Maximizing Cornea and Tissue Donation through Specimen Quality

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Abstract

Purpose: To retrospectively evaluate the efficacy of assays used to screen for transmittable diseases in tissue and cornea donors.

Methods: Three years of data, including donor screening result, confirmatory assay result, specimen quality (hemolysis), and time elapsed between death and specimen procurement, were reviewed. Chi-square analysis was employed to determine statistical significance of findings.

Results: HBsAg, HTLV, and HIV prevalence was noted to be higher than anticipated based on published data. Confirmatory assay results did not support the increased prevalence found in our test population. Instances of reactive screening results for HBsAg, HTLV, and HIV correlated positively with an increase in specimen hemolysis, as well as increased time between death and specimen procurement. Specimen hemolysis showed a positive correlation with increased time between death and specimen procurement.

Conclusions: This retroactive study shows the importance of obtaining a high-quality specimen free of hemolysis when screening for infectious diseases in tissue and cornea donors.

Key Words: Donor screening, hemolysis

Ensuring donated corneas and tissues are free from transmittable diseases is the most important function of the clinical laboratory in the tissue and eye banking industry. Highly sensitive assays approved by the U.S. Food and Drug Administration (FDA) for donor screening are utilized to accomplish this.[1] Screening assays ensure a high confidence level in the ability to detect transmittable diseases, however their increased sensitivity often leads to a high rate of false positive results.[2] In living individuals, a positive screening test result does not confer a diagnosis, it merely reflects the need for additional confirmatory or diagnostic testing and long-term patient monitoring by a physician. In the tissue and eye banking industry, patient monitoring isn't feasible as donors are deceased. As this is the case, federal and industry regulations prohibit the transplantation of tissues or corneas from any donor that exhibits reactivity to the following screening assays.[3]

- Hepatitis B Surface Antigen (HBsAg)
- Hepatitis B Core Total Antibody (HBcT)
- Antibody to Hepatitis C Virus Encoded Antigen (HCV)
- Human Immunodeficiency Virus Types 1 and 2 Plus O (HIV)
- Nucleic Acid Testing (NAT) for HBV, HCV and HIV

In the case of tissues and corneas that will be transplanted outside of the United States, a screening assay for Human T-Lymphotropic Virus (HTLV) Type I and II is often required.

In this retrospective study, we reviewed clinical data over a three-year period from tissue and cornea donors tested in our laboratory to assess the efficacy of the screening assays employed. We identified potential causes leading to the higher-than-expected positive rates of certain screening assays, as well as potential strategies to reduce false-positivity.

MATERIALS AND METHODS

From January 1, 2015 through December 31, 2017 cadaveric specimens were screened in house via FDA approved[3] Enzyme Immunoassay (EIA) methods for hepatitis B surface antigen (n = 4,739), total (IgG and IgM) antibody to hepatitis B core antigen (n = 4,736), antibody to hepatitis C virus encoded antigen (n = 4,739), and antibody to human immunodeficiency virus type 1 and 2 plus O (n = 4,749). In addition, 2,406 specimens were screened for antibody to human T-cell lymphotropic virus type I and II. Confirmatory testing was performed in house for all but one reactive HBsAg specimen (n = 97), as required per the package insert, using a neutralization-based assay. Confirmatory testing was performed at reference laboratories for all but three reactive HIV specimens (n = 9) and all but five reactive HTLV specimens (n = 45) using the Human Immunodeficiency Virus Type 1 Western Blot, the HTLV-I/II Immunoblot, and the HTLV INNO-LIA. HBV NAT results were compared to HBsAg results as both are expected to be concordant in acute infections. Assay information is provided in Table 1. Overall reactivity rates were compared to the published expected prevalence for the United States. It should be noted that reactivity in our test population was expected to be lower than the published expected prevalence due to routine tissue donation screening questions

