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MULTI-CENTRE UK

NANB SURROGATE MARKER STUDY

VOLUME I

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SERUM ALANINE AMINO-TRANSFERASE (ALT) LEVELS IN UK BLOOD DONORS

INTRODUCTION:

In the USA in 1986 the FDA recommended that all blood donations should be screened for ALT as a surrogate marker for non-A, non-B hepatitis (NANBH), and the American Red Cross subsequently initiated a screening programme. On 30th November, 1987 the American Association of Blood Banks reaffirmed its decision to require ALT testing on all blood donations, with rejection of those units with an ALT activity above a specified cut-off (1). There have been no prospective studies in the USA to assess the effect of such a policy on the incidence of post transfusion hepatitis (PTH). The main considerations for adopting ALT screening had emerged from the Transfusion Transmitted Viruses (TTV) study (2) and from another evaluation of patients who underwent open heart surgery and blood transfusion at the National Institute of Health (NIH) Clinical Centre (3). The TTV study showed that of the recipients of at least one unit of blood with an elevated ALT (>60 IU/L), 45% developed NANBH. Similarly the NIH study demonstrated that 29% of the recipients of units with an ALT activity greater than 53 IU/L developed ANBH. It was therefore argued that, in the absence of a specific marker for NANBH, the ALT level in a unit of blood could be used as a "surrogate marker" of infectivity for NANBH.

The TTV and NIH studies were both undertaken in the 1970's. Since then the introduction of more stringent donor deferral policies (including self exclusion of donors at risk of HIV infection) might well have contributed to a reduction in the incidence of PTH (9).

Although NANB hepatitis has traditionally been regarded as a diagnosis of exclusion, this situation may soon change as a result of the achievement of the cloning of the genome of its major causative agent, the Hepatitis C virus (HCV), and the introduction of a specific serological assay for anti-HCV (4-10). However there are still unresolved problems. There are no confirmatory tests available for the new anti-HCV assay, and this assay may have a relatively limited sensitivity (10). Moreover, during the window period before seroconversion, which may last up to 12 months, there is no specific assay available to diagnose infection with hepatitis C virus. Although the relatively low sensitivity of the assay may have been a by-product of the effort to ensure a high specificity, it may also be related to the possibility that other non-A, non-B (and also non-C) hepatitis viruses could conceivably be implicated as the aetiological agents in the transmission of post-transfusion hepatitis. Prior to the discovery of HCV, testing of blood donations for surrogate markers of NANB hepatitis was the only means of reducing the incidence of post transfusion hepatitis. In view of the above considerations, Alter supports the value of retaining ALT testing (9). In addition, van der Poel et al (10), showing an association between HCV seropositivity and raised ALT levels, emphasise the importance of excluding blood donations with raised ALT.

The present D.o.H. multicentre study of surrogate markers in the donor population was conceived and designed just before Chiron Corporation announced that it had cloned the HCV virus. However its original aims are still relevant, particularly as there is a paucity of information about the incidence of post transfusion hepatitis, and the prevalence of positive surrogate markers in the United Kingdom. This study aimed to address the following issues:

1) The incidence of raised alanine aminotransferase activity in the donor population in England.

2) The relevance of ALT screening tests in the context of reducing the incidence of post-transfusion hepatitis in England and Wales.

3) The effect on the donor panel of introducing a deferral policy based on tests for surrogate markers.

Materials and methods:

Three Regional Blood Transfusion Centres (RTCs) participated in this study: North London, Bristol and Manchester, with North London as the coordinating Centre. Approval was obtained from the appropriate ethical committee for each Transfusion Centre. Blood donor sessions at each Centre were selected to represent a mix of Industrial, Public and College donors. All donors at each session were provided with a leaflet requesting their participation and giving them relevant details (see Appendix). An additional blood sample of 10 cm^3 was obtained from the bleed line at the end of the donation, and collected into glass tubes without anticoagulant. Altogether 9741 donors (3036 from North London, 3015 from Bristol and 3690 from Manchester) were tested.

North London:

As soon as the whole blood samples arrived at the Centre they were stored at +4°C and the serum separated within 24 hours of donation. Serum was tested for ALT within 24 hours of donation. An EPOS automated clinical analyser (Eppendorf range) using Merckotest reagents (according to the Scandinavian Committee on Enzymes for ALT) was used for ALT testing at 37°C. Standard sera were tested at the beginning of each run and after each subsequent 30 samples. A repeat measurement was carried out on samples with an ALT activity greater than 45 IU/L.

Manchester:

Whole blood samples were kept at room temperature and separated within 24 hours. The serum samples were kept at room temperature until re-aliquoted on the following day and then stored at +4° C until tested for ALT within the next 5 days. ALT activity was measured at 37°C using the Parallel Analyser (American Monitor Corporation) with a TRIS buffer (pH 7.9) and pyridoxal phosphate.

A further 526 samples from Manchester were separated within 8 hours, and the sera kept at -30° C. These were sent to the North London RTC for ALT testing using the EPOS analyser detailed above.

Bristol:

Blood samples were kept at +4°C and the serum was separated within 24 hours of donation. ALT testing was done within 6 days on the samples which had been stored at +4°C, except for the first 500 samples which were tested within 14 days from the date of donation. The Technicon SMAC System was used for ALT testing based on the automated spectrophotometric glutamic-pyruvic transaminase method, using a TRIS buffer.

REPRODUCIBILITY:

The in-between batch coefficients of variation (CV) for ALT testing at each centre during the period of ALT testing were as follows: 9.9% for North London at 14 IU/L; 7.9% for Manchester at 30 IU/L and 15.4% for Bristol at 39 IU/L.

THE COMPARABILITY PANEL:

Prior to the commencement and during the period of ALT testing three panels of sera (first set 20 ,second set 13 and third set 19 sera) were despatched from North London RTC to the other participating centres. These samples were stored at -20°C and despatched overnight. The ALT activities were measured on the following day at each centre. The results of such testing are contained in figs 11, 12, 13.

RESULTS

A total of 9741 samples were tested for ALT: 3036 from North London (51.9% males), 3015 from Bristol (52.1% males) and 3690 from Manchester (57.7% males). The prevalence of raised ALT (>45 IU/L) was 3.1% overall with 3.06% for North London, 4.56% for Bristol and 1.97% for Manchester.

PRECISION OF THE RESULTS:

NORTH LONDON. While the within batch coefficients of variation (CV) were less than 5% in 7 out of 16 batches (43%), CVs as high as 14% were recorded. The inter-batch CV was 18.4% at 15 IU/L (9.9% when out of the range values were excluded).

BRISTOL. The within batch CV was 3.3% at 108 IU/L. The between batch CVs were as follows: 15.4% at 39 IU/L,and 3.9% at 325 IU/L.

MANCHESTER. These data are not from the period that the original 3169 samples were analysed but are typical of the analytical performance of that time.

The within batch CVs were as follows: at 33 IU/L 7.1%, at 105 IU/L 2.1% and at 309 IU/L 1.2%.

The inter-batch CVs were as follows: at 30 IU/L 7.9%,at 94 IU/L 6.4% and at 315 IU/L 2.3%.

CORRELATION OF THE RESULTS BETWEEN THE PARTICIPATING CENTRES:

For the purposes of correlating the comparability panel results, the values obtained at North London RTC were assumed to represent the "true values". The linearity of relationship between results of the other two centres and of North London was established (see figs 11, 12 and 13).

When considering the panel of sera despatched on 19/7/88: for Bristol and North London the coefficient of correlation (r) was 0.99. The coefficient of correlation between North London and Manchester was 0.85.

*The panel despatched on 4/8/88: the coefficient of correlation between North London and Manchester was 0.98.

For the panel despatched on 17/11/88: the coefficient of correlation between North London and Manchester was 0.95. The coefficient of correlation between North London and Bristol was 0.96.

RESULTS OF ALT TESTS ON SAMPLES FROM BLOOD DONORS:

Figures 1,2,5,8 show the distribution of ALT activities in the donor populations from the three Centres. The second set of results from Manchester (526 samples) was plotted separately as it was obtained using the EPOS analyser at the North London RTC.

As expected none of the histograms showed a normal distribution; however results from Manchester showed a greater degree of skew, particularly in the first set of 3164. Figures 3,4,6,7,9,10 show the gender specific distribution of ALT values. Table III includes the mean ALT of donors in each age group.

As the log of the ALT values in each subgroup did not correspond to a Gaussian curve, non-parametric methods were used to describe these populations.

For the donors at North London, the cut-off value of 45 IU/L represents the 96.75th percentile; this compares with the value of 41.6 IU/L (96th percentile) representing 2.0 SD above the arithmetical mean in a Gaussian population. The point representing the 98.0th percentile was 51 IU/L.

For the first 3164 donors from Manchester whose sera were measured by the Parallel System, 45 IU/L represented the 98th percentile. The 2 S.D. above the arithmethical mean in a Gaussian population would have been equivalent to 40 IU/L. The point representing the 95th percentile is 35 IU/L. The second set of 526 samples analysed by EPOS were described as follows: The cut-off value of 45 IU/L is between the 98th and 98.25th percentiles. Two S.D. above the arithmetical mean was equivalent to 33.4 IU/L in a Gaussian population, (Tables I and II).

*Unfortunately, Bristol RTC did not receive this panel.

