

## The Ausria Test: Critical Evaluation of Sensitivity and Specificity

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Ausria was shown to be considerably more sensitive than CEP by comparing titers of HBAg+ sera and by testing selected proficiency panels, sera from hepatitis patients, and sera from experimentally infected rhesus monkeys; CEP+, Ausria- sera were never encountered. However, in testing NIH donors and personnel, 19 of 20 Ausria+, CEP- sera were shown by neutralization studies to be nonspecific for HBAg, i.e., false positives. Nonspecificity was confirmed by an alternate radioimmunoassay procedure and, in one serum, by density-gradient ultracentrifugation. Testing of 35 additional Ausria+, CEP- sera from New Jersey donors showed 13 specific and 22 (63%) non-

specific reactions. It is mandatory that serum from every Ausria+, CEP- donor undergo specificity testing. In addition to the 14 specific Ausria+, CEP- sera, two sera, subsequently shown to be CEP+, were undetected on the initial CEP test (one was transfused and resulted in hepatitis). These sera were strongly positive by Ausria; sera subject to reader error in CEP are unequivocally positive by Ausria. Ausria tests on CEP- donors suspected of transmitting HBAg failed to implicate a donor in each of eight cases. Some HBAg+ hepatitis will continue to occur despite universal application of Ausria or other tests of equal sensitivity.

**T**HE ADVENT OF a commercially available sensitive radioimmunoassay (Ausria-Abbott Laboratories)<sup>1</sup> for the detection of hepatitis B antigen (HBAg) has placed blood banks under considerable pressure to implement such testing. The two major blood procurement organizations in the nation have responded differently to this pressure. The American National Red Cross has committed itself to screening all donors by Ausria as soon as it is practicable. The American Association of Blood Banks, in a letter to its membership, has stated that while there is some evidence that Ausria is more sensitive than other licensed methods, there is "no evidence that counterelectrophoresis negative, radioimmunoassay positive blood produces disease . . . . Until more scientific data are available and until the practical problems associated with this test

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system are more clearly defined, we feel that the precipitous change to this procedure is not in the best interests of donors or recipients."

In order to establish a scientific base for deciding whether or not to routinely employ Ausria for donor screening, the Clinical Center Blood Bank conducted a series of experiments which compared Ausria sensitivity with that of a well-established counterelectrophoresis (CEP) system, measured duplicate variability and reproducibility, investigated the specificity of Ausria-positive, CEP-negative sera, and assessed the value of Ausria in implicating infectious donors in cases of posttransfusion hepatitis. These studies, described herein, established the increased sensitivity of Ausria for detecting HB<sub>Ag</sub>, but stressed the essential need for specificity testing.

## MATERIALS AND METHODS

### *Sera Tested*

**Donors.** Sera were derived solely from a voluntary donor population representing donations to both the Washington Red Cross (40%) and to the Clinical Center Blood Bank (60%). Both these donor populations contain a large proportion of repeat donors, and each organization had been employing routine donor screening for HB<sub>Ag</sub> by counterelectrophoresis for at least 1 yr at the time this study was initiated.

**Surveillance study.** As part of a parallel study<sup>2</sup> to determine the frequency of HB<sub>Ag</sub> and antibody to HB<sub>Ag</sub> (HB<sub>Ab</sub>) among health care personnel, 4266 coded, duplicate sera were obtained from 2133 health care workers and appropriate controls. These coded sera were then employed in the current study to compare CEP with Ausria for detecting HB<sub>Ag</sub> and to assess duplicate variability of these methods. Sera from persons with a history of hepatitis were excluded to make this sampling more comparable to a normal donor population.

**Donor sera from New Jersey.** Ausria-positive, CEP-negative donor sera in the State of New Jersey are routinely sent to the State Health Department for confirmatory testing. Thirty-five such sera were very kindly supplied to us for specificity testing by Dr. Martin Goldfield, Assistant Commissioner of the New Jersey Department of Health.

