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COMPARISON OF COBAS 6800 AND PROCLEIX PANTHER FOR THE DETECTION OF HCV, HIV AND HBV

Khalid AL Quthami * , Daniah A Alsharif , Suliman A Esmat,
Bassam Oudh Al Johnyi , Waleed S Alwaneen , Fadel Qabbani
The Research Done at King Abdulaziz University
* Email: Kmrqu@hotmail.com ORCID 0000-0002-2055-8728

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Abstract:

Background: *The use of sensitive antibodies has greatly reduced the risk of virus transmission following blood transfusion. There are various versions of NAT assays; they can be performed on individual samples or a pool of samples. Also, they can target individual viral nucleic acid or more than one type of viral nucleic acids simultaneously in the form of triplex or multiplex.*

Objectives: *Compare the sensitivity and specificity of cubes 6800 and Procleix panther for the simultaneous detection of HCV, HIV and HBV and determine the HIV-1, HCV, and HBV analytical sensitivity of these assays.*

Subjects and methods: *Procleix Ultrio assay and Cobas TaqScreen MPX assays were used for the detection of HBC, HCV, and HIV viruses in this study. The fully automated systems used were Procleix panther for Procleix Ultrio assay and Cobas 6800 for Cobas TaqScreen MPX assay. Testing was done according to manufacturer's guidelines of each assay separately. All the tests were performed by the qualified staff of the laboratory. Assay detection limits and statistical difference calculations were carried out with computer software. The 95 percent and 50 percent detection limits, including 95 percent confidence intervals (CIs), were calculated by probit analysis on serial dilutions of reference materials.*

Results: *A total of 9 (0.13%) donations (N = 5000) were found out to be reactive for either of the viral markers tested by Cobas 6800 and Procleix panther. Overall prevalence of viral markers was 1.44% for HBV, 0.4% for HCV, 0.25% for HIV, and 5 (0.05%) co-infections. Of the 7 samples' RR for HBV DNA by both systems, all (100%) remained Procleix reactive when retested in pools of 4 but (94.4%) were Ultrioreactive when tested in a pool size of 8. One of the HBV NAT yield samples (HK5) was reactive in pools of both 4 and 8, but the other yield sample (HK1) was nonreactive in both pool sizes.*

Conclusion: *Efficient function of both techniques were elaborated, however, Procleix Ultrio was slightly superior to Cobas 6800 in few situations, although, new Cobas version is much updated and sensitive from old releases.*

Keywords: HBV, immunoassays, NAT

Corresponding author:
Khalid AL Quthami,
King Abdulaziz University
** Email: Kmrqu@hotmail.com*
ORCID 0000-0002-2055-8728

QR code

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INTRODUCTION:

The availability of safe blood for transfusion is a need of every country. Each unit of donated blood should be screened for major major transfusion transmitted infections (TTIs). Studies have shown that effective blood screening programs have significantly reduced the risk of TTIs (1). The most important of TTIs are Hepatitis B, Hepatitis C, and AIDs which are respectively caused by HBV, HCV, and HIV. In the past and in most of underdeveloped and developing countries, these viruses are screened in blood using serological techniques. Although the use of sensitive antibodies has greatly reduced the risk of virus transmission following blood transfusion. For example, the studies have shown that risk for HCV and HIV-1 transmission is 1 in 493 000 and 1 in 103 000, respectively, in the USA and western Europe (2,3). But still serological techniques are not so sensitive and specific and can lead to virus transmission via blood transfusion.

Nucleic acid test (NAT) is a technique which is highly sensitive and specific for the detection of viral nucleic acids. In this technique, specific sequences of the viral genome (RNA or DNA) are amplified and detected. One of the major benefits of NAT is that it excludes false positive results which is a major concern in serological methods (4). It is really helpful for blood collected during the window period, as well as cases of immuno-silent infections and, possibly, a large spectrum of virus variants. For example, studies show that NAT reduces the window phase to 11 days for HCV and HIV (5). There are various versions of NAT assays; they can be performed on individual samples or a pool of samples. Also, they can target individual viral nucleic acid or more than one type of viral nucleic acids simultaneously in the form of triplex or multiplex (6).