For Bristol donors the cut-off value of 45 IU/L represents the 95.25th percentile. The value for 2.0 SD above the arithmetical mean in a Gaussian population would be 47 IU/L. The point representing the 98.0th percentile was 58 IU/L

BREAKDOWN OF THE ALT RESULTS BY GENDER: (Tables I and II)

For North London female donors, 45 IU/L represents the 98.8th percentile; while for North London male donors 45 IU/L represents the 95th percentile. For Bristol female donors 45 IU/L represents the 98.1 percentile. Although the histogram of the ALT values was not of normal distribution, the logarithmic transformation of the distribution resulted in an essentially Gaussian curve. For Bristol male donors, 45 IU/L represents the 93th percentile. For Manchester female donors 45 IU/L represents the 99th percentile and for male donors 45 IU/L represents the 97th percentile. These Manchester statistics refer to the first 3164 samples. The breakdown by gender of the second set of 526 samples from Manchester would result in sample populations too small to produce reliable statistical results.

BREAKDOWN OF ALT RESULTS BY TYPE OF SESSION: (Table IV)

Although in general it appears that donors from colleges have a lower proportion with raised ALT, this achieves statistical significance only for the donors from Bristol.

BREAKDOWN OF RESULTS BY DONOR TYPE(FIRST TIME/KNOWN DONORS): (Table V)

Examination of the results in Table V reveals that practically no significant differences exist between statistics from first time donors and known donors.

DISCUSSION:

Probably the most obvious conclusion from this observational study is the variability in the ALT statistics, not only from the geographical point of view but also, pertaining to the gender and the session type amongst the donor populations studied (tables 2,3 and 4). American studies have shown that ALT is lowest below age 20, rises to a peak at age 30, and returns to a lower mean by age 65 (16). This pattern was certainly recognisable for male donors from North London (TABLE II); but otherwise not detectable in the other donor populations in the study. The same pattern emerges when analysing the data from ALT estimations carried out earlier at North London (13); while there was a peak mean ALT between 36-45 years, a gradual increase was recorded for females with a peak at 56-65 years. However a larger donor population needs to be studied to be certain that the changes in mean ALT are not fortuitous.

Regional cut-offs versus a national cut-off.

It can be argued that were ALT testing to be adopted in the UK, in the absence of national standardisation with the provision of appropriate reference material, it would be preferable to opt for individual laboratories selecting their own cut-off values for donor deferral. These cut-offs could be based either on the locally derived 2.0 SD above the arithmetical mean, 2.25 SD above the logarithmic mean or preferably the value corresponding to their 95th to 99th percentile. The value of 2.0 SD above the mean was suggested by the TTV study as providing optimal efficacy without incurring in excessive donor loss and is the cut-off most widely used in the United States. However, as the ALT results from donor populations are widely recognised not to conform to a Gaussian distribution, even after the logarithmic transformation of the results, choice of percentiles may be a more rational method of selecting a cut-off. The ALT cut-offs of 2.25 SD or 2.0 SD above the log mean were derived from prospective studies of NANB PTH in the USA which defined the incidence of post-transfusion NANBH. Unfortunately similar studies with stringent protocols have not been reported in the UK and because of the absence of such local data any cut-off chosen would be arbitrary.

It is pertinent to point out that in the USA a policy for a national (as opposed to regional) cut-off was given preference, on the grounds that regional variations in the mean ALT level are mainly due to differences in the prevalence of NANB hepatitis rather than interregional physiological factors (16). However the USA policy did not take into account the differences in social and behavioural patterns (such as alcohol consumption) that are likely to be the major contributing factor to these ALT variations.

Storage and Stability.

A study of this type entails the likelihood of delays in performing ALT estimations. On the basis of the reported data, loss of ALT activity may have occured in the frozen stored samples. Unfortunately it is not possible to uniformly correct for such losses in the ALT activity as the published data on this subject is not entirely consistent, and is also limited in the range of temperatures and storage times studied. (13,17)

Comparability of the results:

Reference to the comparability panels 2 and 3, would lead one to expect lower ALT levels for Manchester (consequently with an increased positive skew). Another explanation for this degree of skew is that, in Manchester, values under 10 IU/L were not specified and thus were entered as 5 IU/L which further skews the histogram positively. An additional factor contributing to the lower mean ALT values in the samples from Manchester, may have been due to the fact that Manchester samples were subject to more prolonged storage at room temperature with resulting loss of ALT activity. In the absence of reproducibility data on a standard sample with an ALT value below 20 IU/L an objective estimate of the instrument precision for the lower range of ALT values cannot be made. However it is the general impression of the users of the Parallel instrument at Manchester that this assay system is designed to ensure higher sensitivity at ranges around 45 IU/L and above with a relative sacrifice of its precision in the range below 15 IU/L.

Gender specific cut-offs:

Another issue for consideration is whether to choose separate cut-offs for male and female donors. Once again any rational decision must await results from current studies of post-transfusion hepatitis/HCV seropositivity in this country. However the present study and previous observations (14) would favour the adoption of different cut-offs, although obviously there might be practical problems. In addition a recently published large study seems not to support gender specific cut-offs (12).

The question of the relevance of ALT testing to the reduction of the incidence of PTH needs further elaboration. In the TTV and NIH studies, hepatitis developed in 10.3% and 12.7% of the transfusion recipients respectively, with widely varying attack rates among the participating centres (from 4.3% in St.Louis to 17.4% in Houston in the TTV study). Furthermore the TTV study showed that 42% of the recipients of single donations with an ALT activity > 45 IU/L developed hepatitis in comparison with 5% who developed hepatitis after receiving a single unit of blood with an ALT activity of <45 IU/L. Overall, 35% of donations with an ALT of 45-59 IU/L, and 47% in the >60 IU/L range were implicated in NANB PTH cases. 35% of the recipients of blood with ALT >45 IU/L developed hepatitis as opposed to 7% of the recipients of blood with ALT <45 IU/L. The NIH study showed that 9.1% of the recipients of blood units with ALT <2.25 SD above the log mean value developed hepatitis as compared with 35.7% when blood with ALT >3.0 SD+log mean value was transfused. Theoretically these results were explained on the basis that carriers of NANBH are more likely to have elevated ALT. Thus in the NIH study, for example, a cut-off of 2.25 SD+log mean would have prevented 41% of the cases of PTH with a donor loss of about 1.5%. Despite these relatively impressive results, ALT testing on a national scale did not immediately follow the publicaton of the TTV and NIH studies in 1981. It was only after the impact of the AIDS epidemic in the USA that pressure for the implementation of surrogate testing gathered momentum. In the final analysis, publication of data pertaining to the chronic sequelae of NANBH probably favoured the adoption of screening for surrogate markers. These data suggested that 10-70% of acute cases of NANBH could develop chronic liver disease

(based on a follow-up of 954 NANB PTH cases as reviewed by Dienstag). Hepatic morphological findings in 95 patients with chronic post transfusion NANBH, evaluated histologically (in 8 series) were compatible with chronic active hepatitis and/or cirrhosis in 44-90% of cases (15). Similar data in this country are lacking with the exception of available information on prevalence and chronicity of NANB hepatitis in haemophilia patients (11).

One further consideration is the question of a single European blood market in the foreseeable future, where issues of blood safety may be interwoven with those of blood product exports and similar questions.

In conclusion, whether or not ALT testing should be considered in the context of reducing the incidence of PTH has become even less clear after the availability of a specific HCV marker. The Consumer Protection Act with the regulations on Product Liability, recently introduced in the UK, also adds new dimensions to this debate. From the consumer's point of view, the important issue is the potential iatrogenic nature of NANB PTH and its chronic sequelae, while the manufacturer has to bear the costs of further microbiological safeguards. The argument that these costs can then be passed on to the consumer has no relevance at the moment in the UK. The subject of cost-effectiveness has recently been reviewed (15), but if the desire to ensure a "minimum risk" product overrides the economical and logistic considerations, ALT testing then becomes a serious contender, among the other aspirants, for entry into the list of microbiologically orientated screening tests.

TABLE I DISTRIBUTION OF ALT VALUES IN BLOOD DONORS

Centre	Gender	Number of donors	Mean ALT (Median) IU/L	S.D.	ALT>45 IU/L
N.London	M	1583	22.2 (19)	12.8	4.82%
	F	1453	13.8 (12)	8.6	1.10%
	Total	3036	18.2 (15)	11.7	3.06%
Bristol	M	1591	24.5 (22)	14.1	6.98%
	F	1424	17.1 (15)	10.9	1.83%
	Total	3015	21.0 (18)	13.2	4.56%
M'chester	M	1816	16.7 (14)	13.3	2.75
	F	1348	12.2 (9)	10.9	0.96
	Total	3164	14.8 (12)	12.6	1.99

TABLE II.PERCENTILES FOR 2SD+MEAN AND THE CUT-OFF OF 45 IU/L

Centre	Gender	PERCENTILE		Mean + 2SD	45 IU/L
		95th	98th	PERCENTILES	
N.London	M	45 IU/L	55 IU/L	95.7th	95.0th
	F	26	37	96.5	98.8
	Total	40	51	96.0	96.75
Bristol	M	51	65	95.2	93.0
	F	33	44	96.5	98.12
	Total	44	58	95.75	95.25
M'chester	M	39	50	96.50	97.0
	F	30	37	96.75	99.0
	Total	35	45	96.75	98.0

TABLE III. MEAN ALT VALUES ACCORDING TO AGE

Centre	Age Group	Number of donors			Mean ALT (IU/L)		
		M	F	Total	M	F	Total
North London	18 - 25 yr	229	298	527	20	12	15
	26 - 35	496	469	965	22	13	18
	36 - 45	445	378	823	23	14	19
	46 - 55	262	206	468	22	13	18
	56 - 65	137	97	234	21	16	19
Bristol	18 - 25	234	326	560	20	16	18
	26 - 35	368	294	663	24	16	20
	36 - 45	393	303	698	24	16	21
	46 - 55	239	165	405	24	18	22
	56 - 65	117	94	212	24	18	22
Manchester	18 - 25	310	344	654	16	11	13
	26 - 35	541	403	944	16	11	14
	36 - 45	521	329	850	16	11	14
	46 - 55	304	189	494	16	13	15
	56 - 65	136	80	216	16	15	15

NB: In North London, 6% of the donors presented with the leaflet requesting their participation in the study refused.