**Hepatitis cases.** Sera were obtained from patients with viral hepatitis hospitalized at the Clinical Center, NIH. Hepatitis sera kept frozen from three prior prospective studies<sup>3,4,5</sup> on posttransfusion hepatitis were also utilized to evaluate the Ausria test.

### *Tests*

**Counterelectrophoresis (CEP).** CEP was performed as previously described.<sup>6</sup> Using this method our laboratory has served as a reference laboratory for panels distributed by the Center for Disease Control, the American Association of Blood Banks, the Bureau of Biologics, and the National Heart and Lung Institute. We have always detected as many or more HB<sub>Ag</sub> positive sera than any other laboratory using a CEP system participating in these trials. There have been no false positive results. These credentials are cited to establish that the CEP method being compared to Ausria in the current study is of proven sensitivity. This CEP method was used for the majority of tests performed, but screening of some routine blood donors employed the commercially available CEP system produced by Spectra laboratories. The Spectra CEP system used was one distributed after January 1973. It was considerably more sensitive than the previous Spectra system and when tested on the above panels of sera, compared favorably with our own CEP system and with all other commercial systems tested.

**Ausria.** Ausria was performed as indicated by the manufacturer. One hundred microliters of the serum to be tested was added to the Ausria tube. After 16-hr incubation and washing, 100  $\mu$ l <sup>125</sup>I-labeled guinea pig HB<sub>Ab</sub> was added and incubation carried out for 90 min. Both incubation periods were performed at room temperature.

A serum was considered positive if the radioactive counts obtained with it were reproducibly greater than 2.1 times the mean of counts obtained with eight negative controls, and if such positive results could be specifically neutralized by unlabeled human antibody to HB<sub>Ag</sub> (see below).

### *Specificity Testing*

Sera which were reproducibly CEP-negative, Ausria-positive were tested for specificity as follows: 100  $\mu$ l of the serum was placed into each of four Ausria tubes. After overnight incubation, the tubes were washed five times. Nothing was added to the first tube. One hundred microliters of human serum containing a strong CEP reactive antibody to HBAG was added to the second tube (it was previously shown that antibodies having anti-ad, anti-ay, or anti-a specificity were equally effective as neutralizing reagents). One hundred microliters of normal guinea pig serum was added to the third tube and 100  $\mu$ l of normal human serum to the fourth; both the normal guinea pig and human sera had been shown to be free of HBAG and HBAb. The tubes were then incubated for 90 min and again washed five times. One hundred microliters of  $^{125}$ I-labeled guinea pig HBAb (as provided in the Ausria test) was added to each of the four tubes and incubation performed for an additional 90 min at room temperature. The tubes were subsequently washed and counted on a Nuclear-Chicago gamma counter. A test was considered to be specific for HBAG if the prior addition of unlabeled HBAb, but not normal guinea pig or human serum, reduced the radioactive counts to less than 1.5 times the mean of negative controls or reduced the radioactive count by at least 50%. A test was considered nonspecific if neutralization with guinea pig serum and/or normal human serum equalled or exceeded that achieved by unlabeled HBAb.

Specificity was also assessed by performing a microtiter solid phase radioimmunoassay,<sup>7</sup> which is approximately equal in sensitivity to Ausria and which used human rather than guinea pig  $^{125}$ I-labeled HBAb.

Because it had been suggested that false positive reactions in the Ausria test are heat labile (personal communication, Dr. Alfred Prince), Ausria tests were also performed at 45°C with incubation times of 2 hr for the test serum and 1 hr for the labeled antibody.