Gen-Probe-Novartis (GPN) and Roche Molecular Systems (RMS) are two main manufacturers of triplet NAT assays for screening of HBC, HCV, and HIV(7). Procleix Ultrio, Procleix Ultrio Plus and Procleix Ultrio Elite assays are three NAT assays offered by GPN and these are performed on Procleix Tigris system and Procleix Panther systems (8, 9, and 10). Cobas Taq Screen MPX and Cobas TaqScreen MPX v2.0 assays are offered by RMS and they are performed on full automated Cobas s201 system. Cobas 6800 is newly developed high throughout platform launched by RMS (11).

The current study will

1. Compare the sensitivity and specificity of cubes 6800 and Procleix panther for the simultaneous detection of HCV, HIV and HBV.
2. Determine the HIV-1, HCV, and HBV analytical sensitivity of these assays.

PATIENTS AND METHODS:

Setting:

The study was conducted in Makkah Regional Lab

Sample:

5000 donors from jan 2017- Dec2017 were included in this study. A sample from each donor was drawn and stored according to guidelines. Each donor signed a consent form for their willingness to enter in the study.

Assays and systems

Procleix Ultrio assay and Cobas TaqScreen MPX assays were used for the detection of HBC, HCV, and HIV viruses in this study. The fully automated systems used were Procleix panther for Procleix Ultrio assay and Cobas 6800 for Cobas TaqScreen MPX assay. Procleix Ultrio is based on transcription Mediated Amplification (TMA) principle and is consisted of three main steps target capture, Amplification and Detection. On the other hand Cobas TaqScreen MPX assay is based on reverse transcription and PCR amplification. It is also composed of three steps; specimen preparation, amplification and detection. Both assays were available with ready to use mixtures

Testing was done according to manufacturer's guidelines of each assay separately. All the tests were performed by the qualified staff of the laboratory. Assay detection limits and statistical difference calculations were carried out with computer software. The 95 percent and 50 percent detection limits, including 95 percent confidence intervals (CIs), were calculated by probit analysis on serial dilutions of reference materials. The statistical differences between detection limits were assessed by estimating the variance of each assay with standard statistical methods and subsequently determining if the mean detection limits between the assays differed with a standard Z test. The false-reactive, invalid sample and failed run rates were compared with the chi-square test and considered significantly different if the p values were less than 0.05**.**

RESULTS:

A total of 9 (0.13%) donations (N = 5000) were found out to be reactive for either of the viral markers tested by Cobas 6800 and Procleix panther. Overall prevalence of viral markers was 1.44% for HBV, 0.4% for HCV, 0.25% for HIV, and 5 (0.05%) co-infections (see Table and Figure 1). The 95 percent and 50 percent detection limits (IU/mL) and their corresponding 95 percent CIs for HIV-1, HCV, and HBV were calculated by probit analysis and are shown in Table 2 and Figure 2 &3. Statistical comparison showed no significant difference between the 95 or 50 percent detection limits between the two assays for HIV-1 and HBV;

however, the Procleix assay showed significantly ($p < 0.05$) lower 95 and 50 percent detection limits for HCV. Two of the 9 discordant samples were RR on the Procleix assay (HBV discriminatory–reactive) but nonreactive by the Cobas 6800 test in a pool of 9 (HK1 and HK5; Table 3 and Figure 4). Both samples were confirmed as HBV DNA–positive as they were reactive by both the Cobas 6800 test with ID-NAT and the Roche TaqMan viral load assay (<6 IU/mL). Both samples were anti-HBc (total immunoglobulin)-positive and HBsAg-negative and were therefore classified as HBV NAT yield samples. An additional sample that was RR on the Cobas 6800 test and later confirmed as a HBV NAT yield sample (HK2) was initially reactive on the Procleix assay, but nonreactive on the discriminatory assays and on the Ultrio assay when retested in duplicate.

Table 1: Viral load descriptive statistics.