TABLE IV. BREAKDOWN OF ALT VALUES BY SESSION TYPE.

	N.LONDON		BRISTOL		MANCHESTER	
	No: (%)*	ALT > 45 IU/L	No: (%)	ALT> 45 IU/L	No: (%)	ALT> 45IU/L
general public sessions	1611 (53%)	41 (2.6%)	1547 (51%)	73 (4.7%)	1092 (34%)	17 (1.5%)
static centres	119 (4%)	6 (5.0%)	465 (15%)	27 (5.8%)	1190 (37%)	26 (2.2%)
Industrial sessions	1122 (37%)	42 (3.7%)	725 (24%)	33 (4.5%)	811 (25%)	20 (2.4%)
Educational institutions	185 (6%)	4 (2.2%)	278 (9%)	4 (1.4%)	71 (2.2%)	0 (0%)
TOTAL	3035	93 (3.1%)	3015	137 (4.5%)	3164	63 (1.9%)

* Of the total

TABLE V. DISTRIBUTION OF THE ALT RESULTS AMONG FIRST-TIME AND REGULAR DONORS.

CENTRE	N. LONDON			BRISTOL			MANCHESTER		
	NO: (%)	MEAN (SD)	ALT> 45	NO: (%)	MEAN (SD)	ALT> 45	NO: (%))	MEAN (SD)	ALT> 45
KNOWN DONORS	2285 (76%)	18 (12)	2.9 %	2621 (87%)	21 (13)	4.6 %	2857 (91%)	14 (12)	2 %
FIRST TIME DONORS	748 (24%)	18 (10)	3.3 %	393 (13%)	20 (12)	4.2 %	287 (9%)	15 (16)	1.4 %

REFERENCES:

1. American Association of Blood Banks reaffirms decision to require ALT and anti-HBc testing. Blood Bank Week, 1987;4:1-2.

2. Aach R D, Szmuness W, Mosley J W, et al: Serum alanine aminotransferase of donors in relation to the risk of non-A, non-B hepatitis in recepients. The transfusion transmitted viruses study. N Engl J Med 1981; 304:989-994.

3. Alter H J, Purcell R H, Holland P V, Alling D W, Koziol D E. Donor transaminase and recipient hepatitis. Impact on Blood Transfusion Service. JAMA 1981;246:630-4.

4. Choo Q-L, Kuo G, Weiner A J, Overby L R, Bradley D W, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A non-B viral hepatitis genome. Science 1989; 244:359-62.

5. Kuo G, Choo Q-L, Alter H J, et al. An assay for circulating antibodies to a major etiologic virus of human non-A non-B virus. Science 1989; 244:362-64.

6. Esteban J I, Esteban R, Viladomiu L, et al. Hepatitis C Virus antibodies among risk groups in Spain. Lancet 1989 ii:294-297.

7. Editorial. Will the Real Hepatitis C Stand Up? Lancet 1989 ii, 307-308.

8. Kuhnl P, Seidl S, Stangel W, Beyer J, Sibrowski W, Flick J. Antibodies to Hepatitis C Virus in German blood donors. Lancet 1989; ii:294-295.

9. Alter J H. Discovery of the Non-A, Non-B Hepatitis Virus : The End of the Beginning or the Beginning of the End. Transfusion Medicine Reviews 1989, Vol III, No 2: 77-81.

10. Van Der Poel C L, Reesink H W, Lelie P N, Leentvaar-Kuypers A, Choo Q L, Koo G, Houghton M. Anti-Hepatitis C antibodies and non-A,

non-B post transfusion hepatitis in the Netherlands. Lancet 1989 ii: 297-298.

11. Hay C R M, Triger D R, Preston F E, et al. Progressive liver disease in haemophilia :An understated problem? Lancet 1985 i:1495-1497.

12. AuBuchon J P, Wilkinson J S, Kassapian S J, Edwards G C. Establishment of a system to standardize acceptability criteria for alanine aminotransferase activity in donated blood. Transfusion 1989 Vol 29:17-22.

13. Mijovic V, Contreras M, Barbara J A J. Serum alanine aminotransferase (ALT) and gamma-glutamyltransferase (gamma-GT) activities in north London blood donors. J Clin Pathol 1987, 40:1340-1344.

14. Brier R O. Serum ALT levels. Effect of sex, race, and obesity on unit rejection rate. Transfusion 1988;28:392-3.

15. Dienstag J L. Non-A, Non-B Hepatitis.I. Recognition, Epidemiology, and Clinical Features. Gastroentrology 1983; 85:439-62.

16. Barbara J A J. Micribiological screening of blood donations:cost versus effectiveness. Medical Laboratory Sciences 1989; 46: 250-254.

17. Zuck T F, Sherwood W C, Bove J R. A review of the recent events related to surrogate testing of blood to prevent non-A, non-B post transfusion hepatitis. Transfusion 1987; 27:203-206.

18. Williams KM,Williams AE,Kilne LM,Dodd RY. Stability of serum alanine aminotransferase activity. Transfusion 1987;27:431-433.

APPENDIX:

The following is the text of the information leaflet distributed among the blood donors in each session selected for inclusion in the study.

"Will You Help Us With Some Important Research?

A liver function test has been developed which may improve the hepatitis (jaundice) testing within the Blood Transfusion Service. Before its introduction for routine use it is important to know the range of liver function tests in healthy people.

This Is Where Your Help Is Needed

With your consent, this test could be carried out on the routine samples collected from you today during your donation. Even if this test proves normal, or in the unlikely event of anything unusual showing up we may wish to contact some people in a few weeks time.

In that event you might be asked to attend for an interview with one of our doctors at some mutually convenient venue.

Further samples might also be required to assist in the evaluation of the new test.

If, however, you do **NOT** wish to be included in this study, please return this leaflet to the clerk at the reception desk with your name and address on the back."

VOLUME II

ANTI-HBc TESTING IN UK DONOR POPULATION

INTRODUCTION:

The subject of testing blood donations for surrogate markers of non-A, non-B hepatitis (NANBH) arouses some controversy. Studies conducted before the introduction of self-exclusion policies (for risk of HIV infection) showed a correlation between donor anti-HBc positive status and the risk of transmitting NANBH (1,2). However a recent Dutch prospective study did not find such a correlation (3). Also when anti-HCV testing was carried out retrospectively on samples in that study, no correlation was found between HCV and HBc antibodies (4). Even when earlier data are considered it is apparent that as a marker of NANBH transmission, anti-HBc has a low predictive value. However a desire to prevent a predicted 43% of cases of NANBH finally prompted its adoption as a mandatory screening test for blood donations in the USA (5). Although a report on the prevalence of anti-HBc in blood donations in England will not directly resolve this controversy, apart from providing useful epidemiological data when integrated with any prospective recipient studies, it should contribute towards clarification of a situation with considerable resource implications.

MATERIALS AND METHODS:

A total of 9742 blood samples were collected from healthy blood donors(age 18-65) at the three participating centres (North London, Bristol and Manchester), between September 1988 and April 1989. Prior consent was obtained for "hepatitis testing"(for further details on sample collection and storage see ALT report, volume I). All sera had been tested for anti-HIV, and HBsAg by standard methods. Sera were despatched from the Manchester and Bristol Regional Transfusion Centres (RTCs) to the North London Blood Transfusion Centre for anti-HBc testing. Wellcome EIA anti-HBc kits were used for initial screening of the 9711 samples in the study. Positive samples were retested in duplicate. Repeatedly reactive samples were sent to a Reference laboratory (Department of Virology, The Middlesex Hospital, London) for further confirmatory testing. In all instances the confirmatory tests included anti-HBc testing using an in-house radioimmunoassay (6), and for 78 samples an additional haemagglutination assay (Green Cross Corp., Japan). Anti-HBs tests were also performed at the Department of Virology, The Middlesex Hospital, on all anti-HBc repeat-reactive samples using an in-house RIA (6). Samples confirmed as anti-HBc positive were sent to the Academic Dept. of Medicine, Royal Free Hospital for HBV DNA investigation.

RESULTS:

Initial screen positive rates were 1.8%, 0.93% and 1.32%, respectively for North London, Bristol and Manchester RTCs, with an overall rate of 1.3%. (Table I)

The overall anti-HBc repeat reactive rate by Wellcome EIA was 0.9% (0.9% for North London, 0.79% for Bristol and 0.94% for Manchester).

Confirmatory testing by RIA and haemagglutination on the repeatedly-reactive samples gave final anti-HBc positive rates of 0.73%, 0.53% and 0.65% for North London, Bristol and Manchester RTCs respectively, with an overall rate of 0.63%. The rate for Manchester may be spuriously low, due to unavailabity of sera for confirmation in 3 cases.

Of 62 anti-HBc positive donations confirmed unequivocally by the Reference Laboratory, 68% were males(Table II), 11% were first time donors(Table IV), and 3.06% showed a raised ALT (>45 IU/L).

The rates of unequivocally confirmed anti-HBc positivity together with elevated ALT (>45 IU/L) for the populations studied were as follows: North London 0.03% (1 out of 3016 donors), Bristol 0.0% (one repeat reactive anti-HBc was not confirmed by the Reference laboratory) and Manchester 0.02% (1 out of 3687 donors, but 3 repeat reactive donors were not tested by the Ref. Lab.)

Tables II and III show the age distribution of anti-HBc positivity which reveals a cumulative acquisition of HBV markers with advancing years.

