HAA (HB Ag) Evaluation—State of the Art*

ALI A. HOSSAINI, Ph.D.

Associate Professor of Clinical Pathology, Medical College of Virginia, Health Sciences Division of Virginia Commonwealth University, Richmond

MARIO R. ESCOBAR, Ph.D.

Associate Professor of Clinical Pathology, Medical College of Virginia, Health Sciences Division of Virginia Commonwealth University, Richmond

Viral hepatitis has been a major problem for public health workers and for blood bankers. There is much to be learned despite recent major advances in the natural history of the disease, in some of its epidemiological characteristics, and particularly in its laboratory diagnosis. These discoveries will significantly reduce the rate of posttransfusion hepatitis. A complete solution of the overall hepatitis problem will be attained only when the causative role of the agent, presumably a virus, has been conclusively demonstrated. This will in turn produce more sensitive and specific diagnostic procedures and lead to the development of effective, preventive and therapeutic measures. Specific laboratory diagnosis of hepatitis became possible with the discovery of Australia antigen—a term used in 1964 (4) by its discoverer Baruch Blumberg. The observation that Australia antigen or Au-1 was intimately related to hepatitis B virus stimulated large numbers of investigators who developed overlapping systems of nomenclature. Some of these terms, including those recommended by the Committee on Viral Hepatitis of the Natural Research Council, are given in Table 1. Henceforth, the symbol HB Ag will be used as the synonym to Au-1.

The discovery of HB Ag has provided a specific marker of infection with serum hepatitis, the most common cause of posttransfusion hepatitis. This in turn has permitted a partial solution to the blood bankers' dilemma, namely, the detection of HB Ag carriers before blood is collected for infusion.

The frequency of HB Ag in different blood donor populations in the U. S. has been estimated to be 0.1-0.5% among volunteer donors, and up to 2% among paid donors of commercial blood banks (6, 9, 14, 15, 16). In other words, blood collected from the latter group may have as high as 20 times the risk of transmitting hepatitis as that obtained from volunteer donors. A list of techniques for the detection of HB Ag is presented in Table 2, including their relative sensitivity in reference to agar gel diffusion (AGD) and the time required for results to be obtained.

AGD was the first method available (3). This method lacks sensitivity. Another disadvantage is that it requires a minimum of one to seven days of incubation before any results are seen. Moreover, it requires that both antigen and antibody be sufficiently potent and also of approximately equivalent concentration; otherwise, the results may be falsely negative. AGD has the significant advantage, however, in that it can discriminate between the components in complex mixtures. It offers a direct demonstration of identity, partial identity or nonidentity between different antigens or antibody reactants. In addition, sensitivity may be increased by certain modifications. Precipitation lines of identity, partial identity, and nonidentity on an agar gel plate are depicted in figure 1.

Counterelectrophoresis (CEP) is the most commonly used version of the immunoelectrophoresis

* Presented by Dr. Hossaini at the 44th Annual McGuire Lecture Series, March 22, 1973, at the Medical College of Virginia, Richmond.

TABLE 1

VIRAL HEPATITIS—NOMENCLATURE

Old Terms	New Terms	Committee on Viral Hepatitis Div. Med. Sc., NAS—National Research Council
Virus B	SH antigen	Hepatitis B virus, HBV
Serum hepatitis (SH)	Au/SH antigen	Hepatitis B antigen, HB Ag
Posttransfusion hepatitis (PTH)	Hepatitis antigen (HA)	Hepatitis B antibody, HB Ag or anti-HB Ag
Long incubation disease	HAA	
Hemologous serum jaundice	MS-2	
Syringe jaundice		
Virus A		
Short incubation disease	MS-1	Hepatitis A virus, HAV
Infectious hepatitis (IH)		
Acute catarrhal jaundice		
Epidemic jaundice		
Epidemic hepatitis		

procedures. The principle of this method is somewhat more difficult to understand than AGD although it is as easy to perform. It has the advantage of requiring only 30 minutes to two hours to obtain results (8, 10). Like AGD, the CEP test utilizes agar gel coated slides and the reactions appear as precipitation lines.