Items		Range	Median(IQR)
Viral Load	HBV	2.43-830	5.1655(298.54)
	HCV	21.59-948.85	80.7750(720.67)
	HIV	15.56-66.4	18.1720(38.47)
Kruskal-Wallis Test	X^2	3	
	P-value	0.223	

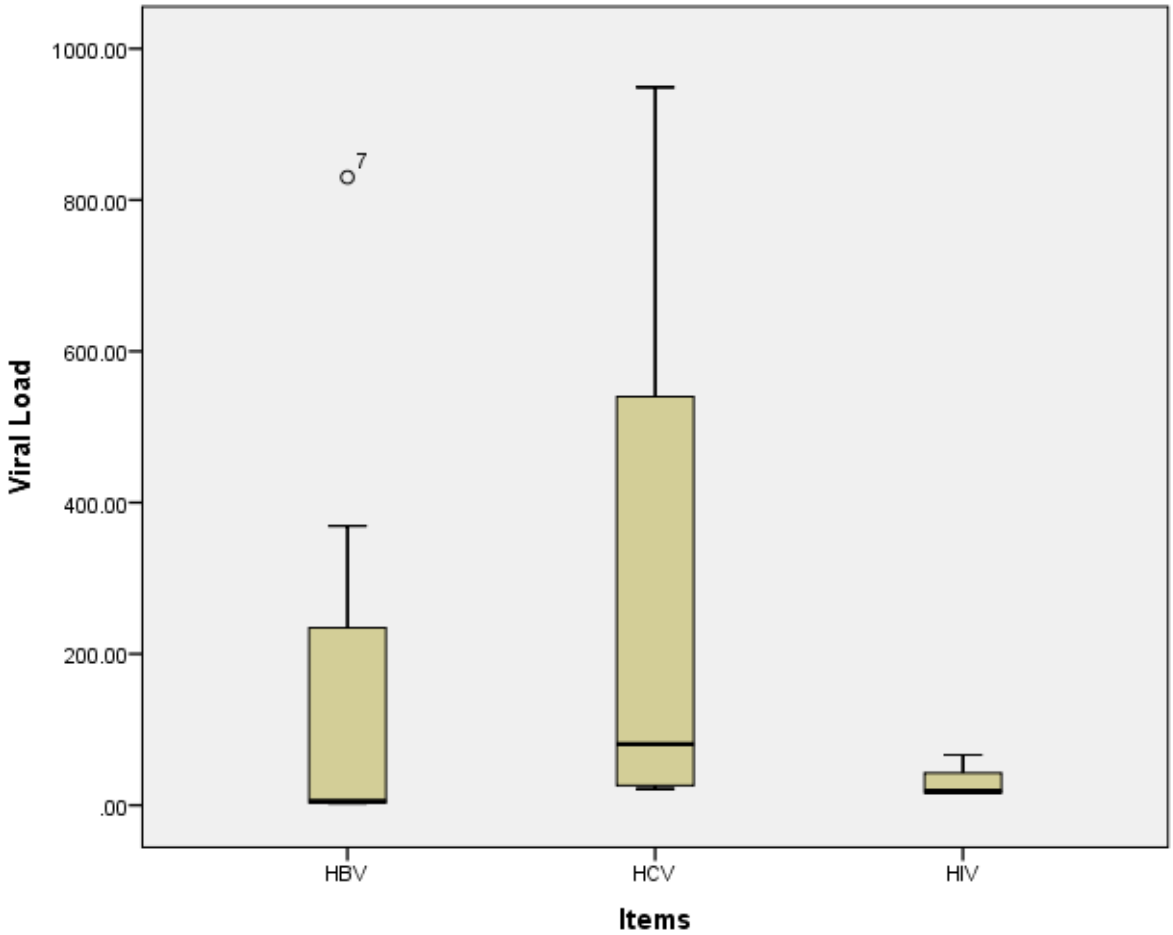

Figure 1: Box-plot showing viral loads

Table 2: Reactivity of viral markers.							
		N/R		R		Total	
		N	%	N	%	N	%
Items	HBV	202	92.7%	16	7.3%	218	100.0%
	HCV	210	96.3%	8	3.7%	218	100.0%
	HIV	210	96.3%	8	3.7%	218	100.0%
Total		622	95.1%	32	4.9%	654	100.0%
Chi-square	X^2	3.976					
	P-value	0.137					

