Validation of Commercial Immunoassays for Detecting HBsAg and HIV Antibodies in Production Pools

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Background/Case Studies

- Plasma fractionators test plasma production pools for HBsAg and HIV antibodies as a qualitative limit test for the control of impurities, to safeguard against errors in donation testing or pooling.
- The European Medicines Agency (EMA) has published guidelines for the validation of immunoassays for the detection of HBsAg and HIV antibodies in production pools.
- The aim was to validate commercial immunoassays for the testing of production pools for HBsAg and HIV antibodies utilizing the EMA guidelines.

Study Design/Methods

- Cutoff values were determined for the Abbott Alinity s HBsAg and HIV Ag/Ab Combo assays by calculating the mean signal-to-cutoff ratio (S/CO) plus 3 standard deviations of two different types of plasma production pool samples.
- Compared the Alinity s determined cutoff values to the previously established calculated cutoffs for the Abbott PRISM® HBsAg and Abbott PRISM® HIV O Plus assays.
- Limit of detection was determined by testing in triplicate, serial dilutions of WHO HBsAg and HIV antibody standards diluted in production pool plasma.
- A normalized detection limit was calculated for the HBsAg assay using production pools containing low, typical and high anti-HBsAg titers.
- Intra-assay variability was determined by testing 20 determinations of a low positive control in 1 run.
- Inter-assay variability was determined by testing at least 3 representative negative production pool samples and a titration series of WHO standard spiked into plasma production samples.
- Runs were performed on six separate days using two different instruments and two different lots of assay reagents.

Results/Findings

Table 1: Cutoff comparison data

Original Instrument Cutoff (S/CO)		New Instrument Cutoff (S/CO)	
HIV Ab	HBsAg	HIV Ag/Ab	HBsAg
0.48	0.72	0.1	0.29

Table 2: Validation data

Validation	Value
HBsAg assay detection limit	0.065 IU/mL
Normalized detection limit	1 hour at 15-250C
Anti-HIV lowest positive dilution	1:10,000 – 1:50,000
Intra-assay variability	<5%
Inter-assay variability	<15%

- The calculated cutoff values for the assays on the Alinity s were all lower than the previously established Prism calculated cutoff values so no need to calculate new cutoff values.
- The HBsAg assay detection limit was 0.065 IU/mL for both source and recovered plasma samples.
- The normalized detection limit study demonstrated that one hour was the maximum amount of time pool samples could sit at 15-25ºC.
- The anti-HIV lowest positive dilution for all replicates varied between 1:10,000 to 1:50,000 depending on subtype and group.
- The % CV of the S/CO values of the replicates of the intra-assay variability validation were less than 5% for both assays.
- The %CV of the S/CO values of the panel of samples of the inter-assay variability validation were less than 15%.

Conclusion

- No need to calculate new cutoff values for the assays on the Alinity s platform.
- These assays are sensitive enough with current cutoff values to meet all the recommendations in the EMA validation guidelines.
- The Abbott Alinity s HBsAg and HIV Combo assays can be utilized to test production pool samples.