Although the competitive RIA, and haemagglutination assay were mostly in agreement, in 4 samples out of a total of 78 where both these assays were performed, divergent results were produced (2 samples were positive only by RIA and 2 other samples by haemagglutination only). In a further 4 samples, a positive anti-HBs result was obtained in the presence of either a negative or divergent anti-HBc result. In another 6 samples due to borderline haemagglutination titres, or to RIA percentage inhibition 6% close to the cut-off, positive anti-HBc status could not be confirmed.

In order to resolve the above discrepancies, the results of confirmatory anti-HBc testing were divided into 3 main subgroups: positive, indeterminate and negative. Of 78 serum samples submitted to the Reference Laboratory, where both RIA and CORECELL assays were performed, 52 gave unequivocally positive results (of these 7 samples were anti-HBs negative i.e. "anti-HBc positive only". In another 16 samples all reference assays were negative and in 10 samples indeterminate results were obtained.

None of the confirmed anti-HBc positive samples had detectable levels of HBV DNA (at a test sensitivity of 0.5 pg HBV DNA per ml of serum, [redacted], personal communication).

The risk factors for HBV infection, as found at interview, are summarised in table V.

DISCUSSION:

Recently, AuBuchon (7) and others reported the American Red Cross experience with routine anti-HBc testing on 2.3 million blood donations using the Corzyme immunoassay (Abbott Laboratories). Their overall positivity rate was 2.54% with an interstate range varying from 1.3% to 4.6%. Eighty five percent of anti-HBc positive donors reacted positively again on their second donation. Although this report provided a mean ALT value for the 4360 positive samples, it did not provide a percentage of raised ALT levels amongst these donors, probably due to the fact that standardisation of ALT testing had not been implemented in the USA during the course of the study. Previously, re-analysis of the Transfusion Transmitted Viruses Study (TTV) had shown that 8.6% of the anti-HBc positive donors had an elevated ALT (1).

Gillon et al found a prevalence of 2% anti-HBc seropositivity among Scottish blood donors (8), while unpublished data from the North London Blood Transfusion Centre (anti-HBc testing carried out in May 1988) showed a 0.95% seroprevalence. Anti-HBc seropositivity was recently reported in 9.7% of Japanese donors (9). In a Swedish study a 1.5% anti-HBc positivity rate has been quoted (10). A report published in 1983 showed 53% of Kenyan donors to be anti-HBc positive(11).

It is generally recognised that there is very little overlap between anti-HBc positive and raised ALT populations. Polesky and Hanson quote 0.03% combined surrogate positivity rate (13), but as the TTV study and the study at the National Institute of Health (NIH) had shown, when such overlap does occur the likelihood of potential NANBH transmission in the recipient of that unit is very high. In this study, 2 donations positive for anti-HBc also had raised ALT levels; one such donation was anti-HCV positive (details in Volume IV of this report).

The TTV study had previously concluded that anti-HBc testing would reduce the incidence of NANB PTH by 21.4%, and the NIH study had shown 4% anti-HBc seropositivity amongst the donors, with 65.7% of cases of post transfusion hepatitis(PTH) having received anti-HBc positive blood. The authors therefore predicted a 43% efficiency in preventing NANB PTH, by excluding anti-HBc positive blood. The objections to using anti-HBc as a surrogate marker were based on its low predictive value, the donor loss incurred, unreliablity of the test results (5) and uncertainty about the correlation between genetically engineered core antigen and stripped Dane particles as used in the assays carried out during the TTV and NIH studies. Perhaps the most important of these consideratons was the question of efficacy and positive predictive value. However, opinions remain divided on this issue. More recent studies reinforcing this low predictive value (apart from the previously

mentioned Dutch study), include a German study which followed 417 recipients; 2.1% of those who received anti-HBc negative blood, developed hepatitis. In comparison, 10.1% of the recipients of anti-HBc positive blood developed PTH. The positive predictive value was calcuated as 10%(14). It is important to point out that this study was carried out between 1980 and 1982, before the impact of self exclusion policies altered the pattern of anti-HBc seropositivity amongst the donor population. However in the study conducted in Sweden mentioned above, only 2.3% of the 129 units involved in 14 cases of PTH were anti-HBc positive. Another recently published report pertains to 110 Japanese recipients of blood with donor ALT values <72 IU/L. NANB PTH developed in 14.5% of the recipients and no correlation was found between the incidence of PTH and the anti-HBc status of the donor(9).

As far as reliability of the anti-HBc assay is concerned, although it may not be entirely certain that RIA provides less false positive results(15), the general impression is that it is a more specific test than EIA. In one study less than 50% of the EIA reactive samples were confirmed when tested by RIA (12). However up to 80% of the repeatedly reactive EIA tests in the present study were confirmed by RIA and haemagglutination.

Another issue associated with the reliability of the assay result is the level of inhibition by RIA. In this study, instead of 50% or 70% inhibition cut-offs (16) as suggested, a variable cut-off was chosen on each batch of samples based on the comparison between a weak positive standard and the negative control.

Alter argues in favour of retaining anti-HBc testing even after the availability of specific marker(s) for NANBH, on the grounds that anti-HBc is not only a surrogate marker for NANBH, but is also a surrogate marker for HIV infection, sexual promiscuity and intravenous

drug abuse as well as being a specific marker for HBV(17). The latter would reduce HBV PTH. Although the number of these cases is admittedly small, there are a few prospective studies available reporting on the incidence of HBV PTH in recipients of anti-HBc positive blood. In the TTV study 1% of the recipients developed markers of HBV infection, although 14 out of 15 such cases became HBsAg negative subsequently. In the NIH study, 13 out of 729 transfused patients developed HBV infection(1.7%), with 4 such patients showing raised transaminase levels. A recent review of results from six other prospective studies showed an overall HBV PTH incidence of 0.7% (18)

A side issue emerging from this study was the prevalence of donors with anti-HBc without other HBV markers. A recent report from the American Red Cross showed that 17-22% of anti-HBc positive samples were "anti-HBc only" (19), while another study reports an"anti-HBc only" rate of 10% (12). In this study 13% of EIA repeat reactive samples were anti-HBs negative and of the confirmed anti-HBc positives 17.7% were"anti-HBc" only.

In conclusion data on anti-HBc seroprevalence among the blood donors in three Regional Transfusion Centres within the UK are presented here. A considerable input from Reference laboratories for confirmation of repeatedly reactive anti-HBc sera would be necessary and even then, about 10% of the results would remain indeterminate. The value of anti-HBc assay as a surrogate marker for NANBH PTH can only be assessed objectively in prospective PTH studies in the country concerned, otherwise a high proportion of donors would be lost without knowing the real significance of a positive test in a given population.

TABLE I. The results of anti-HBc tests and confirmatory assays on 9711 blood donations.

Centre	Total	North London	Bristol	Manchester
Total number of sera tested	9711	3016	3008	3687
Screen positive EIA anti-HBc	133 (1.3%)	56 (1.8%)	28 (0.9%)	49 (1.3%)
Repeat positive EIA anti-HBc	88 (0.9%)	29 (0.9%)	25 (0.83%)	34 (0.92%)
RIA anti-HBs positive	55 (0.5%)	19 (0.6%)	16 (0.5%)	20 (0.5%)
RIA anti-HBc positive	* 63 (0.6%)	25 (0.8%)	16 (0.5%)	22 (0.6%)
CORECELL positive	55 (0.5%)	22 (0.7%)	16 (0.5%)	17 (0.46%)
unequivocally confirm-ed by Reference Lab.	62 (0.63%	22 (0.73%)	16 (0.53%)	24 + (0.65%)
"anti-HBc only"	** 11	4	2	5

* 10 samples which were repeat reactives by EIA were re-tested at the reference lab by RIA only, and of these, 8 were deemed confirmed positive. Thus 71.5% (63/88) of EIA anti-HBc repeat positive results were confirmed by RIA anti-HBc, and of the 78 samples tested by CORECELL assay 70.5% (55/78) were positive while a combination of RIA anti-HBc and CORECELL assay confirmed 79.5% of repeat EIA positive results.

** Consisting 17.71/. of "confirmed" anti-HBc samples

\+ 3 of the 34 repeat reactive samples from Manchester could not be tested by the Reference Lab due to unavailability of serum samples

TABLE II. AGE DISTRIBUTION IN 62 DONORS CONFIRMED AS ANTI-HBc POSITIVE

N. London		Bristol		Manchester		Total	
F	M	F	M	F	M	F	M
8	14	5	11	7	17	20	42

(32%) (68%)

TABLE III . AGE RELATED ANTI-HBc POSITIVITY

AGE(years)	n*	% of total	anti-HBc pos.	
			n	% of *
18-25	1857	(20%)	5	(0.29%)
26-35	2724	(29.5%)	11	(0.40%)
36-45	2509	(27%)	20	(0.79%)
46-55	1430	(15.5%)	14	(0.97%)
56-65	704	(7.6%)	12	(1.70%)
	9239		62	

p = 0.0002

TABLE IV. DISTRIBUTION OF ANTI-HBc POSITIVITY IN FIRST TIME AND REGULAR DONORS.(confirmed anti-HBc)

	First Time Donors				Regular Donors			
	Total		anti-HBc+		Total		anti-HBc+	
	n	%	n	% *	n	%	n	% *
North London	748	24%	4	0.53	2285	76%	18	0.8%
Bristol	393	13%	2	0.50	2621	87%	14	0.5%
Manchester	329	9%	1	0.30	3341	91%	23	0.7%

n=collected samples.

* % of first time or regular donors.

