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NOT FOR PUBLICATION

An investigation of the use of the first generation Ortho and Abbott anti-HCV EIA screening tests in three Regional Transfusion Centres and of confirmatory testing at three reference centres on the repeatedly reactive specimens.

(The results of the investigation were sent to Manchester RTC in January 1991 for data entry and analysed jointly there and at the PHLS Communicable Disease Surveillance Centre)

Summary

Of 10633 blood donor specimens tested at RTCs by the Ortho and Abbott anti-HCV screening assays 65 (0.61%) were repeatedly reactive in one or both assays. Twenty six specimens were repeatedly reactive by Ortho only and 15 by Abbott only, and all these were negative in confirmatory tests at the three reference centres.

Of the 24 specimens reactive in both screening assays, 8 were anti-HCV positive by the Abbott Neutralisation test at all three reference centres. Five of these 8 were positive in Ortho RIBA and one indeterminate. These 6 specimens were PCR positive at all three reference centres. At the two reference centres where all referred specimens were tested by PCR, the 16 Abbott Neutralisation negative specimens were also Ortho RIBA negative and PCR negative.

PCR results were fully concordant at the two reference centres where all 65 specimens were tested. The third centre identified the same 6 specimens as PCR positive and found another PCR positive among the 3 other specimens tested there.

Two candidate screening assays, BHC 11 and UBI, when applied to the 65 referred specimens respectively found 9 and 10 of them reactive. In both cases these included the six concordantly Abbott Neutralisation, Ortho RIBA and PCR reactive specimens.

i)Screening at the RTCs

In September 1990 more than three and a half thousand specimens taken from consecutively processed donations in each of three RTCs were tested by both the Ortho and Abbott EIA anti-HCV screening assays. If specimens were reactive in either test the assays were repeated. The results of this testing are shown in Table 1. Serum and plasma samples of specimens repeatedly positive in one or both of the screening assays (Table 2) were then sent to each of three reference centres.

Table 1) Results of testing at the RTCs

RTC	No. Tested	Ortho Initial Positive No. (%)	Ortho Repeat Positive No. (%)	Abbott Initial Positive No. (%)	Abbott Repeat Positive No. (%)
Glasgow	3516	23 (0.65)	14 (0.40)	18 (0.51)	17 (0.48)
N London	3578	17 (0.48)	15 (0.42)	14 (0.39)	13 (0.36)
Newcastle	3539	25 (0.71)	21 (0.59)	13 (0.37)	9 (0.25)
Total	10633	65 (0.61)	50 (0.47)	45 (0.42)	39 (0.37)

Table 2) Numbers of specimens found repeatedly reactive at the RTC

RTC	Repeatedly reactive by Ortho only	Abbott only	Both assays	Total
Glasgow	6	9	8	23
N London	6	4	9	19
Newcastle	14	2	7	23
Total	26	15	24	65

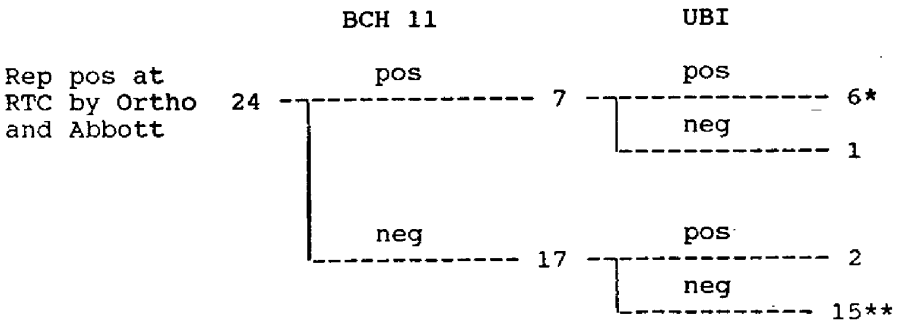
ii) Testing by screening assays at the reference centres

An analysis of the 65 specimens found repeatedly reactive in one or both of the screening assays used in the RTCs forms the rest of this report. The three reference centres were at Ruchill Hospital, University College and Middlesex School of Medicine (UCMSM), and the

PHLS Virus Reference Laboratory (VRL). The results of retesting the 65 specimens by the Ortho and Abbott screening assays were provided by UCMSM and by VRL, where the specimens were tested twice. The results obtained at these centres were in broad agreement with those of the RTCs, and when there were discrepancies the specimen OD / cut-off OD ratios concerned were generally close to 1.