Table 1. Assay Kit and Manufacturer Information

Tradename	Infectious Agent	Format	Manufacturer
Genetic Systems HBsAg EIA 3.0; Genetic Systems HBsAg Confirmatory Assay 3.0	HBV	EIA	Bio-Rad Laboratories Redmond, WA US License 1109
ORTHO HBc ELISA Test System	HBV	EIA	Ortho-Clinical Diagnostics, Inc Raritan, NJ
Ortho HCV Version 3.0 ELISA Test System	HCV	EIA	Ortho-Clinical Diagnostics, Inc Raritan, NJ US License 1236
Genetic Systems HIV-1/HIV-2 Plus O EIA	HIV-1, HIV-2	EIA	Bio-Rad Laboratories Redmond, WA US License 1109
Avioq HTLV-I/II Microelisa System	HTLV-1, HTLV-2	EIA Lysate	Avioq, Inc. Research Triangle Park, NC 27709 US License 1856
Procleix Ultrio Plus Assay	HBV, HCV, HIV-1	Nucleic Acid Test (TMA)	Gen-Probe, Inc., San Diego, CA US License 1592
Genetic Systems HIV-1 Western Blot (WB)	HIV-1	WB	Bio-Rad Laboratories Redmond, WA US License 1109
HTLV Blot 2.4	HTLV-1, HTLV-2	WB	MP Biomedicals Solon, OH
INNO-LIA HTLV I/II	HTLV-1, HTLV-2	LIA	Fujirebio, Europe N.V., Belgium

designed to identify and defer donors with a history of HIV, HBV, and/or HCV. From late 2015 through December 31, 2017 cadaveric specimens (HBsAg and HCV n = 3,545; HTLV n = 2,278; HIV n = 3,555; HBcT n = 3,542) were assessed for hemolysis using the grading scale as shown in Figure 1.[4] Gross hemolysis was defined as a specimen that is opaque to the passage of light. Time elapsed from time of death (TOD) to time of sample collection was assessed on all analytes with available data (HBsAg n = 4,179; HCV

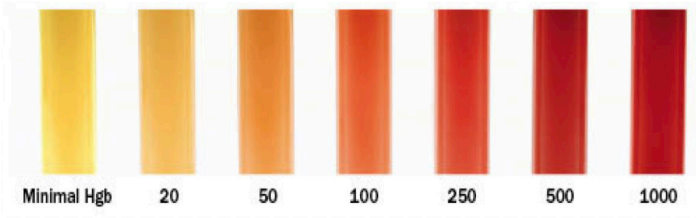

Figure 1

and HBcT n = 4,178; HTLV n = 2,004; HIV n = 4,187), comparing likelihood of a reactive screening assay result with time between TOD and sample collection. Time from TOD to sample collection was also assessed against specimen hemolysis (n = 2,987). Specimen collection site and type of collection tubes were assessed (HBsAg n = 1,485; HCV n = 1,486; HBcT n = 1,482; HTLV n = 1,070; HIV n = 1,484). Chi-square analysis was employed to determine statistical significance at $p < .05$. Chi-square analysis for hemolysis data grouped Minimal hemoglobin (Hgb) through 100 mg/dL Hgb vs. 250 mg/dL Hgb through Gross Hemolysis. Chi-square analysis for time elapsed from death to specimen collection was divided into three groups; 0 minutes – 7 hours and 59 minutes; 8 hours – 15 hours and 59 minutes, and 16 hours -24 hours.

RESULTS

Serology reactive rates obtained over the three-year period are compared with expected rates in the United States population in Table 2. Reactive rates for antibody to HBsAg and HTLV I/II were notably higher than expected. Confirmatory testing results obtained over the three-year period are presented in Tables 3a-c. Comparisons on the degree

Table 2. Observed Reactivity vs. Expected Reactivity (%)

	HBsAg		HBcT		HCV		HIV		HTLV	
Period	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
2015 - 2017	2.07	<1[5]	3.42	<4[6]	1.43	1-2[6]	0.25	<1[7]	2.08	<0.1[8]