Appendix

TABLE V. HEPATITIS RISK FACTORS IN 48 donors
(repeat EIA reactive + RIA or CORECELL positive)

Ethnic origin	
Southern European	2
Indian Asian	1
Occupational hazard	7
Surgery	31
History of Jaundice	6
Hepatitis contact	10
Transfusion history	6
Scarification	
Ear piercing	16
Tattoing	3
Electrolysis	2
Acupuncture	5
Travel to high risk areas	18

REFERENCES:

1. Stevens CE, Aach RD, Hollinger FB, et al. Hepatitis B virus antibody in blood donors and the occurrence of non-A, non-B hepatitis in transfusion recipients. An analysis of the Transfusion-Transmitted Virus Study. Ann Intern Med 1984; 101:733-8

2. Koziol DE, Holland PV, Alling DW, et al. Antibody to hepatitis core antigen as a paradoxical marker for non-A, non-B hepatitis agents in donated blood. Ann Int Med 1984; 104:488-495.

3. Reesink HW, Leentvaar-KuYpers A, Poel CL van der, et al. Non-A, non-B post transfusion hepatitis in open heart surgert patients in the Netherlands: Preliminary results of a prospective study. In: Zukerman AJ(ed) 1988.

4. van der Poel CL, Reesnick HW, Lelie PN, Leentvaar-KuYpers, Choo QL, Kuo G, and Houghton M. Anti-hepatitis C antibodies and non-A, non-B post tranfusion hepatitis in the Netherlands. Lancet 1989; ii:297-298.

5. Menitove JE. Rationale for surrogate testing to detect Non-A,Non-B Hepatitis. Transfusion Medicine Reviews, 1988; Vol 2, No 2, 65-75.

6. Tedder RS, Cameron CH, Wilson-Croome, Howell DR, Colgrove A, and Barbara JAJ. Contrasting patterns and Frequency of Antibodies to the Surface, Core, and e Antigens of Hepatitis B Virus in Blood Donors and in Homosexual patients. Journal of Medical Virology 1980; 6:323-332.

7. AuBuchon JP, Sandler SG, Fang CT, and Dodd RY. American Red Cross experience with routine testing for hepatitis B core antibody. Transfusion 1989; 29:230-232.

8. Gillon J, Hussey AJ, Howe SP, Beckett GA, and Prescott RJ. Post Transfusion Non-A, Non-B Hepatitis: Significance of Raised ALT and Anti-HBc in Blood donors. Vox Sang. 1988; 54:148-153.

9. Hitoshi Ohto, Kagemitsu Ninomiya. Donor anti-HBc and non-A,non-B hepatitis. Transfusion 1989; 3:277-8.

10. Widell A, Sundstrom G, Hansson BG, Fex G, Moestrup T, and Nordenfelt E. Viral hepatitis and liver disease. In Alan R. Liss, New York, pp558-560 Vox Sang 1988; 54:154-159.

11. Bowry TR, Shah MV. A pilot study of hepatitis B viral markers in volunteer blood donors in Kenya, East Africa. Vox Sang, 1983; 44:385-389.

12. Hanson MR, Poleski HF: Evaluation of routine anti-HBc screening of volunteer blood donors: A questionable surrogate test for non-A, non-B hepatitis. Transfusion 1987; 27:107-108.

13. Polesky H, Hanson M. Transfusion-Associated Hepatitis C Virus (non-A,non-B) Infection. Arch Pathol Lab Med 1989; 113:232-235.

14. Zuck TF, Sherwood WC, Bove JR. A review of recent events related to testing of blood to prevent non-A,non-B post transfusion hepatitis. Transfusion 1987; 27:203-206.

15. Troisi CL, Hollinger FB. Current tests for antibody to hepatitis B core antigen used to screen donors for non-A, non-B hepatitis are comparable to the original radioimmunoassay for hepatitis B core antigen. Transfusion 1987; 27:438-440.

16. Hollinger FB. Specific and surrogate screening tests for hepatitis. In: Inslaco SJ and Mentiove JE, eds. Transfusion Transmitted Viruses: Epidemiology and pathology. Arlington, VA: American Association of Blood Banks 1987.

17. Alter HJ. Discovery of the Non-A, Non-B Hepatitis Virus: The end of the Beginning or the Beginning of the End. Transfusion Medicine Reviews. Vol III, No 2. 1989; 77-81.

18. Reesink HW, van der Poel CL . Blood transfusion and hepatitis: still a threat? Blut 1989; 58:1-6.

19. Akins R, Fang C, Mallory D, Zelki D, Sobol D & AuBuchon JA Comparison of three kits for antibodies to hepatitis B core antigen (anti-HBc). Transfusion 1987; 27: 522

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VOLUME III

CLINICAL EVALUATION OF UK BLOOD DONORS WITH RAISED SERUM ALANINE AMINOTRANSFERASE ACTIVITY.

INTRODUCTION:

Serum alanine aminotransferase (ALT) has been used as a surrogate marker of non-A, non-B hepatitis (NANBH) infectivity in blood donors in the USA and some European countries. The case for the relevance of ALT testing for the prevention of non-A, non-B post transfusion hepatitis (NANB PTH) mainly emerged from two large studies conducted in the USA, and published in the early 1980's (1,2). These studies suggested that 40% of donors with raised ALT may be infectious for NANBH. Since then adoption of stringent self exclusion policies for subjects at high risk of HIV infection has not only altered the profile of the blood donor population in the USA and elsewhere, but has also reduced the incidence of NANB PTH. Before any decison is made in the UK to discard blood donations with raised ALT, it is crucial to assess the aetiologic factors contributing to elevated ALT values among the current UK donor population. Apart from some data from the East of Scotland such information is not available.

The present study was aimed at evaluating donors with elevated ALT for identifiable aetiological factors. The question of the prevalence of surrogate markers for NANBH in the UK donor population was also addressed and the data is presented in Volume I of this report.

MATERIALS AND METHODS:

The participating centres in this study were North London, Bristol and Manchester Blood Transfusion Centres. Donors attending preselected sessions were provided with information leaflets on "hepatitis testing", and requested to participiate in the study. The blood donation sessions were selected to be representative of the donor population as a whole. At the completion of donation, 10 ml of blood was obtained from each participating donor. Details of sample storage and testing are given in Volume I. Briefly all samples were tested for ALT and anti-HBc. Other investigations were carried out on selected samples. Aspartate amino-transferrase (AST) and gamma-glutamyl transaminase (gamma-GT) levels were assayed on all the samples from North London. The last 526 samples from Manchester were assayed for gamma-GT. The data for AST and gamma-GT are not included in this report.

Donors with a raised ALT level (>45 IU/L) received a letter soon after the date of donation and were requested to attend an interview. For each donation number with an ALT >45 IU/l, the donation number immediately before, with an ALT<45 IU/L, was chosen as a control, and a similar letter was sent to these donors. Up to three further letters were sent to donors who failed to attend. After failing to respond to the first letter, donors from North London whose current telephone number was available were contacted by telephone.

At the interview a detailed medical questionnaire was administered to test and control donors and data obtained on age, sex, marital status, country of birth, ethnic origin, occupation, history of surgical operations, serious medical illnesses, jaundice, hepatitis, contact with persons known to have had hepatitis, residence or extensive travels abroad, exposure to injections or operations abroad, exposure to scarification or blood transfusion, alcohol intake, level of exercise and medications including herbal remedies. Ethnic origin, country of birth, occupation, medical history and residence abroad of the parents and the sexual partner were also recorded. A brief physical examination was also carried out with special reference to hepatomegaly, splenomegaly, lymphadenopathy and skin (stigmata of chronic liver disease, tattoos etc). All donors were weighed and a verbal account of their height taken. Further blood samples were taken and sera separated within 8 hours for estimation for ALT, AST, gamma-GT, alkaline phosphatase, bilirubin and serum albumin. These tests were carried out at the general hospital connected with the transfusion centre. Sera were also stored at $-30^{o}C$ for testing for cytomegalovirus, Hepatitis A virus and Epstein Barr virus. These assays were carried out at the Public Health Laboratories, Withington Hospital, Manchester.

RESULTS :

Three hundred and three donors with an ALT >45 IU/L, and an equal number of donors with ALT values <45 were invited to attend follow-up interviews. A total of 447 donors attended these interviews (TABLE I), of whom 221 were from the elevated ALT group (41 female,180 male). Thus 72% of the raised ALT group and 74% of the control group attended the recall interview. The mean ALT for the test groups with raised ALT was 63.0 IU/L (SD=7 IU/L) and in the control group was 17.6 IU/L (SD=9 IU/L).

Of the donors seen at interview 81% with a raised ALT were males, compared with 57% in the control group. This male predominance becomes more marked among the donors in the subgroup whose ALT remained elevated >45 IU/L, of whom 91% were males. A full enquiry about other hepatitis risk factors was also made. TABLES II, III and IV summarise the findings elicited at the interviews.

ALCOHOL INTAKE:

A daily alcohol consumption of more than 20 grams was reported by 33% in the control group, 53% in the test groups with raised ALT and 60% in the persistently raised ALT group. Males accounted for 98% (i.e. 64 out of 65) of the donors in the persistently raised ALT group, who consumed more than 20 grams alcohol daily, (TABLE III).

OBESITY:

Moderate obesity (grade 2, as defined by a Body Mass Index >29.9) was found in 27 % of the raised ALT donors and 7% of control donors. Males predominated particularly in the persistently elevated ALT group,

where they constituted 85% (i.e. 30 out of 35) of those with grade 2 obesity.

EXERCISE:

In the raised ALT group, 8% were judged to be taking part in a moderately strenuous physical exercise schedule, compared with 3% in the control group. Of 18 donors in the raised ALT group who exercised strenuously, 17 were males.

OTHER FACTORS:

There was no marked difference in the prevalence of exposure to chemicals and drugs, between the raised ALT and control groups. Likewise hepatitis risk factors appeared not to be significantly different (TABLE IV). However, within the raised ALT group, 6 of the 8 donors who gave a past transfusion history had an elevated ALT on the second blood sample.

