in the specimen which tended to obstruct the pipette tip, this is particularly a problem with frozen sera. These data have been compiled taking into account the results of repeat testing.

Of the 453 samples, 233 (51.43%) were non-reactive in the same immunoassay as that used by the referring RTC's and of these 209 (46.14%) were non-reactive in all three immunoassays at Manchester PHL; the remaining 244 samples were reactive in 1 or more immunoassays.

The first 28 samples received had been screened by the Ortho immunoassay at the Leeds RTC. These samples were tested by all three immunoassays and RIBA II at Manchester PHL. Of these 28 samples, 12 were non-reactive in all three immunoassays and by RIBA II; 11 were reactive only in the Ortho imunoassay, 5 of these were non-reactive by RIBA II and 6 were indeterminate; 4 samples were reactive in both Ortho and Abbott immunoassays and 1 sample was reactive in all 3 immunoassays, all 5 were non-reactive by RIBA II.

On the basis of these results it was decided to perform RIBA II only on samples which were reactive in a single imunoassay with a test/cut-off ratio greater than 2, or which were reactive with a test/cut-off ratio greater than 1 in 2 or more immunoassays. Analysis of the transfusion centre results on initial screening of the 453 referred samples shows that 35 (7.7%) were negative acording to the new cut-off values, 215 (47.5%) had a test/cut-off ratio between 1 and 2, 69 (15.2%) had a test/cut-off ratio of between 2 and 3,

and 134 (29.6%) had a test/cut-off ration of greater than 3. There was a good correlation of the immunoassay results obtained at the transfusion centres with those obtained at Manchester PHL for strongly reactive samples with a test/cut-off ratio of greater than 3. However, many of the weakly reactive samples at the transfusion centres were non reactive at Manchester PHL. (Tables 2,3).

TABLE 2 - SUMMARY OF PHL RESULTS WHEN USING SAME EIA AS ORIGINATING BTC.

		t/c ratio when tested at PHLS				
		<1	1-2	2-3	>3	
t/c ratio at BTC	<1	34 (97.1%)	1 (2.9%)	0	0	35
	1-2	159 (73.9%)	48 (22.3%)	5 (2.3%)	3 (1.4%)	215
	2-3	30 (43.5%)	19 (27.5%)	17 (24.6%)	3 (4.3%)	69
	>3	10 (7.5%)	14 (10.4%)	19 (14.2%)	91 (67.9%)	134
total		233 (51.4%)	82 (18.1%)	42 (9.3%)	97 (21.4%)	453

TABLE 3 - COMPARISON OF RTC AND PHL RESULTS WHEN USING SAME EIA TEST.

		PHLS		
		not-reactive	reactive	
RTC	Ortho	132 (53.2%)	116 (46.8%)	248
	Abbott	42 (43.8%)	54 (56.3%)	96
	UBI	59 (54.1%)	50 (45.9%)	109
total		233 (51.4%)	220 (48.6%)	453

Ortho RIBA II has so far been carried out on 218 samples; of these 24 were

non-reactive in all immunoassays performed at Manchester PHL, and 194 were reactive in 1 or more immunoassays. Of these 218 samples tested by RIBA II at Manchester PHL, 40 (18.3%) were reactive, 40 (18.3%) were indeterminate and 138 (63.3%) were non-reactive. The RIBA II confirmed samples were reactive in all 3 immunoassays at Manchester PHL in all except one sample which was repeatedly non-reactive in the UBI immunoassay. Weak bands were seen in 39 of the 138 non-reactive samples by RIBA II but the intensity was less than the level I IgG control. A response with an intensity equal to or greater than the level I control was seen with the recombinant core antigen band in 21 of the 40 indeterminate samples and in 9 of these the intensity was equal to or greater than that seen with the level II IgG control. (Table 4).

An additional immunoblot assay marketed by Mercia Diagnostics (Inno-lia HCV Ab) was performed on 17 samples which were indeterminate or reactive by RIBA. Of 8 samples which were reactive by RIBA, 5 were reactive by Inno-lia, 2 were indeterminate and 1 was non-reactive. Of the 9 indeterminate samples by RIBA, 1 was reactive by Inno-lia, 1 was indeterminate and 7 were non-reactive.

A summary of all the results for each RTC are shown in Table 5.

TABLE 4 - SUMMARY OF RIBA II RESULTS AT PHL.

		RIBA not reactive	RIBA indeterminate	RIBA reactive	
t/c ratio at RTC	<1	4 (100%)	0	0	4
	1-2	51 (91.1%)	3 (5.4%)	2 (3.6%)	56
	2-3	32 (7.3%)	10 (22.7%)	2 (4.5%)	44
	>3	51 (44.7%)	27 (23.7%)	36 (31.6%)	114
RTC	Ortho	84 (66.1%)	24 (18.9%)	19 (15.0%)	127
	Abbott	28 (53.8%)	12 (23.1%)	12 (23.1%)	52
	UBI	26 (66.7%)	4 (10.3%)	9 (23.1%)	39
PHL	All EIA Neg	24 (100%)	0	0	24
	1 EIA Pos	47 (87.0%)	7 (13.0%)	0	54
	2 EIA Pos	59 (74.7%)	19 (24.1%)	1 (1.3%)	79
	3 EIA Pos	8 (13.1%)	14 (23.0%)	39 (56.5%)	61
total		138 (63.3%)	40 (18.3%)	40 (18.3%)	218

Table 5 summarises the results for each Transfusion Centre by comparing the results on the specimens when tested at Manchester PHL which were negative in the initial EIA test. The results of the RIBA II tests performed so far on samples referred from each Centre are also included.

TABLE 5 - SUMMARY OF PHL RESULTS FOR EACH TRANSFUSION CENTRE.