Table 3a. HBsAg Confirmation

Total Positive HBsAg	98
Total Not Confirmed	82
Total Confirmed	15
Total Unknown	1
Confirmation Rate	15.46%
HBV NAT +	4
HBV NAT + Rate	4.12%

Table 3b. HIV Confirmation

Total Positive HIV	12
Total Not Confirmed	8
Total Confirmed	0
Total Indeterminate	1
Total Unknown*	3
Confirmation Rate	0.00%
Total HIV NAT +	0
HIV NAT + Rate	0.00%
*Samples not sent for confirmatory testing	

Table 3c. HTLV I/II Confirmation

Total Positive HTLV	50
Total Not Confirmed	37
Total Confirmed	0
Total Indeterminate	8
Total Unknown*	5
Confirmation Rate	0.00%
*Samples not sent for confirmatory testing	

of hemolysis for reactive and nonreactive specimens are presented in Tables 4a-e.

Table 4a. Degree of Hemolysis in Non-Reactive HBsAg Specimens vs Reactive HBsAg Specimens

HBsAg NonReactive			HBsAg Reactive		
Hgb mg/dL	n	% Frequency	Hgb mg/dL	n	% Frequency
Minimal	124	3.57%	Minimal	0	0.00%
20	572	16.49%	20	6	7.89%
50	1104	31.82%	50	10	13.16%
100	1153	33.24%	100	12	15.79%
250	327	9.43%	250	15	19.74%
500	124	3.57%	500	15	19.74%
1000	59	1.70%	1000	14	18.42%
Gross	6	0.17%	Gross	4	5.26%
Total	3469		Total	76	
Chi-square Statistic		129.59	The result is significant at $p <$ .05		
P-Value		<.00001			

Table 4b. Degree of Hemolysis in Non-Reactive HBcT Specimens vs Reactive HBcT Specimens

HBcT NonReactive			HBcT Reactive		
Hgb mg/dL	n	% Frequency	Hgb mg/dL	n	% Frequency
Minimal	122	3.57%	Minimal	3	2.48%
20	555	16.22%	20	23	19.01%
50	1073	31.37%	50	41	33.88%
100	1126	32.91%	100	37	30.58%
250	328	9.59%	250	13	10.74%
500	134	3.92%	500	4	3.31%
1000	73	2.13%	1000	0	0.00%
Gross	10	0.29%	Gross	0	0.00%
Total	3421		Total	121	
Chi-square Statistic		0.31	The result is not significant at $p <$ .05		
P-Value		0.578			

Table 4c. Degree of Hemolysis in Non-Reactive HCV Specimens vs Reactive HCV Specimens

HCV NonReactive			HCV Reactive		
Hgb mg/dL	n	% Frequency	Hgb mg/dL	n	% Frequency
Minimal	124	3.55%	Minimal	1	1.96%
20	570	16.31%	20	8	15.69%
50	1096	31.37%	50	18	35.29%
100	1148	32.86%	100	16	31.37%
250	340	9.73%	250	2	3.92%
500	137	3.92%	500	2	3.92%
1000	69	1.97%	1000	4	7.84%
Gross	10	0.29%	Gross	0	0.00%
Total	3494		Total	51	
Chi-square Statistic		0.002	The result is not significant at $p <$ .05		
P-Value		0.965			

Table 4d. Degree of Hemolysis in Non-Reactive HIV Specimens vs Reactive HIV Specimens

HIV NonReactive			HIV Reactive		
Hgb mg/dL	n	% Frequency	Hgb mg/dL	n	% Frequency
Minimal	125	3.52%	Minimal	0	0.00%
20	577	16.27%	20	1	12.50%
50	1114	31.41%	50	1	12.50%
100	1168	32.93%	100	2	25.00%
250	344	9.70%	250	1	12.50%
500	137	3.86%	500	2	25.00%
1000	73	2.06%	1000	0	0.00%
Gross	9	0.25%	Gross	1	12.50%
Total	3547		Total	8	
Chi-square Statistic		6.93	The result is significant at $p <$ .05		
P-Value		0.0085			