Two candidate screening tests were also applied to all specimens, each at one centre only: BCH 11 at UCMSM and UBI at VRL. When applied to the 41 referred specimens which had been repeatedly positive in only one of the screening tests at the RTCs, one was positve by BCH 11 only, another by UBI only, and one by both. The other 38 were negative in both assays and all 41 were negative by Abbott Neutralisation (AN) at all three reference centres, and by Ortho RIBA and PCR in the two centres where they were tested by them. The results of testing by BCH 11 and by UBI the 24 specimens that were repeat positive at the RTCs by both Ortho and Abbott are shown in Figure 1.

Figure 1) Results of using the candidate screening assays to test specimens repeat positive at RTCs by both assays.



* all PCR positive in all 3 centres
** includes one found PCR positive at Ruchill only

iii) Confirmatory tests at the reference centres

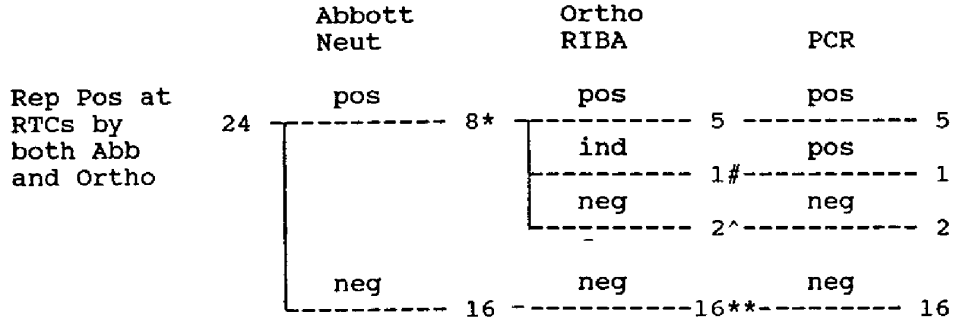
The three reference centres tested all referred specimens by the

Abbott Neutralisation (AN) assay. At UCMSM and VRL all specimens were also tested by Ortho RIBA and PCR: at Ruchill these tests were only applied to the 9 specimens found positive or indeterminate by AN. At Ruchill and VRL the Urea Resistance test was applied to all 65 specimens.

Of the 24 specimens repeatedly reactive in both screening assays at the RTCs, Ruchill found 15 AN negative and these were not investigated there by RIBA or PCR. One specimen was AN equivocal; this specimen was RIBA negative at all three centres and PCR positive at Ruchill only. The other eight specimens were AN positive at Ruchill: six of these were RIBA and PCR positive and two RIBA and PCR negative at all three centres.

The results from UCMSM and VRL of the confirmatory assays on the 24 specimens found repeatedly positive by both screening assays at RTCs are shown in Figure 2.

Figure 2) Results at UCMSM and VRL of the confirmatory tests for the 24 specimens positive by both Ortho and Abbott screening assays.



* includes spec. (332318) AN ind at VRL, RIBA and PCR pos at all centres
\# this spec. (247021) was RIBA ind at all three centres. It was weakly positive in both Ortho and Abbott (specimen OD / cut-off ratios all less than two), but more strongly positive in BHC 11 and UBI. It was PCR positive in all three centres, but with one primer pair only at UCMSM and VRL.
^ includes one RIBA ind at VRL and Ruchill that was PCR neg at all centr
** includes one RIBA ind at VRL, neg at UCMSM.