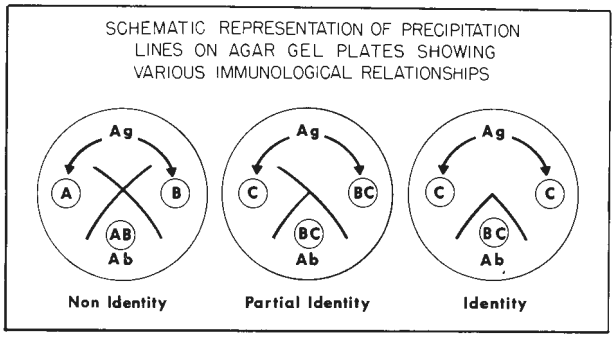

Fig. 1

The complement-fixation (CF) test is considerably more complicated than AGD and CEP technically and in principle. It is not, therefore, as practical for mass screening. Generally it is more sensitive than CEP (11, 12, 18, 20). The sensitivity of certain CEP procedures, however, has been improved to approach that of CF (11, 15, 19). Another disadvantage of CF tests is the scarcity of suitable antisera, since many antisera are of low titer and are anticomplementary (AC).

The hemagglutination-inhibition (HAI) test and the radioimmunoassay (RIA) procedures have been reported to be much more sensitive than the CEP and CF tests for the detection of both antigen and antibody. The comparative sensitivity of CEP and HAI is given in Table 3 as reported by Vyas *et al.* (23). Vyas and Shulman (22) estimated that HAI was 100 times more sensitive than AGD for the detection of HB Ag. In addition to its relatively high sensitivity, HAI has the advantage of simplicity. Not all batches of antigen are satisfactory for coating the reagent red cells, however, and some lots of anti-

TABLE 2

RELATIVE SENSITIVITY OF TESTS FOR AUSTRALIA ANTIGEN IN REFERENCE TO GEL DIFFUSION

Test	Relative Sensitivity	Time	Reference
Agar gel diffusion	1.0	24 hrs.	Blumberg and Alter (4)
Electron microscopy	1.5	2-6 hrs.	Almeida and Waterson (1)
Electroimmunodiffusion	10.0	½-3 hrs.	
a. Immunoelectrodiffusion			Duquesnoy and Becker (8)
b. Immunoelectroosmophoresis			Prince (17)
c. Counterelectrophoresis			Gocke and Howe (10)
Immunoelectronmicroscopy	11.0	2-6 hrs.	Almeida and Waterson (1)
Complement-fixation	100.0	16 hrs.	Shulman and Barker (21)
Hemagglutination inhibition	1000.0	2-4 hrs.	Vyas and Shulman (22)
Radioimmunoassay	1000.0	5 days	Lander, *et al.* (13)
Radioimmunoassay (solid phase)	600.0	2-4 hrs.	Cawley (5)

TABLE 3

COMPARISON OF CEP AND HAI FOR HB AG TESTING ON SERA OF 700 PATIENTS WITH CLINICAL HEPATITIS.*

	CEP+	CEP−
HAI+	201	50
HAI−	9	440

* The same goat anti-HB Ag was used for both CEP and HAI.

serum are not suitable for agglutination and inhibition tests.

A number of RIA techniques have been described. All utilize a radioiodinated marker, usually HB antigen or its antibody labeled with I^{125}. The Ausria-125® of Abbott seemed to be a promising technique because of its high sensitivity with potential screening of blood donors (14, 19). Recent studies, however, have revealed that there may be an inherent serious pitfall in this particular procedure (1). A small percentage of the reactions seems to be falsely positive thus necessitating further tests if mislabeling of individuals as carriers of HB Ag is to be avoided.

Soon after reagents for the detection of HB Ag became commercially available, the Medical College of Virginia Blood Bank began screening donors by the AGD procedure. The Virology Laboratory at the Medical College of Virginia instituted the CF test using antiserum provided by NIH to test patients suspected of having viral hepatitis. When CEP kits became available early in 1971, because of the low sensitivity of AGD, it was decided to conduct a study to evaluate the relative sensitivity of the new method; thus, each of 300 sera were tested by AGD, CF and CEP. The sera were obtained from patients suspected clinically of having viral hepatitis.