Table 4e. Degree of Hemolysis in Non-Reactive HTLV Specimens vs Reactive HTLV Specimens

HTLV NonReactive			HTLV Reactive		
Hgb mg/dL	n	% Frequency	Hgb mg/dL	n	% Frequency
Minimal	63	2.83%	Minimal	0	0.00%
20	346	15.52%	20	3	6.25%
50	731	32.78%	50	7	14.58%
100	771	34.57%	100	15	31.25%
250	206	9.24%	250	2	4.17%
500	69	3.09%	500	13	27.08%
1000	40	1.79%	1000	6	12.50%
Gross	4	0.18%	Gross	2	4.17%
Total	2230		Total	48	
Chi-square Statistic		41.61	The result is significant at $p <$ .05		
P-Value		<.00001			

The degree of specimen hemolysis appeared to have a significant impact on the false reactivity rate of HBsAg ($p <$ .00001), HTLV ($p <$ .00001), and HIV ($p <$ .0085), but not HBcT ($p = .578$), or HCV ($p = .965$). The effect of TOD to time of sample collection on assay reactive rates is shown in Tables 5a-e.

Table 5a. TOD to Specimen Collection in Non-Reactive vs Reactive HBsAg Specimens

	Pos	% Pos	Neg	% Neg	Total
0-7:59	6	0.610%	977	99.390%	983
8-15:59	42	1.975%	2085	98.025%	2127
16-24:00	39	3.648%	1030	96.352%	1069
Chi-square Statistic		23.4283	The result is significant at $p <$ .05		
P-Value		<.00001			

The increase in time between death and specimen procurement exhibits a positive correlation with increased instances of false reactivity for HBsAg ($p <$ .00001), HTLV ($p <$ .00001), and HIV ($p <$ .014), but not HBcT ($p = .954$), or

Table 5b. TOD to Specimen Collection in Non-Reactive vs Reactive HBcT Specimens

	Pos	% Pos	Neg	% Neg	Total
0-7:59	35	3.553%	950	96.447%	985
8-15:59	71	3.355%	2045	96.645%	2116
16-24:00	36	3.343%	1041	96.657%	1077
Chi-square Statistic	0.0941		The result is not significant at $p < .05$		
P-Value	0.954				

Table 5c. TOD to Specimen Collection in Non-Reactive vs Reactive HCV Specimens

	Pos	% Pos	Neg	% Neg	Total
0-7:59	14	1.421%	971	98.579%	985
8-15:59	27	1.276%	2089	98.724%	2116
16-24:00	28	2.600%	1641	152.368%	1077
Chi-square Statistic	1.0614		The result is not significant at $p < .05$		
P-Value	0.588				

Table 5d. TOD to Specimen Collection in Non-Reactive vs Reactive HIV Specimens

	Pos	% Pos	Neg	% Neg	Total
0-7:59	0	0.000%	986	100.000%	986
8-15:59	3	0.141%	2119	99.859%	2122
16-24:00	6	0.556%	1073	99.444%	1079
Chi-square Statistic	0.0941		The result is significant at $p < .05$		
P-Value	0.014				

Table 5e. TOD to Specimen Collection in Non-Reactive vs Reactive HTLV Specimens

	Pos	% Pos	Neg	% Neg	Total
0-7:59	4	0.787%	504	99.213%	508
8-15:59	11	1.053%	1034	98.947%	1045
16-24:00	34	7.539%	417	92.461%	451
Chi-square Statistic	63.4033		The result is significant at $p < .05$		
P-Value	<.00001				

HCV ($p = .588$). The effect of TOD to time of sample collection on specimen hemolysis is shown in Table 6.