Specificity was also assessed by determining the hepatitis B antigen subtype specificity of sera which were Ausria-positive, CEP-negative. Subtyping was kindly performed by Dr. Lacy Overby of Abbott Laboratories using monospecific antisera and a solid-phase radioimmunoassay technique.<sup>8</sup>

### *Density-Gradient Ultracentrifugation*

A serum thought to represent a false positive test by the Ausria method was concentrated and isopycally banded in a cesium chloride (CsCl) density gradient and the distribution of Ausria activity compared with that of known HBAG. A 100-ml aliquot of a unit of serum which assayed 2.2 times the mean negative control was concentrated fourfold by Diaflo ultrafiltration using an XM-100A membrane. This concentrate was then banded for 21 hr at 40,000 rpm in an initially discontinuous CsCl density gradient (1.1 to 1.4 g/cu cm) using the Beckman Ti-14 zonal centrifuge rotor with a 29 liner. The resulting gradient was fractionated into twenty-two 25-ml fractions, and aliquots of each fraction were assayed for Ausria activity and CsCl density. The 1.20 gm/cu cm fraction was subsequently concentrated tenfold by Diaflo ultrafiltration to represent a 40-fold concentrate with respect to the initial serum and again assayed by the Ausria test.

## RESULTS

### *Confirmation of CEP Positive Sera*

Fifty sera known to be HBAG-positive by CEP, but otherwise unselected, were tested by Ausria. All 50 were Ausria-positive. A CEP-positive, Ausria-negative serum has never been detected in our laboratory.

### *Comparative Panels*

Comparative testing for HBAG by CEP and Ausria was performed on panels nationally distributed by the American Association of Blood Banks (AABB), the Bureau of Biologics (BoB) of the Food and Drug Administration, and the Blood Resources Branch of the National Heart and Lung Institute (NHLI). The comparative results are listed in Table 1. Although CEP and Ausria de-

Table 1. Comparative Panels for HBsAg Detection

Source	Number Tested	Maximum Positive for HBsAg*	HBsAg Positives Detected	
			CEP	AUSRIA
AABB 1	20	11	11	11
AABB 2	20	11	11	11
NHLI*	24	18	3	9
BOB*	61	41	30	36

\*Includes dilutions of known positives.

tected the same number of positives in both AABB panels, Ausria detected these positives at much greater dilutions (Table 2). The superiority of Ausria over CEP was better demonstrated in panels with "weaker" sera such as those from NHLI and BoB.

#### Comparative Titers

Serial twofold dilutions were made on the sera from two chronic carriers of HBsAg and the peak titer determined by Ausria and CEP. One carrier had chronic hepatitis and was of the *ad* subtype, the other was an asymptomatic donor of the *ay* subtype. Titers were performed under code on duplicate samples (Table 2). Ausria was 16 and 32 times more sensitive than CEP in detecting the *ay* and *ad* subtypes, respectively.

Comparative titers were also done on the HBsAg-positive sera in the first panel distributed by the AABB (Table 2). The geometric mean titer of the 11 positive sera was 1.8 by CEP and 138 by Ausria; Ausria was thus able to detect HBsAg at dilutions 75 times that which could be detected by CEP.

#### Patients with Hepatitis

To determine the effectiveness of Ausria in detecting HBsAg in patients with hepatitis, sera from three prospective studies on posttransfusion hepatitis<sup>3,4,5</sup>

Table 2. Comparative Titers of HBsAg Positive Sera

Chronic Carriers		CEP Titer	Ausria Titer
Subtype			
<i>ad</i>		1:200	1:6400
<i>ay</i>		1:100	1:1600
AABB Panel 1			
Sample Code No.			
3		1:1	1:64
4		1:1	1:64
5		1:4	1:512
6		1:1	1:256
7		1:8	1:2048
11		1:1	1:64
12		1:1	1:32
13		1:1	1:128
16		1:1	1:64
18		1:1	1:64
19		1:1	1:256
Geometric Mean Titer		1:1.8	1:136

**Table 3. Comparison of Ausria and CEP for Detection of HBAg in Patients with Viral Hepatitis**

	Total	CEP + Ausria +	CEP – Ausria +	CEP – Ausria –
Prospectively studied*	33	9 (27%)	3 (9%)	21 (64%)
Retrospectively studied†	14	10 (72%)	2 (14%)	2 (14%)
Total	47	19 (40%)	5 (11%)	23 (49%)

\*Posttransfusion hepatitis.