	Number Referred by RTC	Number* Reactive at RTC	Number EIA Neg at PHL	RIBA Tests Performed at PHL			
				Number	Neg	Ind	Pos
Leeds	163	160	84 (51.5%)	87	66	15	6
Liverpool	85	85	35 (36.5%)	40	18	9	13
Newcastle	96	96	37 (38.5%)	52	28	12	12
Sheffield	87	62	42 (48.3%)	33	26	3	9
Bristol	22	15	11 (50.0%)	6	5	1	0
total	453	418	209 (46.1%)	218	138	40	40

*using re-defined cut-off levels

Table 6 shows the sero-prevalence of anti-HCV so far obtained in this study for each participating RTC. Assuming RIBA II positive samples indicate true positives.

TABLE 6

RTC	Positive RIBA/total screened	Sero-prevalence
Leeds	6/24582	1/4000
Liverpool	13/10669	1/800
Newcastle	12/26958	1/2100
Sheffield	9/11870	1/1200
Bristol	1/3569	1/3500 (approx)

CONCLUSIONS

On the basis of these results it may be concluded: (1)

That an algorithm can be devised for selection of samples for RIBA II testing on the basis of the immunoassay results. Since no samples which were reactive in only 1 immunoassay were reactive by RIBA II, specimens should only be referred for confirmation by immunoassay and RIBA II if they are reactive with a test/cut-off ratio greater than 1.0 in two or more immunoassays. However, since 7 samples which were only reactive in the Ortho immunoassay were indeterminate by RIBA II, 2 with reactions in the core band, samples should also be referred if they are reactive in a single immunoassay if the test/cut-off ratio is greater than 2.0. This algorithm will be drawn up by the TTD committee and will be validated during the 2 month program of donor screening in September and October.

(2) The patterns of reactive bands with the sample which were indeterminate by RIBA II suggest that further evaluation of these samples by PCR might be useful and this is being arranged.

(3) Since the UBI immunoassay was repeatedly non-reactive for 1 sample which was confirmed positive by RIBA II, caution should be exercised in using this immunoassay in the algorithms devised under conclusion 1 above. Further, this immunoassay needs further investigation.

(4) The improvement in the performance of the RTC's over the period of the trial as they gained in experience with the immunoassays lead to the conclusion that consideration should be given to arranging a workshop for the collaborating centres and other interested RTC's where the implications of these results could be discussed prior to the introduction of formal donor screening.

DR J CRASKE

DR W K PAVER

MANCHESTER PHL - 29th JULY 1991.

APPENDIX

(1)

TABLE 7 - ANALYSIS OF BANDING PATTERNS OF RIBA II INDETERMINATE SAMPLES.

	Number	2	21	13	4
	5-1-1	-	-	-	+
RIBA II	c100-3	-	-	+	-
Banding	c33c	+	-	-	-
Pattern	c22-3	-	+	-	-
	SOD	-	-	-	-

RTC results - t/c ratio

Ortho 1-2	1	1	0	0
Ortho 2-3	0	1	2	0
Ortho >3	0	10	8	1
Abbott 1-2	0	0	1	0
Abbott 2-3	1	2	1	2
Abbott >3	0	4	1	0
UBI 1-2	0	0	0	0
UBI 2-3	0	1	0	0
UBI >3	0	2	0	1

PHL results

1 EIA Pos	0	2	5	0
Ortho 1+, Abbott - , UBI -	0	1	1	0
Ortho 3+, Abbott - , UBI -	0	1	4	0
2 EIA Pos	2	9	7	2
Ortho - , Abbott 1+, UBI 3+	0	0	0	1
Ortho 1+, Abbott 1+, UBI -	1	1	0	0
Ortho 2+, Abbott 1+, UBI -	0	2	1	0
Ortho 2+, Abbott 2+, UBI -	0	1	1	0
Ortho 3+, Abbott 1+, UBI -	1	1	1	1
Ortho 3+, Abbott 2+, UBI -	0	0	1	0
Ortho 3+, Abbott 3+, UBI -	0	3	3	0
3 EIA Pos	0	11	1	2
Ortho 2+, Abbott 1+, UBI 1+	0	0	1	0
Ortho 2+, Abbott 1+, UBI 2+	0	0	0	1
Ortho 2+, Abbott 2+, UBI 2+	0	0	0	1
Ortho 3+, Abbott 1+, UBI 1+	0	2	0	0
Ortho 3+, Abbott 2+, UBI 1+	0	1	0	0
Ortho 3+, Abbott 2+, UBI 2+	0	1	0	0
Ortho 3+, Abbott 3+, UBI 1+	0	2	0	0
Ortho 3+, Abbott 3+, UBI 3+	0	5	0	0

TABLE 8 - ANALYSIS OF BANDING PATTERNS OF RIBA II REACTIVE SAMPLES.

	Number	16	16	1	2	1	2	1	1
	5-1-1	-	+	+	-	+	+	-	+
RIBA II	c100-3	-	+	+	+	+	-	+	-
Banding	c33c	+	+	+	+	-	+	-	-
Pattern	c22-3	+	+	-	+	-	+	+	+
	SOD	-	-	-	-	-	-	-	-
RTC results - t/c ratio									
	Ortho 1-2	0	0	0	0	0	0	0	0
	Ortho 2-3	1	0	0	0	0	0	0	0
	Ortho >3	6	10	1	1	0	0	0	0
	Abbott 1-2	0	0	0	0	1	0	0	0
	Abbott 2-3	1	0	0	0	0	0	0	0
	Abbott >3	3	5	0	0	0	2	0	0
	UBI 1-2	1	0	0	0	0	0	0	0
	UBI 2-3	0	0	0	0	0	0	0	0
	UBI >3	4	1	0	1	0	0	1	1
PHL results:									
	2 EIA Pos	1	0	0	0	0	0	0	0
Ortho 3+, Abbott 2+, UBI -		1	0	0	0	0	0	0	0
	3 EIA Pos	15	16	1	2	1	2	1	1
Ortho 2+, Abbott 1+, UBI 3+		1	0	0	0	1	0	0	0
Ortho 3+, Abbott 3+, UBI 2+		2	0	1	0	0	0	0	0
Ortho 3+, Abbott 3+, UBI 3+		12	16	0	2	0	2	1	1

INTRODUCTION

A large scale evaluation of the Abbott HCV EIA 2nd Generation is currently in progress at the Glasgow and West of Scotland Blood Transfusion Centre. The purpose of this study is to provide information on routine screening prior to the commencement of anti-HCV testing nationally. In addition, data on the performance of confirmation tests at the Reference Laboratory would also be collected. This evaluation commenced on 30.5.91 and is on-going. This report presents, in detail, the results obtained to date.