Some of the results of this initial study are given in Table 4 showing the relationship between CF titers and the reactions of the 300 sera in the AGD and CEP tests. Of the 300 sera tested, 22.3% were positive by CF, whereas 12% and 10.6% were positive by CEP and AGD, respectively. The highest serum dilution giving a positive reaction by AGD was 1:16, while CEP failed to detect dilutions higher than 1:64. This study, like a number of others, indicated that AGD is the least sensitive method and that CF is the most sensitive of the three techniques.

The sensitivity of AGD in this study was somewhat higher than that reported by other workers. This enhancement was probably related to antiserum titer and the placement of patient sera adjacent to wells containing HB Ag-positive serum (reinforcement pattern). Although this study did not provide evidence for a significant difference in the sensitivity of the CEP as compared to AGD, it was decided to abandon the latter procedure in favor of the former because the performance of CEP could be shortened to 60 minutes.

A second study was then started around the middle of 1972 because, at the time, there were no reports in the literature on the relative sensitivity of the various commercial CEP methods that have become available for routine HB Ag screening of donors and patients and because it was felt that a significantly more sensitive, yet relatively simple, technique was needed. Abbott's Ausria-125® was chosen for this purpose because it was reported to meet these criteria (14). It was hoped that the results of this comparative study would serve as a guide in selecting one of the six CEP methods and/or the RIA for donor screening. It was decided that a change from the CEP to the RIA technique should be made if the latter could be shown to be practical and devoid of false positive reactions. Since a reverse-passive hemagglutination (RPHA) technique was available at that time, it was also included in these comparative studies.

The Blood Bank routinely uses the CEP method of Spectra Biologicals to screen donors. Five other CEP methods used in this study were kindly supplied to us by Abbott Laboratories, Ortho Diagnostics, Pfizer, Squibb and Hyland. All CEP tests were performed according to the manufacturers' instructions. In addition to CEP, Abbott kindly supplied us with the reagents for RPHA as well as the reagents and the gamma counter used with their RIA procedure. Again the prescribed protocol of the manufacturer was followed with one modification which consisted of a reduction in the incubation time of test sera with the antibody. The incubation time was reduced from 16 hours to 90 minutes.

Initially 90 sera were tested by the six CEP methods. Of these, 72 were derived from blood secured commercially, 11 were from donors suspected of having Gilbert's disorder and the remaining seven were from individuals known to be HB Ag positive on the basis of previous testing. As seen from Table 5, none of the sera from commercial blood donors or from donors with Gilbert's disorder

TABLE 4

COMPLEMENT-FIXATION (CF) TITERS OF HEPATITIS B ANTIGEN (HB AG) AND REACTIONS OF 300 SERA IN IMMUNODIFFUSION (ID) AND COUNTERELECTROPHORESIS (CEP) TESTS

Total No. of Sera Tested	CF Titer of HB Ag (22.3% positive)	No. of Sera Positive by CEP (12% of total)	No. of Sera Positive by ID (10.6% of total)
233	<1:2	0	0
19	1:2–1:8	0	0
12	1:16–1:32	2	0
16	1:64–1:128	14	12
14	1:256–1:2048	14	14
6	1:4096–1:16384	6	6

gave a positive test. The seven sera previously known to be positive by Spectra CEP technique were positive again on retesting but the other five CEP procedures detected only six of these. Shortly after these tests were performed, kits for RIA and RPHA testing were made available to us. At the same time, and by lucky coincidence, a panel of 20 sera arrived from the American Association of Blood Banks (AABB) for proficiency and quality control testing. CEP and RIA results on these 20 sera are shown in Table 6. AABB subsequently reported that 11 were positive. All 11 were detected by RIA and all but one were detected by Spectra CEP which at the time seemed to be the most sensitive of the CEP methods. Next in sensitivity appeared to be the Ortho and Pfizer CEP procedures, each of which detected nine of the 11 positives. The other methods showed relatively poor sensitivity, failing to detect almost 50% of the positive sera.