†Posttransfusion and sporadic hepatitis.

were tested by Ausria and CEP. Acute phase sera were available from 33 patients with apparent viral hepatitis. The results of this testing, summarized in Table 3, revealed that the majority of posttransfusion hepatitis patients followed prospectively were both CEP-negative and Ausria-negative. In contrast, of 14 cases of either sporadic or posttransfusion hepatitis spontaneously reported to the Blood Bank and investigated retrospectively (Table 3), 72% were CEP-positive.

Five of the total of 47 patients with hepatitis were HBAg positive only by Ausria. Specificity for HBAg was shown in three of these patients either by specific neutralization tests or by antibody seroconversion; in two, no sera were available for determining specificity.

#### *Duration of Positivity*

Serial sera were available from two patients with chronic hepatitis and eight patients with acute hepatitis in which HBAg was detected by CEP in at least one of the serial samples. These sera spanned the time period during which CEP activity was diminishing and eventually disappearing and allowed assessment of possible increased duration of Ausria positivity in excess of CEP activity. In the two cases of chronic hepatitis, HBAg was detected for 9 and greater than 18 wk longer by Ausria than by CEP. In four of the eight cases of acute hepatitis, CEP and Ausria activity disappeared concurrently, but, in the remaining four, HBAg could be detected by Ausria 1.5 to 10 wk longer than by CEP.

#### *Rhesus Monkey Studies*

Serial transmission studies were performed in rhesus monkeys as previously reported.<sup>9</sup> HBAg was transmitted from a human to a rhesus monkey and five successive passages in rhesus monkeys were then achieved. Of 210 monkeys involved in the study, none developed HBAg detectable by CEP, but 15 demonstrated HBAg transiently by radioimmunoassay. HBAg appeared only after an appropriate incubation period and only in animals that had received an HBAg-positive inoculum. Specificity was confirmed in all 15 by neutralization studies.

#### *Duplicate Variability and Reproducibility*

Paired coded samples were available from 2133 individuals entered into a surveillance study (see Materials and Methods). With 2.1 or more times the negative control mean as the index of positivity, there were 20 instances (0.9%) on initial testing in which both members of a duplicate pair were positive, 2100



**Table 4. Reproducibility of the Ausria Test**

Original Test	Number Tested	Final Result †		
		Neg	Equiv.	Pos.
Negative (<1.5)*	117	100%	0	0
Equivocal (1.5–2.1)*	96	80%	15%	5%
Positive (>2.1)*	54	17%	9%	74%

\* Represents ratio of sample counts to the mean of negative controls.

† Represents final interpretation after 1–4 repeat tests on original sample (see text).

instances in which both were negative, and 13 instances (0.6%) in which one member of a pair would have been considered positive and the other member negative. With 1.5 times the negative control mean as the positive cutoff, there were 100 instances (4.7%) in which one member of a pair would have been considered positive and the other member, negative. None of the 4266 duplicate samples disagreed using CEP.

Reproducibility was also examined by taking each sample, which initially was greater than 1.5 times the negative mean, and retesting it from one to four times to establish the final result. One hundred seventeen negative samples were also retested. If a sample was initially less than 2.1 times the mean, and was negative on repeat test, no further testing was performed. If a sample was initially greater than 2.1 times the mean, at least two repeat tests were performed. The results of these reproducibility studies are shown in Table 4 and indicate that a negative result (less than 1.5 times the negative control mean) will again be negative when retested, while a result greater than 2.1 will be reproducible in the majority of cases, but almost 20% of the time will prove to be negative. Sera which are initially equivocally positive (between 1.5 and 2.1 times the mean) will generally prove to be negative, but 15% of the time will remain in the equivocal range and 5% of the time will give a result greater than 2.1.

#### *Voluntary Blood Donors*

Sera from 2341 voluntary donors to the Clinical Center Blood Bank, NIH and to the Washington Red Cross were tested by CEP and Ausria. All of these sera were initially found to be HBAG-negative by CEP; 11 were HBAG-positive by Ausria. The 11 Ausria-positive sera were retested by CEP and two then were found to be CEP-positive (i.e., represented false negative results in the initial CEP test). Thus, nine of the 2341 sera (0.4%) were Ausria-positive, CEP-negative. None of these nine, however, were specifically neutralized by unlabeled HBAb and hence represent false positive results by Ausria (see "specificity" below).

