

Challenges in Testing Post-Mortem Blood Samples

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ABSTRACT

Infectious disease testing of tissue donor samples collected after death can be challenging due to the occurrence of false positive and false negative reactivity. In this presentation we share representative results from cross-sectional and serial sampling studies that convincingly demonstrate the complex relationship between sample hemolysis, post-death collection time, and false positive reactivity in assays for infectious disease analytes. Similarly, we summarize representative published studies showing that false negative results are not due to inhibitors in post-mortem blood, but on rare occasions reflect hemodilution. Lastly, we share our experience with the rare occurrence of invalid nucleic acid test results and its complex association with hemolysis.

Cross-sectional comparison: higher rates in post-mortem vs pre-mortem tissue donors

Analyte	Living donors (BM, egg, sperm N=349,363)	Cornea and tissue donors		Anatomical gift (N=25,417)
		Pre-mortem (N=38,972)	Post-mortem (N=910,089)	
HBsAg	0.19%	0.27%	0.98%	1.71%
HBcore antibody	1.71%	3.24%	4.67%	3.59%
HTLV antibody	0.31%	0.24%	0.64%	N/A
HIV antibody	0.11%	0.19%	0.2%	0.38%
HCV antibody	0.29%	1.55%	2.24%	2.08%
HBV NAT	0.06%	0.11%	0.24%	0.67%
HCV NAT	0.10%	1.12%	1.17%	2.28%
HIV NAT	0.01%	0.00%	0.03%	0.05%

Hale R. 2016. Correlation between sample quality and reactive rates of donor-screening assays. Poster presentation, AATB annual meeting

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Cross-sectional comparison of infectious disease reactive rates: population differences?

Analyte	French population	Living donors (pre-mortem)	Cornea donors (post-mortem, N=560)
HIV antibody	0.16%	0.32%	6.8%
HTLV antibody	0.006%	0.18%	2.9%
HBV surface Ag (HBsAg)	0.68%	1.56%	4.8%
HBcore antibody	8.18%	8.95%	12.1%
HCV antibody	0.86%	2.24%	3.4%

Challine D et al. 2006. Serological viral testing of cadaveric cornea donors. Transplantation 82:788-793

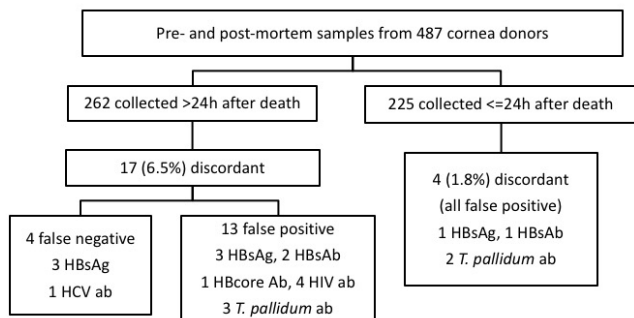
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This table presents results from a representative cross-sectional study comparing infectious disease reactivity rates in post-mortem tissue (cornea in this case) donors to other donor groups. Higher reactivity rates were observed in the post-mortem group, but it is unclear how much of this difference reflected differences in the makeup of the populations.

Our group performed a cross-sectional study of reactivity rates utilizing data from >1.3 million samples tested over a 5-year period. Within the cornea and tissue donor group, the post-mortem collection group exhibited higher reactivity rates compared to the pre-mortem group for most analytes. Further, reactivity rates were generally even high in the post-mortem anatomical gift group. Higher reactivity rates in post-mortem sample are assumed to mostly represent false-positive reactivity. It was noted that within the tissue donor group, post-mortem samples were more likely to be hemolyzed than pre-mortem samples, and hemolysis was even more marked in the anatomical gift group. Also, the post-death collection time was longer for the anatomical gift group compared to the post-mortem tissue donor group. These changes suggest an interplay between hemolysis, post-death sample collection time, and increased reactivity rates.

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Serial samples: reactivity in relation to post-death collection time



Wilkemeyer I et al. 2012. Comparative serology testing of pre- and post-mortem blood samples from cornea donors. Cell Tissue Bank 13:447-452

Increased reactive rates more closely associated with sample quality than post-death collection time

Post-death collection time	HIV antibody reactivity	
	Non-hemolyzed (N)	Hemolyzed (N)
<12h	4% (78)	24% (25)
12-24h	4% (79)	25% (81)
24-36h	0% (36)	26% (47)
>36h	13% (30)	29% (35)

Challine D et al. 2006. Serological viral testing of cadaveric cornea donors. Transplantation 82:788-793

Another approach for assessing post-mortem changes in assay reactivity is serial sampling, where samples are taken from a given donor before death and after death, and the results of infectious disease marker testing compared. These investigators segregated the donors based on the post-death collection time of the post-mortem sample. The proportion of donors with a discordant post-mortem versus pre-mortem result was markedly higher in donors whose post-mortem sample was collected >24 hours after death; further, the discordant patterns included false-positive and false-negative results. These data clearly show an association between post-death collection time and changes in reactivity rates.