Testing of 204 serum samples, representing a mixed population of patients suspected of having viral hepatitis, and "normal" hospital employees, was then done by CF, RPHA, RIA and by the six CEP methods. Results are presented in Table 7. Among the six CEP methods, Ortho's proved to be the most sensitive while Abbott's was the least, failing to detect HB Ag in six sera. There was also a significant difference between Ortho's sensitivity and that of the other four CEP procedures each of which failed to detect the antigen in five sera. These findings and the fact that Ortho's method was nearly as sensitive as the CF test in the present series indicated that when the test conditions were adequate, relatively high sensitivity could be attained with the CEP procedure. Lewis and Coran (14) attributed the higher sensitivity of the Ortho technique to the considerably larger volumes of test sera and antisera used in this method as compared to the others. Nevertheless, these results confirmed our previous findings, namely that generally, CEP methods are less sensitive than CF. These results also revealed that it is possible to get CEP positive–RIA negative results. These findings are in disagreement with those of Lewis and Coran (14).

The present study did not provide evidence that Abbott's RPHA method, a version of hemagglutination, is more sensitive than CF or Ortho's CEP. It was noted that RPHA results were more difficult to interpret, since the differences between weakly positive and negative reactions were not clear and could not provide for a clear-cut diagnostic reading.

Like other studies (5, 13, 24), RIA appeared

TABLE 5

RESULTS OF TESTING 90 SERA BY SIX CEP PROCEDURES (A-F)

Category of Donors	Test Procedure & No. Positive A	B	C	D	E	F	Total No. Samples Tested
Commercial	0	0	0	0	0	0	72
MCV Known Positive	7	6	6	6	6	6	7
Gilbert's	0	0	0	0	0	0	11
CEP Methods:	Spectra	Hyland	Ortho	Pfizer	Abbott	Squibb	

TABLE 6

COMPARATIVE SENSITIVITY OF THE RIA WITH CEP PROCEDURES ON PROFICIENCY SAMPLES FOR DETECTION OF HB AG

Total No. Samples*	CEP System A	B	C	D	E	F	RIA Test	Results
	8	6	7	6	4	5	11	Positive
20	2	1	2	3	2	1	—	W** Positive
	10	7	9	9	6	6	11	Total Positive
CEP Methods	Spectra	Hyland	Ortho	Pfizer	Abbott	Squibb		

* Submitted by the AABB for quality control
** Weakly

to be the most sensitive technique, despite the fact that the CEP and CF procedures could detect a total of five positive sera which were negative by RIA; RIA, however, was the only procedure giving a positive reaction in all 11 sera (Table 7). The higher sensitivity of this technique seemed to be further supported by testing 50 additional sera only by RIA Spectra and Ortho. As seen in Table 8, two sera failed to react by both Ortho's and Spectra's CEP but gave a positive RIA result. In this series, however, none of the sera positive by Spectra's and/or Ortho's CEP methods was negative by RIA.

In order to determine the specificity of the RIA positive reactions, two approaches were taken. First, three sera that were positive by RIA only were concentrated by lyphogel to see if these concentrates would revert from negative to positive CEP on retesting. Only one of the three concentrated sera converted to positive by both CEP procedures; the other two sera remained negative. These results indicated that some of the positive reactions were specific, thus implying that the nonreactivity of unconcentrated sera by CEP was due to the lower sensitivity of this procedure as compared to that of RIA. The second approach consisted of testing double serial dilutions of five sera of known complement-fixing titer by the two CEP methods and by RIA. AB serum known to be negative by Spectra's and Ortho's CEP methods and by RIA was used as a diluent (7). These sera had been frozen at −20°C for variable time periods up to several months. The results (Table 9) indicate that Ortho's CEP and Abbott's RIA are two-to-four times and 16-to-32 times as sensitive as Spectra's CEP, respectively. These findings are in disagreement with those reported by others who showed that RIA is 100-to-1000-fold more concentrative than CEP (5, 14, 24). The lower comparative sensitivity in this study may be due to the shorter incubation period.