Different pairs of primers were used at each centre, and in most cases PCR with two sets of primers was only attempted if the RIBA

result was positive. In three of the 33 PCR investigations where two primer pairs were used the result was positive for the first and negative for the second; in a fourth it was negative for the first and positive for the second.

Detailed results for the 24 specimens repeatedly positive in both Ortho and Abbott screening assays at the RTCs are given in Table 3. In this table all Specimen OD / cut-off OD (T/CO) ratios >= 1 are positive. In the RIBA test a specimen must have at least 2 antigen bands with + intensity or greater to be positive: when only one band passes this criterion the result is indeterminate. All +/- reactions are considered to be non-reactive to the antigen concerned.

The Urea Resistance Test was applied at VRL and Ruchill, and data was reported from VRL (table 4). There was some concordance between resistance to urea washing of the antigen / antibody reaction and RIBA positive / indeterminate status. This correlation was less obvious in tests done at Ruchill (EA Follet, pc).

Table 4 % Resistance to urea wash in Ortho EIA versus RIBA reactivity.

RIBA reactivity	Urea Resistance >= 50%	<50%
Positive/ind	6	2*
Negative	5	52

* values 46.2%, 7.7%

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14.02.91

Table 3) Detailed results for the 24 specimens repeatedly reactive in both Ortho and Abbott