To better understand the interplay between hemolysis, post-death collection time, and reactivity rates, Challine et al further segregated each time group on the basis of hemolysis and assessed the HIV reactivity rate. This analysis clearly showed that for samples collected within 36 hours of death, increased reactivity was associated with hemolysis, and not post-death collection time per se.

Hemolysis AND reactive rates increase with post-death collection time

Post-death collection time (N)	Proportion reactive			Proportion of samples hemolyzed
	HIV ab	HBsAg	HBcore ab	
<12h (103)	4%	2%	10%	24%
12-24h (160)	6%	5%	9%	50%
24-36h (83)	10%	5%	19%	56%
>36h (65)	8%	9%	18%	55%

Challine D et al. 2006. Serological viral testing of cadaveric cornea donors. Transplantation 82:788-793

False positive reactivity: take-home messages

- Hemolysis serves as a visual measure of post-mortem absence of osmotic regulation, resulting in cell rupture and release/generation of the factors actually causing false positive reactivity.
- Limited research on physiological differences in pre-mortem and post-mortem blood indicates a reduction in total protein concentration post-mortem, but similar levels of albumin, IgG, and IgM.
- Studies are needed to identify the factors in post-mortem serum/plasma responsible for false positive reactivity in infectious disease serologic assays.
- Similarly, serial studies are needed to further investigate post-mortem false positive reactivity in nucleic acid detection assays, and the relationship to specimen quality and time of collection

Regarding the first bullet point, leaders in this area think that the compound responsible for visual assessment of hemolysis, namely free hemoglobin, is not actually responsible for false positive results. Rather, hemolysis is a surrogate marker for cell rupture, which releases or generates the factor directly causing false positive reactivity. This factor remains undefined.

In their cross-sectional study, Challine et al evaluated reactivity rates of post-mortem samples in relation to post-death collection time. For all 3 analytes evaluated, reactivity rates increased with collection time, reaching peak levels >24 hours after death. They also found that the proportion of samples exhibiting hemolysis increased with post-death collection time, but peaked earlier than the reactivity rates did.

Possible causes of false negative reactivity: inhibitors?

Spiking studies show that post-mortem blood does not inhibit detection of infectious disease antibodies, antigens, or nucleic acid.

- Antibodies: Baleriola C et al. 2012. Infectious disease screening of blood specimens collected post-mortem provides comparable results to pre-mortem specimens. *Cell Tissue Bank* 13:251-258
- Antigen: Kitchen AD & Newham JA. 2011. Qualification of serological infectious disease assays for the screening of samples from deceased tissue donors. *Cell Tissue Bank* 12:117-124
- Nucleic acid: Gubbe K et al. 2012. Validation of virus NAT for HIV, HCV, HBV and HAV using post-mortem blood samples. *Transfus Med Hemother* 39:381-385

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Test failures: NAT invalid samples

- At our Los Angeles facility, approximately 2% of samples give an invalid HBV/HCV/HIV NAT result when initially tested, due to a low internal control signal
- Sample must be diluted and retested, increasing turnaround time
- >95% of such samples are severely hemolyzed
- But <10% of severely hemolyzed samples yield an invalid result when initially tested

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As shown in slide 4, false negative results occur less often than false positive results, but are of particular concern from a safety standpoint due to the possibility of donor-derived transmission of an unrecognized infection. Although the most straight-forward explanation for false negative results is the presence of inhibitors, many studies (representative studies listed here) provide no evidence for an inhibitor of antibody, antigen, or NAT reactivity in post-mortem serum/plasma.

A small proportion of samples yield invalid results in NAT assays. Nearly all such samples are severely hemolyzed, but most hemolyzed samples do not yield invalid results. This finding again points to the complex relationship between hemolysis and results in infectious disease assays.

Possible causes of false negative reactivity: hemodilution

- Hemodilution requires massive blood loss AND transfusion of crystalloids, colloids, or blood products prior to death; neither alone causes hemodilution.
- Titration studies indicate that blood would need to be diluted >20-fold to give a false negative result in routine infectious disease serology assays. Hemodilution rarely leads to such high levels of dilution.
- However, false negative results due to hemodilution do appear to occur, albeit rarely. HCV ab false negative described*:
 - Pre-mortem RIBA+ sample had a clearly visible IgG control band
 - Post-mortem RIBA- sample had no IgG control band, indicating an extremely low level of IgG.

*Heim A et al. 1999. Evaluation of serological screening of cadaveric sera for donor selection for cornea transplantation. *J Med Virol* 58:291-295

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Summary

- Infectious disease assay reactivity rates are increased in post-mortem blood specimens compared to pre-mortem specimens.
- Increased reactivity rates are most significantly associated with hemolysis, which in turn is associated with increasing time between death and specimen collection.
- Hemolysis is most likely a surrogate marker for cell destruction, resulting in release/generation of the unknown factor(s) causing false positive reactivity in infectious disease assays.
- False negative assay reactivity in post-mortem blood specimens is rare compared to false positive reactivity. Although some false negative results represent hemodilution, the mechanisms responsible for most false-negative results remain unclear.
- Test failures are uncommon, but are nearly always associated with severe hemolysis.

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Studies suggest that hemodilution is an extremely unlikely cause of false negative results, but cases have indeed been described.