TABLE 7

COMPARATIVE SENSITIVITY OF THE RIA WITH CEP, CF AND RPHA PROCEDURES FOR DETECTION OF HB AG (204 samples*)

CEP Methods: A = Spectra B = Hyland C = Ortho D = Pfizer E = Abbott F = Squibb

CEP System A	B	C	D	E	F	CF Test	RPHA Test	RIA Test	Results
19	17	22	15	15	18	28	28	38	Positive
3	5	5	7	6	4	—	—	—	W** Positive
22	22	27	22	21	22	28	28	38	Total Positive
1	2	0	4	0	0	—	—	—	Nonspecific
1	1	1	0	0	0	0	1	1	Positive by one method only

Proportion positive by one or more methods: 41/204
* From a mixed population: "normal" hospital employees and patients suspected of having hepatitis.
** Weakly

No correlation was found among CF, CEP and RIA titers. For example, serum #5 with a CF titer of 1:8,192 had an RIA titer only one dilution higher than that of serum #1 which had a CF titer of 1:128. Environmental factors, such as temperature and duration of storage as well as differences in handling of sera, might have been responsible for some of these changes. A second study was then conducted in which five freshly drawn samples (three known positive and two known negative) were tested in a similar fashion. The CF titer of the three positive sera was determined (Table 10).

Unfortunately, the RIA results were erratic and difficult to explain. For example, positive serum #2

TABLE 8

COMPARISON OF RESULTS OBTAINED BY RIA AND ORTHO AND SPECTRA CEP METHODS (50 samples)

No. of Tests	% of Tests	RIA	Ortho CEP	Spectra CEP
41	82	0	0	0
4	8	+	+	+
3	6	+	+	0
2	4	+	0	0
% Total	100	18	14	8

reacted first, at dilutions 1:64 and 1:128. Negative results followed for seven serial dilutions to appear positive again at a dilution of 1:32,768. Analogous results were obtained with the presumably negative sera #4 and #5. Serum #4 was negative when tested, undiluted and at 1:2 and 1:8 dilutions, but it was positive at 1:4 and 1:16. Serum #5 reacted at a 1:4 dilution but was negative undiluted and when tested at 1:2, 1:8 and 1:16.

It has been reported that when normal human sera are used as diluent they may enhance reactivity of positive sera (6). This, however, does not explain the erratic behavior of the RIA test in this series. A technical error cannot be excluded entirely as the possible cause and would suggest that this technique may be too exacting to be used as a screening procedure. This could conceivably be responsible for at least some of the false positive reactions described in the last series.

Since not all RIA positive–CEP negative sera became positive after concentration and in view of the erratic results obtained in the last series, it is impossible to ascertain the specificity of all RIA positive reactions. This cautious statement is consistent with a recent report which described the occurrence of false positive reactions using Abbott's RIA technique. It was demonstrated that reactivity of an antibody present in human serum directed against a guinea pig protein was responsible (2).