The results obtained are presented in four parts:-

1 Abbott HCV EIA 2nd Generation initial screen and repeat test results up to 22.7.91.

2 Abbott HCV EIA 2nd Generation initial screen and repeat test results up to 13.7.91, including Reference Laboratory RIBA-2 results.

3 Abbott HCV EIA 2nd Generation initial screen and repeat test results up to 25.6.91, including Reference Laboratory RIBA-2 and PCR results.

4 Analysis of the results obtained by dividing all donors into male and female groups.

(AH) Disk 1: HCVEIAS 1/8/91

RESULTS

SECTION ONE

Up to 22.7.91, a total of 23,644 donors have been tested (Table 1). Ninety four samples were found to be initial screen positive, of which 89 (0.38%) were repeatable and forwarded to the Reference Laboratory.

Two kit batches were in use at this time and the results obtained with each are shown on Tables 2(a) and 2(b). It can be seen that there is batch variation in the incidence of repeat positive results (0.45% compared with 0.29%). Variation could be significant in testing large numbers of donors.

SECTION TWO

Up to 13.7.91, a total of 20,986 donors were tested (Table 3). Eighty five samples were found to be initial screen positive, of which 81 (0.38%) were repeatable and therefore sent to the Reference Laboratory. RIBA-2 results are available on all these samples (Table 4). Forty one were found to be RIBA-2 negative (50.6%). Of the remaining 40, a total of 22 were confirmed positive (27.2%) and 18 (22.2%) gave indeterminate results. These results show that 0.105% of our donor population (1/954) are confirmed as anti-HCV positive by RIBA-2. A further 0.085% (1/1166) are classified as indeterminate.

SECTION THREE

A complete set of Reference Laboratory results, including PCR, are available on all samples tested up to 25.6.90. At that time, 12,425 screening tests had been performed, of which 56 were found repeatable positive (Table 5) and sent to the Reference Laboratory.

Table 6 presents the RIBA-2 and PCR results on these 56 EIA repeat positive samples. Thirty one samples were RIBA-2 negative. Of the remaining 25, a total of 14 were confirmed as RIBA-2 positive and 11 were classified as indeterminate. These 25 samples were further PCR tested. Thirteen (93%) of 14 RIBA-2 positive samples were also PCR positive. Of the 11 indeterminate samples, 3 (27%) were found to be PCR positive.

The 56 samples described above have been tested with the Ortho 2 assay and the results are detailed in Table 7. Forty two (75%) of the 56 Abbott repeat positives were also Ortho 2 positive. Of particular interest is donation 461368. This Abbott repeat positive donation is negative in the Ortho 2 assay, RIBA-2 indeterminate, but was found to be PCR positive.

A complete results profile on each of the 56 repeat Abbott positives is detailed in Table 8.

Table 9 provides a breakdown of the RIBA-2 results and relates them to PCR positivity. The highest percentage of PCR positive results are obtained from donors RIBA-2 positive to both C33c and C22 bands. Only 1 donor reactive to both these bands has been found to be PCR negative to date (468676).

SECTION FOUR

The results of dividing all donors tested up to 22.7.91 (see Table 1) into male and female are shown on Table 10. No difference is noted between groups.

The results of also dividing all donors tested up to 25.6.91, for which complete Reference Laboratory results are available, are presented in Table 11. Again, no significant differences between males and females is found.

CONCLUSION

A repeat positive rate of 0.45% was obtained on screening 12,425 donor samples with the Abbott HCV EIA 2nd Generation (Table 5). This figure is similar to that obtained in an earlier evaluation of this test.

At the Reference Laboratory 14 (0.11%) donors were confirmed as RIBA-2 positive and a further 11 donors gave indeterminate results. PCR testing of these RIBA-2 positive and indeterminate samples revealed 16 (0.13%) to be PCR positive (Table 6).

Two results are of particular interest. Donation 468676 was found to be Abbott 2 and Ortho 2 positive, was reactive to 3 bands in RIBA-2, yet was negative in PCR (Table 8). Donation 461493 was Abbott 2 positive, RIBA-2 indeterminate and PCR positive, but was negative in the Ortho 2 assay (Table 8).

A slight batch variation was noted (Tables 2a and 2b) and this will be monitored in future. It is important that manufacturers minimise batch variation as it could have a major effect on the daily operations of the transfusion service.

Examination of both male and female donors revealed no obvious statistical differences.

This report presents detailed results obtained so far in this evaluation. The information contained within must now be analysed in greater depth before firm conclusions can be drawn.

We would like to acknowledge Dr A Robinson (Regional Transfusion Centre, Bridle Path, Leeds) for the assistance received in performing all Ortho 2 tests on our behalf.