TABLE I. DETAILS OF DONOR FOLLOW-UP

CENTRES	N'Lond	B'stol	M'ches	Total
donors screened	3036	3015	3690	9741
donors with ALT level >45 IU/L	93	137	73	303
donors attending follow-up	65	100	56	221
second ALT >45 IU/L	34	42	32	108
second ALT <45 IU/L	31	58	24	113
"control"donors with ALT <45 IU/L	93	137	73	303
donors attending follow up	56	110	60	226
second ALT >45 IU/L	2	6	1	9
second ALT <45 IU/L	54	104	59	217

TABLE II. FACTORS ASSOCIATED WITH RAISED ALT.

	TEST GROUP ALT>45 n %	CONTROL GROUP n %
ALCOHOL INTAKE >20 GRAMS/DAY	118 (53.3%)	75 (33%)
BODY MASS INDEX >2	60 (27.1%)	17 (7%)
EXERCISE	18 (8.1%)	7 (3%)
EXPOSURE TO CHEMICALS	14 (6.3%)	13 (6%)
ORAL CONTRACEPTIVES	6 ** (14.6%)	15 * (42.5%)
DRUGS	5 (2.2%)	8 (4%)
HERBAL MEDICINE	5 (2.2%)	3 (1%)

* of 35 females
** of 41 females

TABLE III. DONORS WITH PERSISTENTLY ELEVATED ALT vs. DONORS WITH ELEVATED ALT ONLY ON THE FIRST SAMPLE.

	first ALT >45 IU/L					
	second ALT>45			second ALT <45		
	F	M	total	F	M	total
Number	10	99	109	31	81	112
AGE(MEAN)	(36)	(36)	(36)	(40)	(38)	(39)
ALT (MEAN) (SD)	(82) (31)	(65) (19)	(67) (21)	(66) (32)	(55) (9)	(58) (19)
ALCOHOL CONSUMPTION > 20 Gram/day	1	64	65	7	46	53
OBESITY GRADE>2	5	30	35	6	19	25
EXERCISE >GRADE 3	0	7	7	1	10	11

TABLE IV.HEPATITIS RISK FACTORS.

	first ALT >45 IU/L		
	N:109 second ALT>45	N:112 second ALT<45	N:226 first ALT <45 IU/L
Transfusion history	6 (5.5%)	2 (2%)	8 (30%)
Previous surgery	57 (52%)	64 (57%)	140 (61%)
Tattooing	7 (6%)	5 (4%)	7 (3%)
Occupational hazard (health workers etc)	10 (9%)	8 (7%)	27 (12%)
Contact with hepatitis	18 (17%)	18 (16%)	37 (16%)
History of jaundice	3 (3%)	3 (3%)	16 (7%)
Extensive travel to high hepatitis risk areas	16 (15%)	16 (14%)	46 (20%)
Residence abroad	22 (20%)	25 (22%)	50 (22%)
Injection or operations abroad	10 (9%)	12 (11%)	24 (10%)
History of I.V.drugs abuse	1 (1%)	0	1 (0.4%)

DISCUSSION:

This study confirms the previous reports on the general characteristics of donors with raised ALT (3,4). By far the major contributing factors to ALT elevation in the donor population are alcohol consumption and obesity. We did not follow the "best guess" approach for the evaluation of donors with raised ALT. However, it is interesting to note that the authors of one study which followed the "best guess" approach emphasised that "The mere documentation of obesity or alcohol abuse does not prove that these factors cause the elevation in ALT level "(3). The availability of HCV markers should supply a ready answer to the vexed question of whom among the donors with raised ALT are the ones most likely to transmit NANB hepatitis (8). Anti-HCV testing however will not exclude the risk of hepatitis transmission by as yet unidentified viruses.

Others (3) have reported, and we agree, that an invitation to meet medical staff from the Blood Transfusion Service generates a certain unavoidable degree of anxiety in the donors. This may relate to apprehensions that they are to be informed of a serious condition such as AIDS or hepatitis. Many who did not respond to the intial letter, gave anxiety as the reason. Others may have merely felt apprehensive that the projected interview may infringe upon their privacy. Although the interviewers endeavoured to reassure all volunteers, we feel that most of the donors seen were relieved to learn that future follow up was not planned.

At the clinical evaluation interview, donors were advised to reduce alcohol intake and weight as appropriate. This measure will be of value in terms of health education, as both excessive alcohol consumption and obesity are major risk factors for cardiovascular

disease (6). It could be argued that in providing such counselling the Blood Transfusion Service is fulfilling its obligation to ensure the health and safety of blood donors. On the basis of the results obtained in this study, we recommend that deferral of donors based on a raised ALT should be replaced by interview, counselling and repeat ALT estimation after 6 months. This study shows that half of these donors are likely to have a normal ALT on their return visit. For those donors whose ALT remains elevated above a significant level, additional counselling or referral to their general practitioners should be considered. With a significant and persistent elevation of ALT it may well be that a temporary deferral is in the best interest of the donor (in some it may provide the stimulus to alter their life styles, and in others a period for diagnostic evaluation, and follow up may clarify the underlying cause for the ALT elevation). Such deferral can remain in force until the G.P. informs the transfusion centre of the return of ALT to acceptable levels.

Gillon et al recently published their data on the prevalence and clinical evaluation of donors with raised ALT in the East of Scotland (4). They chose an alcohol consumption of 40 grams daily as the value above which alcohol was considered the cause of ALT elevation. At this level, 33% of the donors with raised ALT in their study were consuming alcohol excessively and the raised ALT was attributed to this factor. We found however that the level of 20 grams alcohol consumption was more relevant to our sample population, in addition to being consistent with previously defined levels for excessive alcohol intake (10). There are of course wide regional variations in alcohol consumption and a lower threshold for alcohol excess in East Scotland may have encompassed almost all their subjects and rendered such a criteria devoid of statistical discrimination. On the other hand Gillon

et al were less lenient with regard to obesity, using a definition of obesity of 10% above the ideal weight, which is a lower threshold for obesity than a Body Mass Index (BMI) of 29.9 as selected in our study. A recent Consensus Conferennce in the USA suggested that a BMI of more than 27.8 in males and 27.3 in females represented the levels (corresponding to 20% above desirable body weight) at which obesity was associated with ill-health, and weight reduction recommended (6). Epidemiological surveys undertaken in the USA in the early 1980's showed raised ALT to be mainly a male phenomenon (5). Similar epidemiological studies demonstrated that hepatitis B markers were also more prevalent in the male population (11). This difference in prevalence of hepatitis B markers was attributed to higher IV drug abuse and sexual promisicuity in males. By similar reasoning it was assumed that a higer raised ALT prevalence in males, presumably free of HBV markers reflected the prevalence of NANB infection in this sex. Population studies using specific NANBH markers may well confirm a higher prevalence in males, but even then such seroprevalence will probably account for only a small proportion of the males with raised ALT, with the majority of such ALT elevations in countries such as the UK being attributable to high alcohol intake.

Although our study had the benefit of a control group, we are unable to confirm the significance of exposure to chemicals and drugs in causing elevated ALT This observation should not detract from the previously established causal relationships between hepatotoxic agents and liver damage(12). Had this study been designed to pinpoint the cause of each individual ALT elevation using all diagnostic facilities available,then perhaps a more accurate estimation would have been availble of the true contribution of chemical agents and drugs to the raised ALT levels.

In this study evaluation of exercise lacks an objective grading. However there is some evidence of ALT elevation in endurance athletes (7). Our observations are consistent with this general premise but it is difficult to quantify the effect of exercise on an individual ALT estimation.

Of the test donors with raised ALT, 51% had a normal ALT level on their second sample, this compares with 19% of plasmapheresis donors with persistently raised ALT levels in Gillon's series, and 17% in donors followed up in Alter's series(13). However our results are nearer to those of Freidman et al who reported that approximately two thirds of subjects had persistently or intermittently elevated ALT levels on follow-up (3).

In conclusion, this study confirms the previous reports that the vast majority of donors with raised ALT are obese and consume alcohol in excess. However from the findings of this study the issue of justifiability of ALT testing cannot be resolved. The main arguments for adoption of this test in the post HCV era are the "window of infectivity", and the possibility of other NANB viruses causing PTH. Against ALT testing are the problems of non-specificity of the test, finance, donor loss and donor counselling. The introduction of other specific viral markers to narrow the "window of infectivity" and exclude other viruses may, in due course, render the subject of ALT testing of only academic interest. In the meantime, the desirability of ALT testing or otherwise remains an issue of health economics.

REFERENCES:

1. Aach R D, Szmuness W, Mosley J W, Hollinger B, Kahn R A, Stevens C E, Edwards V M, Werch J. Serum alainie aminotransferase of donors in relation to the risk of non-A,non-B hepatitis in rcepients. The Transfusion Transmitted Viruses Study. New Engl. J. Med. 304a:989-994 (1981).

2. Alter H J, Purcell R H , Holland P V, Alling D W, Koziol D E . Donor transaminase and recepient hepatitis : impact on blood transfusiion services.-B hepatitis agents in donated Blood. Ann Intern Med . 104: JAMA.1981;246-630-4.

3. Freidman S L, Dienstag J L, Watkins E, Hinkle C A, Spiers J A, Rider S V, Huggins C E. Evaluation of blood donors with elevated alanine aminotransferase levels. Annals of Internal Medicine.1987; 107:137-144.

4. Gillon J, Hussey A J, Howe S P, Beckett G J, Prescott R J. Post-transfusion Non-A, Non-B hepatitis : Significance of raised ALT and anti-HBc in blood donors. Vox Sang. 54: 148-153 (1988)

5. Kahn R A, Johnson G, Aach R D, Hines A, Ellis F, Miller W V. The distribution of serum alanine aminotransferase levels in a blood donor population. American Journal of Epidemiology Vol.115, NO.6. 929-939. (1982)

6. National Institute of Health Consensus Developement Conference Statement. Health implications of obesity. Annals of Internal Medicine. 1985;103:147-151.