TABLE 9

TITER OF FIVE HB AG POSITIVE SERA* BY CEP (SPECTRA & ORTHO) AND BY RIA

Serum Dilution	Method 1 CEP A	Method 1 CEP C	Method 1 RIA	Method 2 CEP A	Method 2 CEP C	Method 2 RIA	Method 3 CEP A	Method 3 CEP C	Method 3 RIA	Method 4 CEP A	Method 4 CEP C	Method 4 RIA	Method 5 CEP A	Method 5 CEP C	Method 5 RIA
Undiluted	+	+	ND**	+	+	ND	+	+	ND	+	+	ND	+	+	ND
1:2	+	+	ND	+	+	ND	+	+	ND	+	+	ND	+	+	ND
1:4	+	+	ND	+	+	ND	W+	+	+	+	+	ND	+	+	ND
1:8	+	+	ND	+	+	ND	−	+	+	+	+	ND	+	+	ND
1:16	+	+	+	W+***	+	+	−	−	+	+	+	ND	+	+	ND
1:32	−	+	+	−	+	+	−	−	+	+	+	ND	+	+	ND
1:64	−	+	+	−	−	+	−	−	+	+	+	ND	W+	+	+
Undiluted	−	−	+	−	−	+	−	−	+	W+	+	+	−	+	+
1:256	−	−	+	−	−	+	−	−	−	−	+	+	−	−	+
1:512	−	−	+	−	−	−	−	−	−	−	−	+	−	−	+
1:1024	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+
1:2048										−	−	+	−	−	+
1:4096										−	−	−	−	−	−
1:8192										−	−	−	−	−	−
1:16384										−	−	−	−	−	−
1:32,768										−	−	−	−	−	−
CF Titer	1:128			1:512			1:512			1:8192			1:8192		

* The five sera in this experiment have been frozen for various time periods at −20°C. The above CF titers were those obtained on the fresh samples prior to freezing.

** ND = not done.

*** W+ = weakly positive.

TABLE 10

TITERS OF FIVE FRESH HB AG POSITIVE SERA BY CEP (SPECTRA & ORTHO) AND BY RIA

Serum Dilution	Serum 1 CEP A	Serum 1 CEP C	Serum 1 RIA	Serum 2 CEP A	Serum 2 CEP C	Serum 2 RIA	Serum 3 CEP A	Serum 3 CEP C	Serum 3 RIA	Serum 4 CEP A	Serum 4 CEP C	Serum 4 RIA	Serum 5 CEP A	Serum 5 CEP C	Serum 5 RIA
Undiluted		+	ND*	+	+	ND	+	+	ND	−	−	−	−	−	−
1:2	+	+	ND	+	+	ND	+	+	ND	−	−	−	−	−	−
1:4	+	+	ND	+	+	ND	+	+	ND	−	−	+	−	−	+
1:8	W+**	+	ND	+	+	ND	+	+	ND	−	−	−	−	−	−
1:16	−	+	+	+	+	ND	W+	+	+	−	−	+	−	−	−
1:32	−	−	+	+	+	ND	−	+	+						
1:64	−	−	+	W+	+	+	−	+	+						
1:128	−	−	+	W+	+	+	−	−	+						
1:256	−	−	+	−	−	−	−	−	+						
1:512	−	−	−	−	−	−	−	−	+						
1:1024	−	−	−	−	−	−	−	−	+						
1:2048				−	−	−	−	−	+						
1:4096				−	−	−	−	−	−						
1:8192				−	−	−	−	−	−						
1:16384				−	−	−	−	−	−						
1:32,768				−	−	+	−	−	−						
CF Titer	1:128			1:512			1:512			2			2		

* ND = not done.
** W+ = weakly positive.

Until further tests can be performed to demonstrate the presence of HB Ag in the RIA positive–CEP negative sera by other methods designed to eliminate the human antiguinea pig protein interference, the positivity of these sera must remain suspect.

In conclusion, this study supports the claim that the RIA is the most sensitive of all assay methods for detection of HB Ag in use today. Because of the inability to verify the presence of HB Ag in those sera positive by RIA alone, however, this claim must be taken only at face value. Further studies are needed to confirm the specificity of these positive reactions.

REFERENCES

1. ALMEIDA, J. AND WATERSON, A. P. Immune complexes in hepatitis. *Lancet* 1:909, 1971.

2. ALTER, H. J., POLESKY, H. F. AND HOLLAND, P. V. False positive tests for hepatitis-associated antigen in blood donors caused by antibodies to ruminant serum proteins. *J. Immunol.* 108:358, 1972.

3. BLUMBERG, B. S. AND ALLISON, A. C. Studies on the isoprecipitin-determined human serum polymorphism. *Proc. Second Int. Congr. Human Genetics,* Rome, 1961, pp. 733–736.

4. BLUMBERG, B. S. AND ALTER, H. J. Precipitating antibodies against a serum protein ("Australia antigen") in the serum of transfused hemophilia patients. *J. Clin. Invest.* 44:1029, 1965.