#### *Implicated Donors*

All the donor sera to four cases of HBAG-positive posttransfusion hepatitis, six cases of anamnestic antibody response to HBAG, and one case of primary immune response to HBAG were available for testing by Ausria. All donors had

**Table 5. Suspect Donors to Patients With Serologic Evidence of Exposure to HBAG  
(All Donor Sera Available; All CEP-Negative on Initial Screen)**

	Patient	Number Donors	Number Ausria +	Specifically Neutralized by HBAb
HBAG + post-transfusion hepatitis	MW	22	0	
	KG	3	1	QNS*
	PL	11	0	
	EL	19	1†	Yes†
HBAb Sero-conversion	JL	14	0	
Anamnestic HBAb Response	SA	13	0	
	WB	14	2	QNS
	LH	10	0	
	RE	16	0	
	CH	4	1	No
	WS	16	1	No

\* QNS, Quantity not sufficient for specificity testing.

† Found to be CEP + when retested after Ausria result known.

been found to be CEP-negative prior to transfusion. As seen in Table 5, in six of the 11 episodes none of the donors were positive by the Ausria test. This was true even if the positive cutoff was reduced to 1.5 times the mean.

Although in five instances, it appeared a donor was implicated by the Ausria test, in two of these cases neutralization studies showed these to be false-positive Ausria tests and in two others there were insufficient serum samples available for specificity testing. Thus, in only one of 11 instances (case EL) could a CEP-negative donor be absolutely identified as being HBAG-positive by Ausria; in this case, although the donor was CEP-negative when tested at the time of his blood donation, his serum was retrospectively shown to be CEP-positive. This thus represented an initial failure in reading the CEP plate, rather than a failure of the CEP system itself.

### *Specificity*

Using neutralization with specific antibody as the measure of HBAG specificity in the Ausria test, 14 CEP-positive sera were tested and all found to be specific as was the "Positrol" sample distributed with the Ausria kit. In contrast, the majority of Ausria-positive, CEP-negative sera demonstrated patterns of neutralization which indicated nonspecificity. These consisted of either specific neutralization with guinea pig serum rather than HBAb or of equal neutralization with HBAb, guinea pig serum, and normal human serum (Table 6).

Of the 20 Ausria-positive, CEP-negative sera detected in the surveillance study and among NIH volunteer donors, only one was shown to be specific for HBAG by neutralization tests (Table 7). Nonspecificity of these 19 Ausria-positive, CEP-negative sera was also indicated by the following facts: (1) None were HBAG positive using a sensitive microtiter radioimmunoassay<sup>7</sup> which employed human <sup>125</sup>I-labeled HBAb; (2) none could be subtyped by the Ausria test using monospecific subtype antibodies; and (3) 12 of 19 became HBAG

**Table 6. Patterns of Neutralization in HBAg Specificity Testing of Ausria+, CEP- Sera**

	Standard Ausria Test (cpm)	HBAb Added (cpm)	GPS Added (cpm)	NHS Added (cpm)	Interpretation
Pattern 1	2125	622	2350	2720	HBAg specific
Pattern 2	1947	1893	917	1890	Non specific; anti guinea pig
Pattern 3	3643	3184	3033	3281	Nonspecific; unknown cause

HBAb, unlabeled human antibody to HBAg. GPS, guinea pig serum without HbAg or HBAb. NHS, normal human serum without HBAg or HBAb. cpm, counts per minute.

negative by Ausria when incubated at 45°C and the remaining seven showed diminished radioactive counts; specific positives, in contrast, showed enhanced counts after such high-temperature incubation.

Because the frequency of Ausria-positive, CEP-negative sera which were specific for HBAg appeared to be inordinately low, an additional 35 Ausria-positive, CEP-negative sera were obtained from the New Jersey Department of Health. Of these, 13 (37%) were specific for HBAg (Table 7).