7. Apple F C, Mcgue M K. Serum enzyme changes during marathon running. Am J Clin Pathol,1983;79:716-719.

8. Editorial. Will the real hepatitis C stand up? Lancet. 1989; ii: 307-308.

9. Alter J H. Discovery of Non-A, Non-B hepatitis virus : the end of the beginning or the beginning of the end. Transfusion Medicine Reviews. Vol III, 1989:77-81.

10. Paton A. ABC of alcohol (second edition 1988). British Medical Association.

11. Szmuness W, Hirsch Rl, Prince AM, et al. Hepatitis B surface antigen in blood donors : further observation. J Infect Dis 1975; 131:111-8.

12. Sherlock S. Diseases of liver and biliary system. 6th ed(1981) Blackewell Scientific Publications.

13. Alter, H.J. Post-transfusion hepatitis: clinical features, risk and donor testing; in Barker, Dodd, Infection, immunity and blood transfusion, pp. 47-61 (Liss, New York 1985).

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VOLUME IV

REPORT ON EVALUATION OF ORTHO HCV ELISA TEST SYSTEM

INTRODUCTION:

Post transfusion hepatitis (PTH), as a clinical entity, has been well recognised since the 1950's. The introduction of screening of blood donations for the surface antigen of Hepatitis B virus (HBV) reduced the incidence of PTH by about 25%(1,2,3). But it was also realised that Hepatitis A virus (HAV), Cytomegalovirus (CMV) and Epstein Barr Virus (EBV) collectively contributed to no more than a very small percentage of the remaining cases of PTH. The term non-A, non-B hepatitis (NANBH) was introduced to define the cases of PTH, where HBV, HAV, CMV and EBV had already been excluded as the causative agents. Thus NANBH has so far been a diagnosis of exclusion. There was some evidence that the presence of raised alanine aminotransferase (ALT) activity and antibody to the core antigen of hepatitis B (anti-HBc) in donor blood may be associated with the transmission of NANBH to the recipients of such units of blood (4,5). Hence ALT and anti-HBc were termed surrogate markers for NANBH. The studies linking donor raised ALT and positive anti-HBc status to PTH in the recipient, were conducted before the introduction of self exclusion policies for donors at high risk of HIV infection. Since the introduction of these policies the blood donor profile has dramatically changed contributing to a reduction in the incidence of post-transfusion NANBH.

In the UK a national multicentre study, sponsored by the Department of Health, was initiated in September 1988, to define the prevalence of these surrogate markers in the donor population, (UK NANB surrogate marker study). Three Regional Blood Transfusion Centres (Bristol, North London and Manchester RTCs) participated in this study. In addition this project aimed to clinically evaluate the donors with positive surrogate markers. The detailed results of these studies are the subject of volumes I, II and III of this report.

Recently, researchers at Chiron Corporation succeeded in cloning the genome of a viral agent named hepatitis C virus (HCV), which appears to be responsible for the majority of the cases of transfusion associated and sporadic NANBH(6,7). In June 1989, Dr H.H.Gunson

arranged with Ortho Diagnostics, the manufacturers of the new specific serological assay for HCV (based on the genetically engineered viral antigen produced by Chiron Corp.), for the supply of sufficient assay kits to enable testing of the sera already available from the UK NANB surrogate marker study. It was also agreed to choose the North London Blood Transfusion Centre as the testing site for all serum samples.

MATERIAL AND METHODS:

Samples were collected from healthy volunteer blood donors (age 18-65). Consent for "hepatitis testing" was obtained and 3036, 3014 and 3691 donors were recruited to the study respectively from North London, Bristol and Manchester Transfusion Centres (a total of 9742 donors, 54% male). The following were first-time donors: 24%, 13%, and 8.8% respectively from NLBTC, Bristol and Manchester. Sera were tested for ALT and anti-HBc (for details see the ALT and anti-HBc reports).

A total of 9684 samples have been tested with the ELISA HCV ANTIBODY TEST: 3010 from the North London, 3032 from Bristol and 3642 from Manchester. Satisfactory results were obtained with the Reproducibity and Proficency Panels as supplied by the manufacturer. All initially reactive sera were retested in duplicate. Testing of sera for anti-HCV was completed by early October 1989.

RESULTS:

ALT AND ANTI-HBc TESTING:

The rate of raised alanine aminotransferase activity (ALT>45 IU/L) was 3.18% overall, with 3.03%, 4.54% and 2.19% for donors from North London, Bristol and Manchester respectively.

Overall anti-HBc positivity rate was 0.9% by Wellcome ELISA anti-HBc assay (0.88% for North London, 0.79% for Bristol and 0.94% for Manchester). Subsequent testing at the reference laboratory (Middlesex Hospital, Department of Virology) gave an anti-HBc "confirmed" rate of 0.73%, 0.53% and 0.65% for North London, Bristol and Manchester RTCs respectively.

ANTI-HCV TESTING.

Initial screen reactivity rates were as follows: North London 1.06%, Bristol 0.52% and Manchester 0.98% (overall 0.86%; see table I).

Repeat reactive HCV seropositivity rate were 0.83% for North London, 0.36% for Bristol and 0.68% for Manchester (overall 0.62%; see table I).

HCV seropositivity was 4.5% in the North London anti-HBc positive group and 0% in the corresponding groups from Bristol and Manchester, with an overall rate of 1.6%.

Relationships between anti-HCV and ALT are shown in tables II, III and IV.

The HCV seroprevalence in the raised ALT group was 3.2% for North London, 0.72% for Bristol, 1.36% for Manchester and overall 1.65%. On the other hand, 8.1% of all anti-HCV repeat reactive samples had an ALT>45 IU/L.

Relationships between anti-HCV and anti-HBc are shown in tables V, VI and VII. Analysis of anti-HCV in donors with elevated ALT, with or without anti-HBc, is shown in table VIII.

The percentages of new donors amongst the HCV seropositive donors from NLBTC, Bristol and Manchester were 24%, 16% and 13% respectively.

Relevant data for the clinical history of the anti-HCV positive donors is shown in table IX. Summaries of the findings in anti-HCV positive donors and their samples are shown in Tables X, XI and XII for Bristol, Manchester and NLBTC respectively. In addition, typical titres seen in HCV seropositive donors are shown in table XIII.

DISCUSSION:

This study shows a geographical variation in the prevalence of HCV seropositivity ranging from 1 in 277 for Bristol (rural base), to 1 in 120 for North London (metropolitan area).

There is a correlation between a raised ALT and HCV seropositivity, with 8.1% of HCV seropositive donors having an ALT>45 IU/L. Alternatively 1.6% of the donors with raised ALT were HCV seropositive. This correlation is strengthened when both surrrogate markers are positive (50%) although only two donors had abnormal results for both surrogate markers.

In separate studies on NANB PTH carried out at North London RTC, it has been shown that the sera from one HCV seropositive donor known to have transmitted NANBH, has a titre of approximately 1000. (Sample 3, Table XIII). This is the highest titre observed there and usually titres are <100.

Results of testing fresh and frozen sera for anti-HCV did not demonstrate a difference in the distribution of optical density of any significance.

The male preponderance as observed with HBV markers also appears to hold true for HCV seroprevalence.

One essential criticism of the assay system is the lack of a weak positive control. The other feature which could be improved upon is the length of the shelf life of the kit (only 3 months). There is room for slight modifications of the procedure manual e.g. bold headings, etc. The test system is acceptably user-friendly.

Although from the results obtained so far it appears that the Ortho HCV ELISA has an accepatable specificity and sensitivity, these issues can not be definitively addressed as part of this evaluation, as there were no samples with well established links with NANBH tested in this study. However, this first report on screening Uk donors sera for anti-HCV will serve as the basis for the future implementation of this screening test in the UK Blood Transfusion Service.

TABLE I.ANTI-HCV INITIAL SCREEN AND REPEAT REACTIVE RESULTS

	Total n	INITIAL REACTIVE n	%	REPEAT REACTIVE n	%
NLBTC	3010	32	1.06%	25	0.83%
Bristol RTC	3032	16	0.52%	11	0.36%
Manchester RTC	3642	36	0.988%	25	0.686%
TOTAL	9684	84	0.86%	61	0.62%

TABLE II. HCV SEROPOSITIVITY IN DONORS WITH RAISED ALT

	ALT >45 n	HCV+ ALT>45	rate of HCV+ in raised ALT
NLBTC	93	3	3.2%
Bristol RTC	137	1	0.72%
Manchester RTC	73	1	1.36%
TOTAL	303	5	1.65%

TABLE III.
RATE OF COMBINED ANTI-HCV POSITIVITY AND RAISED ALT IN DONOR POPULATION

CENTRE	TOTAL TESTED FOR ALT	RAISED ALT		RAISED ALT+HCV POS	
		n	%	n	% *
NLBTC	3036	93	3.0	3	0.099
Bristol RTC	3015	137	4.56	1	0.032
Manchester RTC	3690	73	1.97	1	0.027
TOTAL	9741	303	3.1	5	0.051

* % of the total in each centre.