5. CAWLEY, L. P. A seminar on basic immunology. Presented at the American Association of Blood Banks, Chicago, 1971, p. 111.

6. CHALMERS, T. C. *Hepatitis and Blood Transfusion,* eds. G. N. Vyas, H. A. Perkins and R. Schmid (Grune and Stratton, 1972), p. 277.

7. DREESMAN, G. R., HOLLINGER, S. B. AND MELNICK, J. L. Detection of hepatitis B antigen by counterimmunoelectrophoresis: Enhancing role of homologous serum dilutions. *Appl. Microbiol.* 24:1001, 1972.

8. DUQUESNOY, R. J. AND BECKER, G. A. Rapid screening for hepatitis antigen by immunoelectrodiffusion using paper discs. *Transfusion* 10:221, 1970.

9. GOCKE, D. J., GREENBERGH, H. B. AND KAVEY, N. B. Hepatitis antigen. Detection of infectious blood donors. *Lancet* 2:248, 1969.

10. GOCKE, D. J. AND HOWE, C. Rapid detection of Australia antigen by counterimmunoelectrophoresis. *J. Immunol.* 104:1031, 1970.

11. HOLPER, J. C. AND JAMBAZIAN, A. Comparative sensitivity of complement-fixation, counterimmunoelectrophoresis, radial, and double diffusion for detection of Australia antigen. *Transfusion* 2:157, 1971.

12. HOSSAINI, A. A. AND ESCOBAR, M. R. The incidence of Au-HAA among donors and recipients. Presented at annual meeting of the Virginia Society of Hematology, June 18–19, 1971.

13. LANDER, J. J., ALTER, H. J. AND PURCELL, R. H. Frequency of antibody to hepatitis-associated antigen as measured by a new radioimmunoassay technique. *J. Immunol.* 106:1166, 1971.

14. LEWIS, J. H. AND CORAN, J. E. Australia antigen detection. *Transfusion* 12:301, 1972.

15. OKOCHI, K. AND MURAKAMI, S. Observations on Australia antigen in Japanese. *Vox Sang.* 15:374, 1968.

16. OKOCHI, K., MURAKAMI, S., NINOMIYA, K. AND KANEKO, M. Australia antigen, transfusion and hepatitis. *Vox Sang.* 18:289, 1970.

17. PRINCE, A. M. An antigen detected in the blood during the incubation period of serum hepatitis. *Proc. Natl. Acad. Sci.* 60:814, 1968.

18. PURCELL, R. H., HOLLAND, P. V., WALSH, J. H., WONG, D. C., MORROW, A. G. AND CHANOCK, R. M. A complement-fixation test for measuring Australia antigen and antibody. *J. Infect. Dis.* 120:383, 1969.

19. Report of test results, Panel 2. American Association of Blood Banks HB Ag Proficiency Testing Program, October 24, 1972.

20. SCHMIDT, N. J., GEE, P. S. AND LENNETTE, E. H. Relative sensitivity of gel diffusion, complement-fixation and immunoelectroosmophoresis tests for detection of hepatitis-associated antigen and antibody. *Appl. Microbiol.* 22:165, 1971.

21. SHULMAN, N. R. AND BARKER, L. F. Virus-like antigen, antibody and antigen-antibody complexes in hepatitis measured by complement-fixation. *Science* 165:304, 1969.

22. VYAS, G. N. AND SHULMAN, N. R. Hemagglutination assay for antigen and antibody associated with viral hepatitis. *Science* 170:323, 1970.

23. VYAS, G. N., WILLIAMS, E. W. AND BOND, H. E. Serologic specificity of hemagglutination assay for hepatitis associated antigen. Presented at 24th Annual Meeting of the American Association of Blood Banks, Chicago, Sept. 12–17, 1971.

24. WALSH, J. H., YALOW, R. AND BERSON, S. A. Detection of Australia antigen and antibody by means of radioimmunoassay technique. *J. Infect. Dis.* 121:550, 1970.