W Hughes
I Macvarish
A Barr
R Mitchell

1 August 1991

TABLE 1 Abbott HCV EIA 2nd Generation Results From 30.5.91 To 22.7.91

DONORS TESTED	INITIAL SCREEN POSITIVE	REPEAT POSITIVE
23644	94 (0.40%)	89 (0.38%)

TABLE 2 Abbott HCV EIA 2nd Generation Results From 30.5.91 to 22.7.91 (Batch Analysis)

(a) Batch 52798HP

DONORS TESTED	INITIAL SCREEN POSITIVE	REPEAT POSITIVE
12311	60 (0.49%)	56 (0.45%)

(b) Batch 53901HP

DONORS TESTED	INITIAL SCREEN POSITIVE	REPEAT POSITIVE
11333	34 (0.30%)	33 (0.29%)

TABLE 3 Abbott HCV EIA 2nd Generation Results From 30.5.91 to 13.7.91

DONORS TESTED	INITIAL SCREEN POSITIVE	REPEAT POSITIVE
20986	85 (0.40%)	81 (0.38%)

TABLE 4 RIBA-2 Results on EIA Repeat Positives Found Up To 13.7.91

RIBA-2	NO OF DONORS	% SAMPLES TESTED	% ALL DONORS
Negative	41	50.6%	0.195%
Indeterminate	18	22.2%	0.085%
Positive	22	27.2%	0.105%
TOTAL	81	100%	0.38%

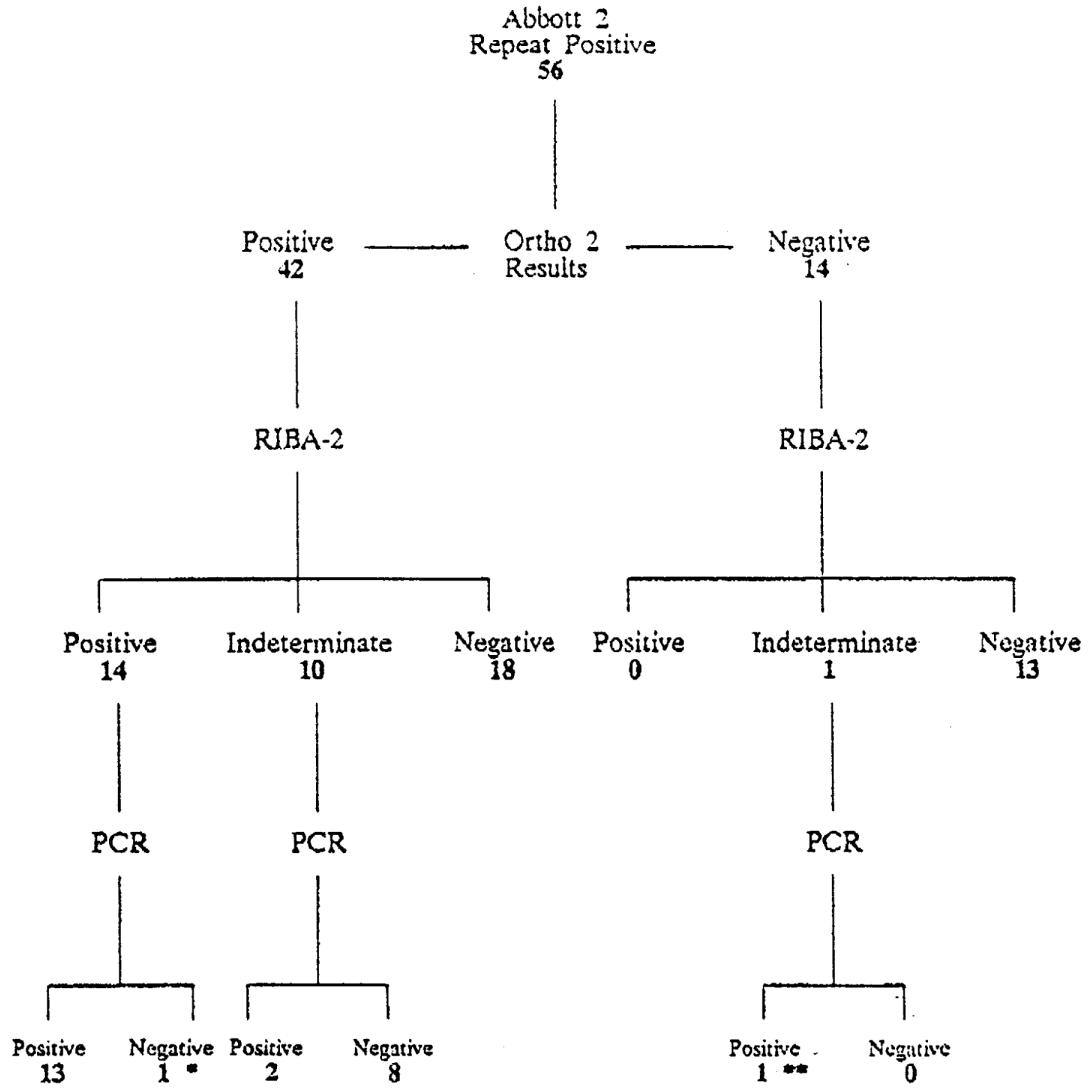
TABLE 5 Abbott HCV EIA 2nd Generation Results From 30.5.91 To 25.6.91

DONORS TESTED	INITIAL SCREEN POSITIVE	REPEAT POSITIVE
12425	60 (0.48%)	56 (0.45%)

TABLE 6 RIBA-2 and PCR Results on EIA Repeat Positive Samples Found Up To 25.6.91

RIBA-2	NO OF DONORS	%	PCR	
			Positive	Negative
Negative	31	55	NT	NT
Indeterminate	11	20	3	8
Positive	14	25	13	1
TOTAL	56	100	16	9

TABLE 7



* Donation No 468676 (see Table 8)