Overall, of the 55 Ausria-positive, CEP-negative sera (Table 7), 25% were specific for HBAg, 33% were nonspecific due to antibody which reacted with the guinea pig reagent used in the Ausria test, and 42% were nonspecific for reasons which are currently undetermined.

Lastly, 14 persons with nonspecific reactions in the Ausria test were retested 1 yr or more from the time of their initial sample; eight still showed nonspecific reactivity.

#### *Density-Gradient Ultracentrifugation*

Hepatitis type B antigen is fully retained by ultrafiltration using the Diaflo XM-100A membrane and bands with a buoyant density of 1.20 g/cu cm in a CsCl density gradient.<sup>10</sup> With a suspected false-positive serum, Ausria activity was concentrated by Diaflo ultrafiltration, but did not distribute as particulate HBAg upon density-gradient centrifugation. The 1.20 g/cu cm fraction was negative for Ausria activity as was a tenfold concentrate of this fraction. In-

**Table 7. Specificity of Ausria**

	Number Tested	Ausria+ CEP-	Specific for HBAg	Nonspecific	
				Guinea Pig Related	Cause Unknown
NIH donors	2341	9*	0	3	6
Surveillance study	2133	11	1	5	5
New Jersey donors†	35	35	13	10	12
Total		55	14(25%)	18(33%)	23(42%)

\*Two additional sera, initially found to be Ausria-positive, CEP-negative, were CEP-positive when retested after Ausria result known.

†These represent selected sera from a large number of commercial and voluntary donors in the state of New Jersey. They were known to be Ausria-positive, CEP-negative when received in our laboratory and were used solely to determine the degree of HBAg specificity of such sera.



stead, Ausria activity was recovered at a density of 1.28 g/cu cm, well separated from the 1.20 g/cu cm region; a buoyant density of 1.28 g/cu cm would be compatible with that of a serum protein.

#### DISCUSSION

The increased sensitivity of Ausria when compared with CEP is clearly demonstrated in this study. Ausria detected all sera HBAg-positive by CEP, was 15 to 75 times more sensitive than CEP based on comparative titers, detected additional positive sera in selected panels, detected HBAg more frequently in cases of clinical hepatitis (plus allowed for detection over a longer period of time), and allowed for the specific detection of HBAg in rhesus monkeys whose sera were consistently negative by CEP. These findings confirm the sensitivity data reported by others.<sup>11,12,13</sup>

Coded, duplicate samples demonstrated that this increased sensitivity was achieved without an inordinate degree of non-reproducible results. Using the manufacturer's suggested 2.1 cut off as indicative of HBAg positivity, duplicate variability was only 0.6%. Further, 74% of sera positive at the 2.1 level were again positive on retest, and an additional 9% were at least greater than 1.5 times the negative mean when retested. Nonreproducibility will result in some waste of resources and laboratory effort but the degree of such for the Ausria test seems to be within quite acceptable limits.

It is important to distinguish between nonreproducible (nonrepeatable) positive Ausria results and those which are consistently reproducible but which are, none the less, nonspecific. Although there have been several reports<sup>11,12,13</sup> attesting to the sensitivity of Ausria as compared with CEP, few have dealt with the question of specificity. In our study, of 2341 blood donors and of 2133 persons involved in an epidemiologic surveillance study, 19 of 20 consistently Ausria-positive, CEP-negative samples proved to be false positives. There is little doubt that these were false positive reactions; they could not be specifically neutralized, they could not be confirmed in another radioimmunoassay system, they could not be HBAg subtyped, they were largely heat labile and, on density-gradient ultracentrifugation of one such serum, Ausria positivity resided in the region of the serum proteins rather than the region characteristic of HBAg. The problem of false positives in this test was originally recognized by Sgouris,<sup>14</sup> and recently reemphasized by Prince.<sup>15</sup> These false reactions are in part due to antibodies in normal human sera which are either directed against, or cross-react with, guinea pig proteins. As seen in this study, however, not all the false positive reactions can be attributed to anti-guinea pig antibody; the cause of these other nonspecific reactions has not as yet been determined.