Table 6. TOD to Specimen Collection in Hemolysis Levels (Minimal – 100mg/dL vs 250 mg/dL – Gross Hemolysis)

	00:00-07:59		08:00:00-15:59		16:00-24:00	
Hgb mg/dL	n	% Frequency	n	% Frequency	n	% Frequency
min	39	5.28%	54	3.47%	19	2.73%
20	158	21.41%	286	18.40%	81	11.65%
50	276	37.40%	483	31.08%	173	24.89%
100	211	28.59%	501	32.24%	236	33.96%
250	44	5.96%	131	8.43%	107	15.40%
500	7	0.95%	57	3.67%	52	7.48%
1000	3	0.41%	38	2.45%	24	3.45%
gross	0	0.00%	4	0.26%	3	0.43%
Total	738		1554		695	
Chi-square Statistic	104.21		The result is significant at $p < .05$			
P-Value	<.00001					

The increase in time between death and specimen procurement exhibits a positive correlation with increased instances of hemolysis ($p < .00001$). Specimen collection site and specimen collection tube were assessed and found to show no significant difference (data not included).

DISCUSSION:

Review of our three-year serology reactive rates compared to published references suggest acceptable performance for the HBcT, HCV, and HIV assays when compared to the anticipated disease prevalence within the population. Reactivity rates for HBsAg and HTLV are higher than expected suggesting an elevated incidence of false positivity for the assays. Additional support of false positive results for the HBsAg and HTLV assays is the lack of concordance between the screening assays and the confirmatory assays. Of reactive HBsAg results 84.54% failed to confirm via the neutralization assay and 95.88% failed to show reactivity for hepatitis B nucleic acid. Of the HTLV results sent for confirmatory testing, all failed to be confirmed by the confirmatory assay, with 17% deemed "indeterminate". Review

of concordance between the degree of specimen hemolysis and serology reactivity suggests a positive correlation between hemolysis and serology reactivity in the HBsAg ($p < .00001$), HTLV ($p < .00001$), and HIV ($p = .00797$) assays. Over the duration of the study it was observed that 63.16% of reactive HBsAg specimens exhibited hemolysis ≥ 250 mg/dL vs. 14.87% of nonreactive HBsAg specimens. This relationship was exhibited in the HTLV assay as well, with 47.92% of reactive HTLV specimens exhibiting hemolysis ≥ 250 mg/dL vs. 14.30% of nonreactive HTLV specimens. The relationship between hemolysis and HIV serology reactivity may need further follow up due to a low incidence of HIV reactive specimens (n = 8) with hemolysis data. Concordance between hemolysis and the HBcT and HCV assays was not statistically significant. Specimen reactivity exhibited a significant relationship between prolonged time between death and specimen collection for the HBsAg assay ($p < .00001$), the HTLV assay ($p < .00001$), and the HIV assay ($p = .014$). The correlation with the HIV assay may need additional investigation due to a low incidence of HIV reactivity overall (n = 9). Review of elapsed time between death and specimen collection suggests a significant correlation between time elapsed and increased rates of hemolysis with 7.3% of specimens collected within 8 hours of the time of death exhibiting ≥ 250 mg/dL of hemolysis, 14.8% of specimens collected between 8 and 16 hours post-mortem exhibiting ≥ 250 mg/dL of hemolysis, and 26.8% of specimens collected between 16 and 24 hours post-mortem exhibiting ≥ 250 mg/dL of hemolysis ($p < .00001$).

CONCLUSIONS

Our investigation into the performance of screening assays suggest suboptimal performance when used as a screening method for tissue and cornea donation. Our higher than anticipated reactive rates when compared to expected disease prevalence, coupled with our low confirmatory rates and lack of NAT positive concordance, suggest an increased rate of false reactive results for the HBsAg, HTLV, and possibly HIV assays. Furthermore, our investigation demonstrates that poor specimen quality, particularly hemolysis, is a contributing factor to the poor performance of the HBsAg and HTLV screening assays. Our data suggests that specimens for testing should be procured as soon as possible following death to avoid specimen hemolysis. In instances in which specimen hemolysis cannot be avoided it may be prudent to try to obtain a pre-mortem specimen from the hospital. Improving the efficacy of the screening assays will not only drive efficiencies in the tissue and eye banking industry, it will ensure organizations are able to maximize the gift of donation through utilization of all suitable tissues for transplant.

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