** Donation No 461368 (see Table 8)

TABLE 8 Complete Results Profile on Samples Found Up To 25.6.91

DONATION NUMBER	ABBOTT RESULTS				RIBA-2 RESULTS					PCR	ORTHO 2	
	Serum	Serum	Serum	Pigtail	5-1-1	C100	C33c	C22	SOD			+/-
GRO-A	1.087	1.029	0.959	0.796	-	1+	-	-	-	-	2.340	+
	0.436	0.446	0.446	0.402							0.751	
	2.200	2.200	2.200	2.200	4+	4+	4+	4+	-	+	2.328	+
	0.518	0.446	0.446	0.402							0.751	
	0.619	0.554	0.525	0.629	-	-	-	-	-		1.604	+
	0.447	0.402	0.402	0.447							0.751	
	0.478	0.447	0.459	0.438	-	-	-	-	-		0.094	-
	0.447	0.402	0.402	0.447							0.751	
	0.725	0.434	0.492	0.338	-	-	-	-	-		0.569	-
	0.447	0.402	0.402	0.447							0.751	
	2.200	1.991	2.082	2.200	-	-	-	4+	-	-	2.407	+
	0.446	0.402	0.402	0.447							0.751	
	0.639	0.634	0.680	0.529	-	-	-	-	-		1.657	+
	0.446	0.402	0.402	0.447							0.751	
	2.200	2.200	2.200	2.200	4+	4+	4+	4+	-	+	2.442	+
	0.402	0.447	0.447	0.431							0.751	
	0.421	0.409	0.467	0.422	-	-	-	-	-		0.127	-
	0.412	0.447	0.447	0.431							0.751	
	1.051	1.264	1.127	1.222	-	-	-	1+	-	-	2.213	+
	0.412	0.447	0.447	0.431							0.751	
	0.465	0.525	0.585	0.503	-	-	-	-	-		0.455	-
	0.447	0.431	0.431	0.468							0.751	
	0.476	0.519	0.508	0.375	-	-	-	-	-		0.840	+
	0.403	0.431	0.431	0.468							0.751	
	2.200	2.200	2.200	2.200	-	-	2+	4+	-	+	2.684	+
	0.403	0.431	0.431	0.468							0.751	
	2.200	2.200	2.200	2.200	4+	4+	4+	4+	-	+	2.503	+
	0.431	0.468	0.468	0.396							0.751	

TABLE 8 Cont'd

DONATION NUMBER	ABBOTT RESULTS				RIBA-2 RESULTS					PCR	ORTHO 2	
	Serum	Serum	Serum	Pigtail	5-1-1	C100	C33c	C22	SOD			+/-
GRO-A	1.834	1.653	1.835	1.655	-	-	-	-	-		1.591	+
	0.431	0.468	0.468	0.396							0.751	
	2.200	2.200	2.200	2.200	-	-	-	4+	-	+	1.896	+
	0.404	0.396	0.396	0.427							0.751	
	0.821	0.816	0.725	0.744	-	-	-	-	-		1.229	+
	0.468	0.396	0.396	0.427							0.751	
	0.488	0.518	0.482	0.467	-	-	-	-	-		0.486	-
	0.396	0.427	0.427	0.427							0.751	
	2.200	2.200	2.200	2.200	-	-	4+	4+	-	+	2.698	+
	0.427	0.407	0.407	0.407							0.751	
	2.200	2.200	2.200	2.200	-	-	-	4+	-	+	2.759	+
	0.427	0.467	0.467	0.467							0.751	
	2.200	2.200	2.200	2.200	3+	4+	4+	4+	-	+	2.744	+
	0.412	0.467	0.467	0.467							0.751	
	2.200	2.200	2.200	2.200	-	-	4+	4+	-	+	2.446	+
	0.412	0.467	0.467	0.467							0.751	
	1.280	1.078	1.103	0.982	-	-	-	-	-		0.272	-
	0.467	0.444	0.444	0.444							0.751	
	0.521	0.530	0.421	0.493	-	-	-	-	-		0.120	-
	0.417	0.444	0.444	0.444							0.751	
	0.832	0.708	0.662	0.683	-	-	-	-	-		1.913	-
	0.467	0.444	0.444	0.414							0.751	
	0.464	0.403	0.453	0.372	-	-	-	-	-		1.045	+
	0.444	0.414	0.414	0.414							0.751	
	1.228	1.004	0.980	0.945	-	-	-	-	-		1.753	+
	0.444	0.414	0.414	0.414							0.751	
	0.534	0.440	0.407	0.424	-	-	-	-	-		0.097	-
	0.441	0.414	0.414	0.414							0.751	

TABLE 8 Cont'd

DONATION NUMBER	ABBOTT RESULTS				RIBA-2 RESULTS					PCR	ORTHO 2	
	Serum	Serum	Serum	Pigtail	5-1-1	C100	C33c	C22	SOD			+/-
GRO-A	1.684	1.742	1.582	1.629	-	-	-	-	-		1.286	+
	0.441	0.414	0.414	0.414							0.751	
	2.200	2.200	2.200	2.200	-	-	4+	4+	-	-	2.580	+
	0.441	0.414	0.414	0.414							0.751	
	2.200	2.200	2.200	2.200	-	4+	2+	4+	-	-	2.363	+
	0.441	0.414	0.414	0.414							0.751	
	0.578	0.506	0.502	0.503	-	1+	-	-	-	-	1.267	+
	0.444	0.414	0.414	0.414							0.751	
	0.571	0.484	0.487	0.423	-	-	-	-	-		1.655	+
	0.444	0.414	0.414	0.414							0.751	
	0.740	0.848	0.637	0.739	-	1+	-	-	!	-	1.712	+
	0.444	0.414	0.414	0.414							0.751	
	0.672	0.591	0.535	0.598	-	-	-	-	-		2.609	+
	0.444	0.414	0.414	0.414							0.751	
	0.475	0.337	0.426	0.418	-	-	-	-	-		0.195	-
	0.444	0.414	0.414	0.414							0.751	
	0.702	0.618	0.645	0.545	-	-	-	-	-		0.089	-
	0.444	0.414	0.414	0.414							0.751	
	0.499	0.453	0.523	0.462	1+	-	-	-	-	+	0.229	-
	0.414	0.397	0.397	0.397							0.751	
	0.527	0.435	0.492	0.306	-	-	-	-	-		0.197	-
	0.414	0.397	0.397	0.397							0.751	
	2.200	2.200	2.200	2.200	-	1+	3+	4+	-	+	2.303	+
	0.414	0.397	0.397	0.397							0.751	
	1.274	1.023	1.198	1.083	-	1+	-	-	-	-	2.646	+
	0.499	0.420	0.420	0.420							0.751	
	0.522	0.563	0.574	0.573	-	-	-	-	-		0.781	+
	0.473	0.377	0.377	0.377							0.751	