From a practical standpoint, the problem of nonspecificity is not a major one and should not seriously limit the usefulness of this test. What is essential is that the existence of false positives be recognized and that no individual be labeled as being HBAg positive on the basis of the Ausria test alone until the reaction has been confirmed as being specific. Such specificity testing is quite simple and easily incorporated into routine screening measures. The number of false positive tests can undoubtedly be reduced by technologic changes in the Ausria method itself, and such modifications are currently under evaluation. It

must be stressed, however, that no matter what modifications are introduced, every positive result by Ausria which cannot be confirmed by another immunologic technique must be confirmed by appropriate neutralization studies. The implications of falsely labeling an individual as HBAg-positive are considerable, and may become still more significant in the future if persons are excluded from job opportunities based on their HBAg carrier state.

Because specificity has not been determined in most published reports, one cannot at present estimate the number of "true" Ausria-positive, CEP-negative donors that exist on a national basis. It is clear that in our laboratory this number is small. The fact that Ausria provides such a small advantage in our laboratory is probably related to the fact that we use solely noncommercial blood, that we have a limited donor population that tends to donate repeatedly, that we have an active hepatitis surveillance program that excludes implicated donors even if they do not have measurable HBAg in their blood, and that we have a technical staff experienced in reading weak precipitin lines in the CEP tests. The value of the Ausria test for any given laboratory would undoubtedly increase as they deviated from these principles, particularly if they used commercial donors or suboptimal technique in reading CEP plates.

Despite the fact that the number of specific Ausria-positive, CEP-negative donors is disappointingly small in our donor population, we believe that this test, or one of equal sensitivity, should supplant CEP as soon as is practical. We base this on two premises: First, specific Ausria-positive, CEP-negative donors *do* exist and, second, sera that are weakly positive by CEP tend to be missed in routine laboratories, whereas, because of the inherent objectivity of the Ausria test, these same sera would be readily detected by Ausria. In the hands of inexperienced personnel, CEP may lead to false negative results, whereas Ausria may lead to false positive results. The latter is wasteful, but tolerable; the former endangers the blood recipient.

Unresolved at this time is the infectivity of blood which is Ausria-positive, CEP-negative. Since it is known that blood which is Ausria-positive, CEP-positive is infectious<sup>4</sup> and since blood which is Ausria-negative, CEP-negative can, none the less, cause HBAg-positive hepatitis,<sup>5</sup> it seems reasonable to assume that blood which has an intermediate amount of HBAg, namely that which is specifically Ausria-positive, CEP-negative, would also be infectious. Proof of this, however, will have to await the results of several prospective controlled studies of posttransfusion hepatitis which are now in progress.

Although it is anticipated that these prospective studies will indicate that it is more dangerous to receive specific, Ausria-positive, CEP-negative blood than blood which is negative by both tests, it is also anticipated that the overall impact of the Ausria test (or others of equal sensitivity) on posttransfusion hepatitis will be definite, but relatively small.

It is evident in testing donors involved in cases of posttransfusion hepatitis that some HBAg positive hepatitis will continue to occur even if the Ausria test were universally applied. In addition, a significant proportion of posttransfusion hepatitis is due to virus(es) unrelated to HBAg<sup>5</sup> and hence unaffected by tests for HBAg, no matter how sensitive they are. It seems unlikely that tests more sensitive than radioimmunoassay are on the immediate horizon, so that

total prevention of HBsAg-positive hepatitis will have to depend upon some form of biologic or physical inactivation or removal of the hepatitis B virus or of immunization of the blood recipient. Such measures will be independent of testing.

Lastly, it is important to emphasize that the impact of HBsAg testing on post-transfusion hepatitis is minimal when compared with the potential impact of eliminating the use of commercial blood.<sup>4,16</sup> By far, the single most significant measure for the reduction of posttransfusion hepatitis is the total exclusion of the commercial donor.

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