TABLE IV. RATE OF RAISED ALT IN ANTI-HCV POSITIVE DONORS

	HCV+	Raised ALT in HCV+	rate of raised ALT in HCV+
NLBTC	25	3	12%
Bristol RTC	11	1	9%
Manchester RTC	25	1	4%
TOTAL	61	5	8.1%

TABLE V. COMBINED ANTI-HCV + ANTI-HBc POSITIVITY

CENTRE	NUMBER TESTED	ANTI HBc POS		HCV+ in HBc+	
		n	%	n	% *
NLBTC	3016	22	0.73	1	0.03
Bristol RTC	3008	16	0.53	0	0
Manchester RTC	3687	24	0.65	0	0
TOTAL	9711	62	0.63	1	0.01

* % of of the total in each centre

TABLE VI. RATE OF ANTI-HCV POSITIVITY IN ANTI-HBc POS. DONORS

	anti-HBc +	HCV POS IN HBc POS DONORS	
		n	%
NLBTC	22	1	4.5
Bristol RTC	16	0	0
M'chester RTC	24	0	0
TOTAL	62	1	1.6

TABLE VII. RATE OF ANTI- HBc POSITIVITY IN ANTI-HCV POSITIVE DONORS

	HCV POSITIVE	ANTI HBc+ IN HCV+	
		No.	%
NLBCT	25	1	4%
Bristol RTC	11	0	0
Manchester RTC	25	0	0
TOTAL	61	1	1.6%

TABLE VIII. RELATIONSHIP BETWEEN HCV POSITIVITY AND COMBINED "HBc+, RAISED ALT" in 303 donors with ALT>45 IU/L

ANTI-HBc STATUS	total n	HCV+ DONORS IN n	%
POS	2	1	50%
NEG	301	4	1.3%
TOTAL	303*	5	1.6%

*All 303 had ALT >45 IU/L

TABLE IX. CLINICAL EVALUATION

	ANTI-HCV + DONORS*			
	Normal Alt		Raised Alt	
	anti-HBc neg	anti-HBc pos	anti-HBc neg	anti-HBc pos
No:	30	0	2	1
M/F	23/7	0	1/1	1/0
Marital Status:		0		
Married	12	0	1	1
Divorced	5	0	1	0
Single	9	0	0	0
Widowed	1	0	0	0
Born outside UK	1	0	0	0
History of jaundice	0	0	0	1
History of contact with hepatitis	4	0	2	0
Previous surgery	22	0	2	0
Residence abroad	10	0	2	1
Ext. travel abroad	5	0	1	0
Inj/operation abroad	7	0	2	0
Previous transfusion	3	0	1	0
Scarification	14	0	2	1

* all the donors included above were British Caucasians

TABLE X. NATIONAL DONOR STUDY - BRISTOL
RESULTS OF ANTI-HCV POSITIVE DONORS

Donation Number	Age	Gender	anti-HCV optical densities Initial cutoff		Repeat cutoff		ALT	Anti-HBc
057 545	27	F	.667	.483	.617 .416	.470	17	Neg
011 587	21	M	1.886	.479	1.360 1.081	.469	39	Neg
009 415	35	M	.444	.487	.560 .477	.469	33	Neg
004 252	57	M	.625	.467	.615 .480	.469	24	Neg
062 227T3	57	M	1.506	.477	1.606 1.279	.475	58	Neg
006 062	36	M	>2.5	.515	>2.5 >2.5	.475	11	Neg
075 955T4	37	F	>2.5	.468	2.092 1.701	.475	16	Neg
065 857	59	M	1.763	.512	1.829 1.187	.475	14	Neg
062 761	41	M	>2.5	.473	>2.5 >2.5	.478	31	Neg
062 771	50	M	>2.5	.473	>2.5 >2.5	.478	29	Neg
070 040	57	F	1.178	.478	1.345 1.484	.499	15	Neg

TABLE XI. NATIONAL DONOR STUDY - MANCHESTER
RESULTS OF ANTI-HCV POSITIVE DONORS

Donation Number	Age	Gender	anti-HCV results (O.D) Initial	cutoff	Repeat	cutoff	ALT	Anti-HBc
210 813	65	M	.543	.453	1.363 1.076	.469	15	Neg
213 030	48	M	.396	.453	.272 .417	.469	22	Neg
192 694	33	M	1.208	.453	1.781 1.889	.469	6	Neg
670 895	38	F	.558	.481	.594 .578	.469	26	Neg
669 739	42	F	2.189	.483	2.334 2.190	.466	19	Neg
176 884	27	M	>2.5	.444	>2.5 >2.5	.475	NT	Neg
176 885	20	M	>2.5	.444	>2.5 >2.5	.475	200	Neg
673 314	39	M	1.297	.462	.935 .997	.475	12	Neg
682 913	25	F	.769	.472	.632 .569	.483	11	Neg
673 910	26	M	.574	.476	.407 .504	.483	18	Neg
679 910	65	F	.685	.477	.647 .606	.483	36	Neg
673 896	55	M	.429	.417	.809 .812	.483	5	Neg
672 172	46	M	.554	.477	.787 .744	.483	11	Neg
664 455	28	M	.690	.511	.751 .865	.478	4	Neg
213 057	25	M	.481	.504	.411 .343	.478	14	Neg
213 173	55	M	.504	.504	.500 .644	.478	5	Neg
664 499	39	F	.675	.489	.730 .791	.478	4	Neg
665 326	45	M	1.167	.517	1.316 1.193	.499	21	Neg
665 291	21	M	.468	.517	.426 .524	.478	11	Neg
664 139	52	F	.474	.517	.431 .454	.478	5	Neg
665 305	29	M	.973	.517	1.070 1.045	.478	17	Neg
665 286	49	M	.789	.517	.814 .826	.478	6	Neg
670 842	30	F	1.784	.426	1.657 1.803	.434	4	Neg
680 027	51	M	>2.5	.493	>2.5 >2.5	.443	28	Neg
682 264	40	M	1.226	.455	1.674 1.848	.479	4	Neg

TABLE XII. NATIONAL DONOR STUDY - NLBTC
RESULTS OF ANTI-HCV POSITIVE DONORS

Donation Number	Age	Gender	HCV Result (O.D) Initial	cutoff	Repeat	cutoff	ALT	Anti-HBc
133 504.9	47	F	.968	.471	.414 .467	.470	23	Neg
846 980.6	37	F	.536	.491	.703 .844	.470	14	Neg
127 299.3	37	M	.749	.488	.982 .887	.470	55	Neg
858 578.4	51	F	1.134	.480	.802 .892	.488	11	Neg
126.750	21	F	.513	.487	.548 .705	.488	23	Neg
842 981.2	36	F	.998	.456	1.559 1.413	.488	12	Neg
835 814.1	51	M	1.01	.497	1.537 1.538	.489	17	Neg
836.673WX	37	M	>2.5	.536	>2.5 >2.5	.489	59	Pos
843 070.5	56	F	.637	.463	1.132 1.237	.469	18	Neg
854 100.0	27	F	.405	.463	.519 .469	.469	27	Neg
854 119.1	29	M	.751	.463	.795 .797	.469	33	Neg
847 286.6	60	F	.968	.471	1.666 1.419	.469	6	Neg
862 193.4	29	M	.501	.483	.890 .889	.478	24	Neg
855 082.4	23	F	1.312	.483	1.597 1.465	.478	10	Neg
128 794WX	38	M	.641	.485	1.351 1.445	.478	12	Neg
131 596WX	31	M	.798	.484	.783 .851	.489	14	Neg
847 712.4	55	M	.694	.506	.668 .683	.485	19	Neg
843 791.2	41	F	>2.5	.506	>2.5 >2.5	.506	132	Neg
862 198.5	23	M	.522	.489	1.059 1.063	.485	14	Neg
847 710.8	39	M	.484	.469	1.709 2.132	.478	12	Neg
843 105.1	59	F	1.41	.494	1.469 1.349	.485	16	Neg
133 204WX	45	F	1.516	.478	.838 .945	.463	19	Neg
858 050.2	28	M	.634	.478	.346 .568	.468	18	Neg
836 668.3	51	F	1.817	.452	1.815 1.844	.463	14	Neg
858 037.5	47	M	.648	.452	.800 .796	.463	14	Neg

TABLE XIII. RESULTS OF TESTING ANTI-HCV REACTIVE SAMPLES AT VARIOUS DILUTIONS

	Sample No.1	Sample No.2	Sample No.3
	OD	OD	OD
Neat	>2 +	>2 +	>2 +
1:5	1.131 0.859 +	>2 +	>2 +
1:10	0.525 0.560 +	>2 +	>2 +
1:20	0.153 - 0.160	1.819 1.780 +	>2 +
1:50	0.045 .042 -	0.267 - 0.371	>2 +
1:100	0.031 - 0.034	0.132 - 0.172	>2 +
1:200	NT	NT	2.248 1.177 +
1:400	NT	NT	1.024 0.989 +
1:1000	NT	NT	0.501 0.496 +
1:2000	NT	NT	0.172 - 0.220
Cut off	0.470	0.470	0.470

REFERENCES:

1. Dienstag JL. Non-A,Non-B Hepatitis.I. Recognition, Epidemiology and clinical features. Gastroenterology 1983, 85:439-62.

2. Gocke DJ, Greenberg HB, Kavey NB. Correlation of Australia antigen with post-transfusion Hepatitis. JAMA 1970;212/:877-9.

3. Alter HJ, Holland PV, Purcell RH, et al. Post-transfusion hepatitis after exclusion of commercial and hepatitis-B antigen positive donors. Ann Intern Med 1972;77:691-9.

4. Aach RD, Szmuness W, Mosley JW, et al. Serum alanine aminotransferase of donors in relation to the risk of non-A,non-B hepatitis in recipients. The transfusion transmitted viruses study. N Engl J Med. 1981; 989-1035.

5. Koziol DE, Holland PV, Alling DW, et al(1986). Antibody to hepatitis coreantigen as a paradoxical marker for non-A,non-B hepatitis agents in donated blood . Ann Intern Med 104:488-495.

6. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A,non-B viral hepatitis genome. Science 1989;244:359-62.

7. Kuo G, Choo Q-L, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A,non-B hepatitis. Science 1989;244:362-4.

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