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Performance of the UltraQual Multiplex PCR Assay when Screening Pooled Source Plasma Donations

Jeffrey Albrecht[1], Francoise Gala[1], Claire House[1], Jur Jang[1], Monica Byrd[2], Alice Stewart[2]
1. National Genetics Institute, Laboratory Corporation of America® Holdings 2. Octapharma Plasma, Inc.

National GENETICS INSTITUTE
LabCorp Specialty Testing Group

I. Introduction

Nucleic Acid testing of pooled source plasma donations is a critical quality control step for ensuring manufactured product safety and this testing is strictly regulated by the Food and Drug Administration (FDA).[1] A new nucleic acid test, the UltraQual® Multiplex PCR Assay (multiplex assay), was recently developed by National Genetics Institute (NGI) for simultaneously screening pooled source plasma donations for Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), HIV-1, and HIV-2. This new test was licensed by the FDA on May 30, 2018.[2] The performance of the multiplex assay was compared to that of individual NGI assays for HBV, HCV, and HIV-1 that were previously licensed by the FDA and are currently being used. The objective of this study was to demonstrate the ability of the multiplex assay to accurately and specifically detect target virus nucleic acids in individual plasma donations that are included in pools of up to 512.

II. Methods

The multiplex assay is carried out only and entirely at NGI's laboratories. Multiplex assay nucleic acid extraction is performed using Chemagen Chemagic[3] Viral DNA/RNA Kit reagents and the Chemagen Chemagic MSM I automated extractor. PCR amplification includes primers specific for the HCV, HIV-1, HIV-2 and HBV targets. Detection of target amplicons is performed using a Bio-Rad Bio-Plex[4] Suspension Array system which employs Luminex[5] bead and flow cytometry technology.

This study was performed in collaboration with multiple donor centers owned or operated by Octapharma Plasma, Inc. Over a six-month period, a total of 1,534,648 individual plasma donations from 161,037 different donors from 54 donor centers were tested in 3,000 master pools containing up to 512 donations each. NGI's FDA-approved pooling and testing algorithm (Biological License BL 103868/5002) was used for pooling aliquots of individual plasma donations and subsequently identifying any positive donations. Per the algorithm, a positive donation is identified by first obtaining a positive result for the master pool containing the donation. Additional testing leads to the identification of positive primary pools (subset pools containing up to 64 donations), which ultimately allows matrix resolution and confirmation of a positive donation.

Plasma pools were tested in parallel with the multiplex assay and NGI's individual FDA-licensed UltraQual® HCV, UltraQual® HIV-1, and UltraQual® HBV Assays. Clinical specificities and sensitivities of the multiplex assay and the individual assays were compared using McNemar's Test for matched pairs (p-value)

III. Results

The number of confirmed positive donations (sorted by virus) and the number of those donations identified as positive separately by the multiplex assay and the individual assays, following NGI's pooling and testing algorithm, are presented in Table 1. The table also lists assay clinical sensitivities for each viral type and the upper and lower 95% confidence intervals for those sensitivities. The multiplex assay HIV-2 sensitivity could not be calculated since no donations positive for HIV-2 were identified in this study.

Table 1: Determined Assay Clinical Sensitivities

Virus	Number of Confirmed Positive Donations	Multiplex Assay		Current Assays	
		Number Determined as Positive*	Clinical Sensitivity (95% Confidence Intervals)	Number Determined as Positive*	Clinical Sensitivity (95% Confidence Intervals)
HCV	254	254	100.0% (98.6% - 100.0%)	238	93.7% (90.0% - 96.4%)
HIV-1	30	30	100.0% (88.4% - 100.0%)	29	96.7% (82.8% - 99.9%)
HIV-2	0	0	Not calculated [b]		
HBV	21	15	71.4% (47.8% - 88.7%)	16	76.2% (52.8% - 91.8%)
HBV & HIV-1	2	2**	100.0% [a]	2**	100.0% [a]

* Identified positive following NGI's pooling and testing algorithm
** Each donation was positive for at least one of the viruses
[a] Confidence intervals not calculated, given the small number of samples
[b] Not calculated, as no positives were identified

When the number of positive results obtained were compared using McNemar's Test for matched pairs (see Table 2), the multiplex assay and the individual assays were similar at detecting the HIV-1 and HBV targets, with a p-value >0.05. However, the two assays were statistically different at detecting HCV, indicating that the multiplex assay is superior to the individual assay for this virus.