TABLE 8 Cont'd

DONATION NUMBER	ABBOTT RESULTS				RIBA-2 RESULTS					PCR	ORTHO 2	
	Serum	Serum	Serum	Pigtail	5-1-1	C100	C33c	C22	SOD			+/-
GRO-A	0.621	0.638	0.597	0.586	-	-	-	-	-		0.096	-
	0.473	0.377	0.377	0.377							0.751	
	0.642	0.811	0.789		-	1+	-	-	-	-	2.136	+
	0.391	0.390	0.390								0.751	
	0.417	0.488	0.434	0.406	-	-	-	-	-		0.888	+
	0.377	0.390	0.390	0.390							0.751	
	2.175	2.200	2.200	2.200	-	-	-	4+	-	-	2.688	+
	0.421	0.421	0.421	0.421							0.751	
	2.200	2.200	2.200	2.200	-	1+	4+	3+	-	+	2.372	+
	0.390	0.421	0.421	0.421							0.751	
	0.414	0.524	0.460	0.448	-	-	-	-	-		0.798	+
	0.390	0.421	0.421	0.421							0.751	
	0.532	0.397	0.427	0.355	-	-	-	-	-		1.563	+
	0.411	0.406	0.406	0.425							0.751	
	0.530	0.536	0.563	0.536	-	-	-	-	-		0.880	+
	0.406	0.431	0.431	0.431							0.751	
	0.908	0.696	0.832	0.814	-	-	-	-	-		1.881	+
	0.459	0.444	0.444	0.444							0.751	
	0.488	0.496	0.425	0.501	-	-	-	-	-		0.222	-
	0.459	0.444	0.444	0.444							0.751	
	0.493	0.459	0.415	0.490	-	-	-	-	-		1.023	+
	0.459	0.444	0.444	0.444							0.751	
	2.200	2.200	2.200	2.200	-	-	4+	4+	-	+	2.680	+
	0.459	0.444	0.444	0.444							0.751	
	2.200	2.200	2.200	2.200	4+	4+	4+	4+	-	+	2.411	+
	0.459	0.444	0.444	0.425							0.751	
	2.200	2.200	2.200	2.200	-	-	2+	4+	-	+	2.375	+
	0.422	0.424	0.424	0.424							0.751	

TABLE 9 Breakdown of RIBA-2 and PCR Results

RIBA-2 BANDS	NUMBER OF DONATIONS	PCR	
		Positive	Negative
5.1.1	1	1	0
C100	5	0	5
C33c	0	0	0
C22	5	2	3
5.1.1 C100	0	0	0
5.1.1 C33c	0	0	0
5.1.1 C22	0	0	0
5.1.1 C100 C33c	0	0	0
5.1.1 C100 C33c C22	5	5	0
C100 C33c	0	0	0
C100 C33c C22	3	2	1
C33 C22	6	6	0
No Bands	31	0	0
TOTAL	56	16	9

TABLE 10 Results Up to 22.7.92 Divided Into Male and Female Groups

	DONORS TESTED	MALE	FEMALE
	23644	11911 (50.4%)	11733 (49.6%)
Abbott 2 Repeat Positive	89 (0.38%)	45 (0.38%)	44 (0.38%)

TABLE 11 Results Up To 25.6.91 Divided Into Male and Female Groups

	DONORS TESTED	MALE	FEMALE
	12425	6240 (50.2%)	6185 (49.8%)
Abbott 2 Repeat Positive	56 (0.45%)	30 (0.24%)	26 (0.21%)
RIBA-2 Negative	31	17	14
RIBA-2 Indeterminate	11	6	5
RIBA-2 Positive	14	7	7
PCR Positive	16	8	8

ABBOTT HCV EIA 2nd GENERATION BATCH VARIATION

Introduction

The Abbott HCV EIA 2nd Generation evaluation report dated 1/8/91 revealed batch variation in the number of repeat positive results obtained with the 2 batches used. The first batch, 52798HP, gave a repeat positive rate of 0.45% (56 samples) compared to 0.29% with batch 53901HP. To investigate this further 56 samples repeatable positive in batch 52798HP were retested in duplicate with batch 53901HP.

Results

Table 1 shows that 45 of the 56 samples tested were also positive in batch 53901HP. Five of these 45 samples were positive in only one of the duplicate tests performed.

Detailed results have been appended to Table 8 of the original report and are presented here as Table 2. It can be seen that:-

a) No strongly reactive samples in batch 52798HP were negative in batch 53901HP.

b) All 5 samples, reactive in only 1 of the duplicate tests performed using batch 53901HP had previously given similar results with batch 52798HP. All 5 samples were RIBA-2 negative.

c) All 11 samples found negative with batch 53901HP gave higher optical density readings than the normal negative donor population (ie closer to the cut-off).

d) All 11 samples found negative with batch 53901HP had previously given positive results, close to the cut-off, with batch 52798HP. Six of these 11 had actually given both positive and negative results when originally repeat tested with batch 52798HP.

e) Only 1 sample found negative on retesting with batch 53901HP showed any reactivity in RIBA-2. This sample (461142) gave an indeterminate (1+) reaction to band C100 in RIBA-2 and was PCR negative.

f) One sample found repeat positive in both batches had a sample/cut-off ratio < 2 and was RIBA Indeterminate, PCR Pos (461493). This sample is known to be Ortho 2 negative.