HCV TRIAL SHOWING TEST OD/CUT OFF OD RATIOS AND CONFIRMATION TESTING

SERUM NUMBER	RTC SCREEN/REPEAT RESULTS: ABBOTT T/CO SCREEN	ABBOTT T/CO REPEAT	ORTHO T/CO SCREEN	ORTHO T/CO REPEAT	REF LAB	RESULTS OF REFERENCE LABORATORIES CONFIRMATION TESTS: BHC11 T/CO	UBI T/CO	ABB Neut	ORTHO RIBA 5-1	c100	c33	c22	P.C.R 203	209	NCR2	J622	NS5	5'
312129	2.867	2.997	3.523	3.944	RUCHILL			POS	-	+/-	++++	++++	POS	POS				
					COLINDALE		12.330	POS	-	+	++++	++++					POS	POS
					TEDDER	4.926		POS	+/-	+	++++	++++			POS	NEG		
332318	6.434	5.822	4.990	4.960	RUCHILL			POS	+++	+	++++	++++	POS	POS				
					COLINDALE		11.421	EQU	++	+	++++	++++					POS	POS
					TEDDER	4.096		POS	++	+++	++++	++++			POS	POS		
332838	1.601	1.432	1.813	2.096	RUCHILL			NEG										
					COLINDALE		0.081	NEG	-	+/-	-	-					NEG	NEG
					TEDDER	0.146		NEG	-	+/-	-	-			NEG			
338013	1.334	1.159	2.154	1.882	RUCHILL			POS	+	+/-	-	-	NEG	NEG				
					COLINDALE		1.747	POS	+	+/-	-	-					NEG	NEG
					TEDDER	0.192		POS	+/-	-	-				NEG			
338284	1.056	1.254	1.843	2.084	RUCHILL			POS	-	+/-	-	-	NEG	NEG				
					COLINDALE		0.109	POS	-	+/-	-	-					NEG	NEG
					TEDDER	0.144		POS	-	+/-	-	-			NEG			
338402	3.056	3.282	3.165	3.165	RUCHILL			NEG										
					COLINDALE		0.072	NEG	-	+/-	-	-					NEG	
					TEDDER	0.188		NEG	-	+/-	-	-			NEG			
343551	2.417	2.398	2.230	3.205	RUCHILL			NEG										
					COLINDALE		0.059	NEG	-	-	-	-					NEG	
					TEDDER	0.697		NEG	+/-	+/-	+/-	+/-			NEG			
349268	1.141	1.178	1.478	1.232	RUCHILL			NEG										
					COLINDALE		0.050	NEG	-	-	-	-					NEG	
					TEDDER	0.166		NEG	-	+/-	-	-			NEG			
203746	3.950	4.581	4.419	4.603	RUCHILL			POS	+/-	+/-	++++	++++	POS	POS				
					COLINDALE		12.616	POS	+	++	++++	++++					POS	POS
					TEDDER	4.713		POS	+	++	++++	++++			POS	POS		
247021	1.677	1.738	1.158	1.191	RUCHILL			POS	-	-	+/-	++++	POS	POS				
					COLINDALE		11.389	POS	-	-	-	++++					NEG	POS
					TEDDER	3.986		POS	+/-	+/-	+/-	++++			POS	NEG		
247109	1.096	1.263	1.695	1.511	RUCHILL			NEG										
					COLINDALE		0.130	NEG	-	-	-	-					NEG	
					TEDDER	0.109		NEG	+/-	+/-	+/-	+/-			NEG			
718559	6.527	6.510	5.495	5.682	RUCHILL			POS	++++	++++	++++	++++	POS	POS				
					COLINDALE		13.622	POS	++++	++++	++++	++++					POS	POS
					TEDDER	4.532		POS	+++	+++	++++	++++			POS	POS		
790184	1.778	1.663	3.140	3.116	RUCHILL			NEG										
					COLINDALE		0.151	NEG	+	-	-	-					NEG	NEG
					TEDDER	0.097		NEG	-	+/-	-	-			NEG			
793833	1.238	1.395	4.895	3.884	RUCHILL			NEG										
					COLINDALE		0.146	NEG	-	-	-	-					NEG	
					TEDDER	0.837		NEG	+/-	+/-	-	-			NEG			
797548	1.227	1.175	1.440	1.922	RUCHILL			EQU	-	+/-	-	-	POS	POS				
					COLINDALE		0.141	NEG	-	+/-	-	-					NEG	NEG
					TEDDER	0.361		NEG	-	+/-	-	-			NEG			
812399	1.015	1.031	1.497	1.356	RUCHILL			NEG										
					COLINDALE		0.124	NEG	-	-	-	-					NEG	
					TEDDER	0.087		NEG	-	+/-	-	-			NEG			
812400	4.976	4.471	5.669	5.682	RUCHILL			NEG										
					COLINDALE		0.168	NEG	-	-	-	-					NEG	
					TEDDER	0.202		NEG	-	-	-	-			NEG			
256771	2.280	1.857	1.019	1.032	RUCHILL			NEG										
					COLINDALE		3.163	NEG	-	-	-	-					NEG	
					TEDDER	0.792		NEG	-	-	-	-			NEG			
267420	3.029	3.566	2.912	3.108	RUCHILL			NEG										
					COLINDALE		0.100	NEG	-	-	-	-					NEG	
					TEDDER	0.157		NEG	+/-	+/-	-	-			NEG			
291941	5.721	4.931	5.187	5.252	RUCHILL			POS	+	+	++++	++++	POS	POS				
					COLINDALE		11.249	POS	+	+	+++	++++					POS	POS
					TEDDER	4.687		POS	++	++	++++	++++			POS	NEG		
315701	2.528	3.362	4.448	4.189	RUCHILL			NEG										
					COLINDALE		0.118	NEG	-	-	-	-					NEG	
					TEDDER	0.140		NEG	+/-	+/-	-	-			NEG			
342238	2.414	1.501	5.365	5.656	RUCHILL			NEG										
					COLINDALE		0.118	NEG	-	++	-	-					NEG	NEG
					TEDDER	0.074		NEG	-	-	-	-			NEG			
342564	5.263	4.536	5.112	5.252	RUCHILL			NEG										
					COLINDALE		0.072	NEG	-	-		-					NEG	
					TEDDER	0.106		NEG		-		-			NEG			
342618	3.586	4.282	4.633	4.742	RUCHILL			NEG										
					COLINDALE		0.172	NEG	-			-					NEG	
					TEDDER	0.455		NEG			-	-			NEG			