Table 2: Differences in Assay Sensitivity

Virus	P-value*
HCV	0.0002
HIV-1	1.0000
HBV	1.0000

* A significant difference is indicated when the p-value is < 0.05

The number of confirmed negative/not implicated (NI) donations (sorted by virus) and the number of those donations identified as negative/NI separately by the multiplex assay and the individual assays following NGI's pooling and testing algorithm are presented in Table 3. The clinical specificities, with lower-bound 95% confidence intervals for the specificities, are also shown in the table. Both assays demonstrated 100% specificity for each viral target.

Table 3: Assay Clinical Specificities

Virus	Confirmed Negative or Not Implicated Donations	Multiplex Assay		Current Assays	
		Number Negative or Not Implicated*	Clinical Specificity**	Number Negative or Not Implicated*	Clinical Specificity**
HBV	1,534,625	1,534,625	100.0%	1,534,625	100.0%
HCV	1,534,394	1,534,394	100.0%	1,534,394	100.0%
HIV-1	1,534,616	1,534,616	100.0%	1,534,616	100.0%
HIV-2	1,534,648	1,534,648	100.0%	N/A ***	N/A ***

* Identified as negative or not implicated following NGI's pooling and testing algorithm
** Lower bound 95% confidence interval is 99.995% ≤ x < 100%
*** NGI does not perform an FDA-licensed individual HIV-2 Assay

IV. Conclusion

The multiplex assay has been evaluated and found to be equivalent to the individual assays for identifying donations positive for HIV-1 and HBV and found to be superior to the individual assay for detecting HCV. The difference in the HCV results can be explained, at least in part, by the dramatically improved analytical sensitivity (95% detection limit) of the multiplex assay compared with the licensed individual assay when testing master pools for HCV. The sensitivities for the three targets are listed in Table 4.[6]

Table 4: Master Pool 95% Detection Limits

Virus	Average 95% Detection Limit (95% Confidence Intervals)	
	Multiplex Assay	Individual Assays
HCV	1.72 IU/mL (1.41 – 2.35)	6.61 IU/mL (5.06 – 10.57)
HIV-1 Group M	9.95 IU/mL (8.30 – 12.89)	8.57 IU/mL (7.01 – 11.68)
HBV	0.66 IU/mL (0.54 – 0.90)	0.90 IU/mL (0.64 – 2.11)

The multiplex assay has a substantially lower (approximately four-fold) detection limit for HCV compared to the individual assay. This difference appears to be significant, given that the 95% confidence intervals for the sensitivities did not overlap for the two assays. This difference most likely accounted for the ability of the multiplex assay to detect matrix pools containing the 16 HCV-positive donations not detected by the licensed assay.

During the course of this study, there were five and six confirmed HBV-positive donations that were not identified by the individual assay and the multiplex assay, respectively, following NGI's pooling and testing algorithm. In these cases, the HBV viral loads in these donations were low[7] (data not shown), which is consistent with the very early stages of infection or with a chronic infection. When these donations were diluted to generate master and primary pools, the HBV viral concentrations fell near or below the detection limit of the assays. At these low viral loads, HBV is not consistently detected and the variance in results between the multiplex and individual assays would be expected (as would variance from run to run using the same procedure).

No HIV-2 positive donations were identified during the course of this study. Therefore, this study does not provide evidence that HIV-2 can be detected in clinical samples when using the multiplex assay. However, NGI has shown through analytical validations that the multiplex assay can efficiently detect HIV-2.

In conclusion, this clinical study demonstrates that the multiplex assay is a suitable replacement for the NGI's currently used FDA-licensed assays.

V. References

1. Code of Federal Regulations, Title 21 Part 610, Section 610.40, *Test Regulations*
2. FDA BLA Approval letter. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/ucm609352.htm
3. Registered trademark of PerkinElmer
4. Registered trademark of Bio-Rad Laboratories, Inc.
5. Registered trademark of Luminex Corporation
6. NGI PV618 Amendment 1, Determination of the Analytical Sensitivity and Precision of the NGI
7. UltraQual® Multiplex Assay
8. NGI PV658, National Genetic Institute's UltraQual® Multiplex PCR Assay for HCV, HIV-1, HIV-2, and HBV

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