NB It should be noted that CPD A_1 plasma samples were tested in this exercise. Dilution with anticoagulant may have contributed to the results obtained.

W Hughes
I Macvarish
A Barr
R Mitchell

(AH) DN1 HCVEIAS3 (7/8/91)

TABLE 1

Abbott 2 Batch 52798HP Repeat Positive	Abbott 2 Batch 53901HP Positive
56	45 * (80%)

* 5 Samples were positive in only one of the two duplicate tests performed

TABLE 2 Complete Results Profile on Samples Found Up To 25.6.91

DONATION NUMBER	ABBOTT RESULTS BATCH 52798HP				RIBA-2 RESULTS					PCR	ORTHO 2		ABBOTT 2*	
	Serum	Serum	Serum	Pigtail	5-1-1	C100	C33c	C22	SOD			+/-	Batch 53901HP	
GRO-A	1.087	1.029	0.959	0.796	-	1+	-	-	-	-	2.340	+	0.764	+
	0.436	0.446	0.446	0.402							0.751		0.671	+
	2.200	2.200	2.200	2.200	4+	4+	4+	4+	-	+	2.328	+	2.2	+
	0.518	0.446	0.446	0.402							0.751		2.2	+
	0.619	0.554	0.525	0.629	-	-	-	-	-		1.604	+	0.379	-
	0.447	0.402	0.402	0.447							0.751		0.442	-
	0.478	0.447	0.459	0.438	-	-	-	-	-		0.094	-	0.357	-
	0.447	0.402	0.402	0.447							0.751		0.485	+
	0.725	0.434	0.492	0.338	-	-	-	-	-		0.569	-	0.283	-
	0.447	0.402	0.402	0.447							0.751		0.260	-
	2.200	1.991	2.082	2.200	-	-	-	4+	-	-	2.407	+	2.2	+
	0.446	0.402	0.402	0.447							0.751		2.108	+
	0.639	0.634	0.680	0.529	-	-	-	-	-		1.657	+	0.590	+
	0.446	0.402	0.402	0.447							0.751		0.533	+
	2.200	2.200	2.200	2.200	4+	4+	4+	4+	-	+	2.442	+	2.2	+
	0.402	0.447	0.447	0.431							0.751		2.2	+
	0.421	0.409	0.467	0.422	-	-	-	-	-		0.127	-	0.274	-
	0.412	0.447	0.447	0.431							0.751		0.278	-
	1.051	1.264	1.127	1.222	-	-	-	1+	-	-	2.213	+	0.886	+
	0.412	0.447	0.447	0.431							0.751		0.908	+
	0.465	0.525	0.585	0.503	-	-	-	-	-		0.455	-	0.442	-
	0.447	0.431	0.431	0.468							0.751		0.410	+
	0.476	0.519	0.508	0.375	-	-	-	-	-		0.840	+	0.456	+
	0.403	0.431	0.431	0.468							0.751		0.415	-
	2.200	2.200	2.200	2.200	-	-	2+	4+	-	+	2.684	+	2.2	+
	0.403	0.431	0.431	0.468							0.751		2.2	+
	2.200	2.200	2.200	2.200	4+	4+	4+	4+	-	+	2.503	+	2.2	+
	0.431	0.468	0.468	0.396							0.751		2.2	+

Cut-Off 0.447

TABLE 2 Cont'd

DONATION NUMBER	ABBOTT RESULTS BATCH 52798HP				RIBA-2 RESULTS					PCR	ORTHO 2		ABBOTT 2*	
	Serum	Serum	Serum	Pigtail	5-1-1	C100	C33c	C22	SOD			+/-	Batch 53901HP	
GRO-A	1.834	1.653	1.835	1.655	-	-	-	-	-		1.591	+	1.702	+
	0.431	0.468	0.468	0.396							0.751		1.650	+
	2.200	2.200	2.200	2.200	-	-	-	4+	-	+	1.896	+	2.2	+
	0.404	0.396	0.396	0.427							0.751		2.2	+
	0.821	0.816	0.725	0.744	-	-	-	-	-		1.229	+	0.677	+
	0.468	0.396	0.396	0.427							0.751		0.816	+
	0.488	0.518	0.482	0.467	-	-	-	-	-		0.486	-	0.550	+
	0.396	0.427	0.427	0.427							0.751		0.468	+
	2.200	2.200	2.200	2.200	-	-	4+	4+	-	+	2.698	+	2.2	+
	0.427	0.407	0.407	0.407							0.751		2.2	+
	2.200	2.200	2.200	2.200	-	-	-	4+	-	+	2.759	+	2.2	+
	0.427	0.467	0.467	0.467							0.751		2.2	+
	2.200	2.200	2.200	2.200	3+	4+	4+	4+	-	+	2.744	+	2.2	+
	0.412	0.467	0.467	0.467							0.751		2.2	+
	2.200	2.200	2.200	2.200	-	-	4+	4+	-	+	2.446	+	2.2	+
	0.412	0.467	0.467	0.467							0.751		2.2	+
	1.280	1.078	1.103	0.982	-	-	-	-	-		0.272	-	1.297	+
	0.467	0.444	0.444	0.444							0.751		1.380	+
	0.521	0.530	0.421	0.493	-	-	-	-	-		0.120	-	0.549	+
	0.417	0.444	0.444	0.444							0.751		0.717	+
	0.832	0.708	0.662	0.683	-	-	-	-	-		1.913	-	0.582	+
	0.467	0.444	0.444	0.414							0.751		0.583	+
	0.464	0.403	0.453	0.372	-	-	-	-	-		1.045	+	0.472	+
	0.444	0.414	0.414	0.414							0.751		0.437	-
	1.228	1.004	0.980	0.945	-	-	-	-	-		1.753	+	1.242	+
	0.444	0.414	0.414	0.414							0.751		1.281	+
	0.534	0.440	0.407	0.424	-	-	-	-	-		0.097	-	0.439	-
	0.441	0.414	0.414	0.414							0.751		0.481	+

Cut-Off 0.447

TABLE 2 Cont'd

DONATION NUMBER	ABBOTT RESULTS BATCH 52798HP				RIBA-2 RESULTS					PCR	ORTHO 2		ABBOTT 2°	
	Serum	Serum	Serum	Pigtail	5-1-1	C100	C33c	C22	SOD			+/-	Batch 53901HP	
GRO-A	1.684	1.742	1.582	1.629	-	-	-	-	-		1.286	+	1.699	+
	0.441	0.414	0.414	0.414							0.751		1.617	+
	2.200	2.200	2.200	2.200	-	-	4+	4+	-	+	2.580	+	2.2	+
	0.441	0.414	0.414	0.414							0.751		2.2	+
	2.200	2.200	2.200	2.200	-	4+	2+	4+	-	-	2.363	+	2.2	+
	0.441	0.414	0.414	0.414							0.751		2.2	+
	0.578	0.506	0.502	0.503	-	1+	-	-	-	-	1.267	+	0.366	-
	0.444	0.414	0.414	0.414							0.751		0.397	-
	0.571	0.484	0.487	0.423	-	-	-	-	-		1.655	+	0.468	+
	0.444	0.414	0.414	0.414							0.751		0.500	+
	0.740	0.848	0.637	0.739	-	1+	-	-	!	-	1.712	+	0.664	+
	0.444	0.414	0.414	0.414							0.751		0.714	+
	0.672	0.591	0.535	0.598	-	-	-	-	-		2.609	+	0.683	+
	0.444	0.414	0.414	0.414							0.751		0.731	+
	0.475	0.337	0.426	0.418	-	-	-	-	-		0.195	-	0.325	-
	0.444	0.414	0.414	0.414							0.751		0.445	-
	0.702	0.618	0.645	0.545	-	-	-	-	-		0.089	-	0.639	+
	0.444	0.414	0.414	0.414							0.751		0.581	+
	0.499	0.453	0.523	0.462	1+	-	-	-	-	+	0.229	-	0.492	+
	0.414	0.397	0.397	0.397							0.751		0.539	+
	0.527	0.435	0.492	0.306	-	-	-	-	-		0.197	-	0.357	-
	0.414	0.397	0.397	0.397							0.751		0.475	+
	2.200	2.200	2.200	2.200	-	1+	3+	4+	-	+	2.303	+	2.2	+
	0.414	0.397	0.397	0.397							0.751		2.2	+
	1.274	1.023	1.198	1.083	-	1+	-	-	-	-	2.646	+	0.892	+
	0.499	0.420	0.420	0.420							0.751		0.811	+
	0.522	0.563	0.574	0.573	-	-	-	-	-		0.781	+	0.521	+
	0.473	0.377	0.377	0.377							0.751		0.626	+

Cut-Off 0.447

ABLE 2 Cont'd

ONATION UMBER	ABBOTT RESULTS BATCH 52798HP				RIBA-2 RESULTS					PCR	ORTHO 2		ABBOTT 2'	
	Serum	Serum	Serum	Pigtail	5-1-1	C100	C33c	C22	SOD			+/-	Batch 53901HP	
GRO-A	0.621	0.638	0.597	0.586	-	-	-	-	-		0.096	-	0.579	+
	0.473	0.377	0.377	0.377							0.751		0.576	+
	0.642	0.811	0.789		-	1+	-	-	-	-	2.136	+	0.653	+
	0.391	0.390	0.390								0.751		0.592	+
	0.417	0.488	0.434	0.406	-	-	-	-	-		0.888	+	0.414	-
	0.377	0.390	0.390	0.390							0.751		0.438	-
	2.175	2.200	2.200	2.200	-	-	-	4+	-	-	2.688	+	2.103	+
	0.421	0.421	0.421	0.421							0.751		2.098	+
	2.200	2.200	2.200	2.200	-	1+	4+	3+	-	+	2.372	+	2.2	+
	0.390	0.421	0.421	0.421							0.751		2.2	+
	0.414	0.524	0.460	0.448	-	-	-	-	-		0.798	+	0.328	-
	0.390	0.421	0.421	0.421							0.751		0.333	-
	0.532	0.397	0.427	0.355	-	-	-	-	-		1.563	+	0.334	-
	0.411	0.406	0.406	0.425							0.751		0.335	-
	0.530	0.536	0.563	0.536	-	-	-	-	-		0.880	+	0.579	+
	0.406	0.431	0.431	0.431							0.751		0.584	+
	0.908	0.696	0.832	0.814	-	-	-	-	-		1.881	+	0.550	+
	0.459	0.444	0.444	0.444							0.751		0.527	+
	0.488	0.496	0.425	0.501	-	-	-	-	-		0.222	-	0.341	-
	0.459	0.444	0.444	0.444							0.751		0.369	-
	0.493	0.459	0.415	0.490	-	-	-	-	-		1.023	+	0.288	-
	0.459	0.444	0.444	0.444							0.751		0.382	-
	2.200	2.200	2.200	2.200	-	-	4+	4+	-	+	2.680	+	2.2	+
	0.459	0.444	0.444	0.444							0.751		2.2	+
	2.200	2.200	2.200	2.200	4+	4+	4+	4+	-	+	2.411	+	2.2	+
	0.459	0.444	0.444	0.425							0.751		2.2	+
	2.200	2.200	2.200	2.200	-	-	2+	4+	-	+	2.375	+	2.2	+
	0.422	0.424	0.424	0.424							0.751		2.2	+

Cut-Off 0.447