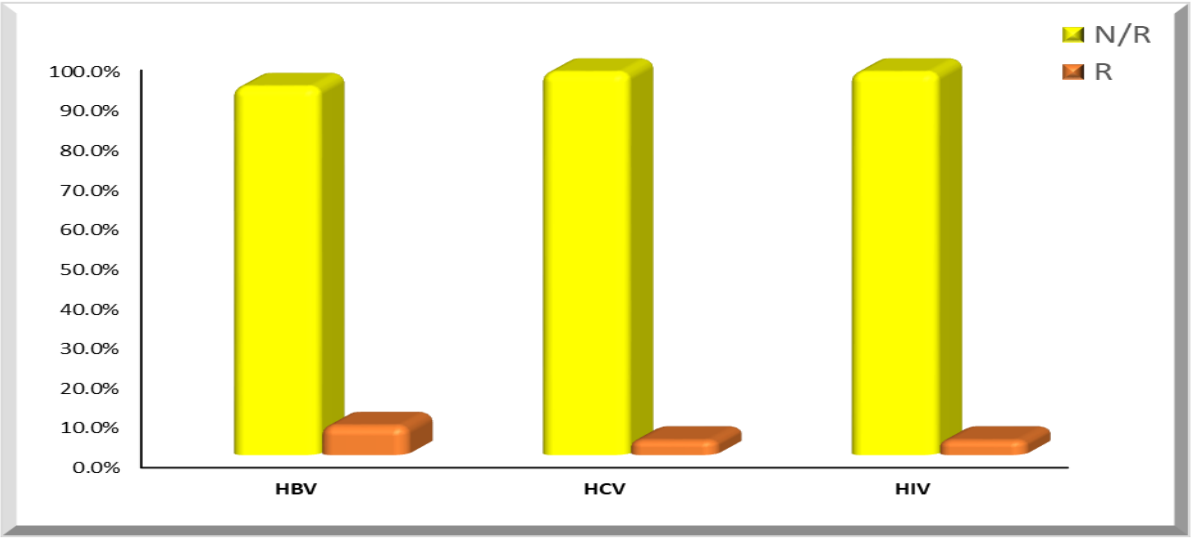

Figure 2: Bar chart of viral markers between reactive and non-reactive results.

Table 3: Comparison between 5 dilutes and their viral loads.					
Continue to		Items			
		HBV	HCV	HIV	Total
Dilute 4	N	1	0	0	1
	%	12.50%	0.00%	0.00%	6.30%
Dilute 5	N	0	0	2	2
	%	0.00%	0.00%	50.00%	12.50%
Dilute 6	N	0	1	1	2
	%	0.00%	25.00%	25.00%	12.50%
Dilute 7	N	1	2	1	4
	%	12.50%	50.00%	25.00%	25.00%
Dilute 8	N	6	1	0	7
	%	75.00%	25.00%	0.00%	43.80%
Total	N	8	4	4	16
	%	100.00%	100.00%	100.00%	100.00%
Chi-square	X^2	16.439			
	P-value	0.037*			

Table 4: Mean and SD of viral markers.								
	HBV		HCV		HIV		ANOVA	
	Mean	SD	Mean	SD	Mean	SD	F	P-value
Dilute 0	20.519	6.283	22.965	2.916	25.875	1.592	1.634	0.233
Dilute 1	23.689	6.145	26.458	2.718	26.943	4.891	0.667	0.530
Dilute 2	26.368	6.017	29.868	2.652	32.060	1.149	2.196	0.151
Dilute 3	28.080	5.803	33.768	3.128	34.123	1.447	3.302	0.069
Dilute 4	30.139	5.879	33.890	2.047	36.043	1.453	2.554	0.116
Dilute 5	30.820	4.504	35.683	2.948	37.445	1.530	5.013	0.026*
Dilute 6	33.123	4.573	37.908	2.776	38.350	1.556	2.611	0.122
Dilute 7	34.783	3.718	38.360	1.975	39.270	0.0	1.651	0.251
Dilute 8	36.327	2.891	39.720	0.00			1.181	0.327
Friedman test	23.154		36.787		45.640			
P-value	0.002*		<0.001*		<0.001*			

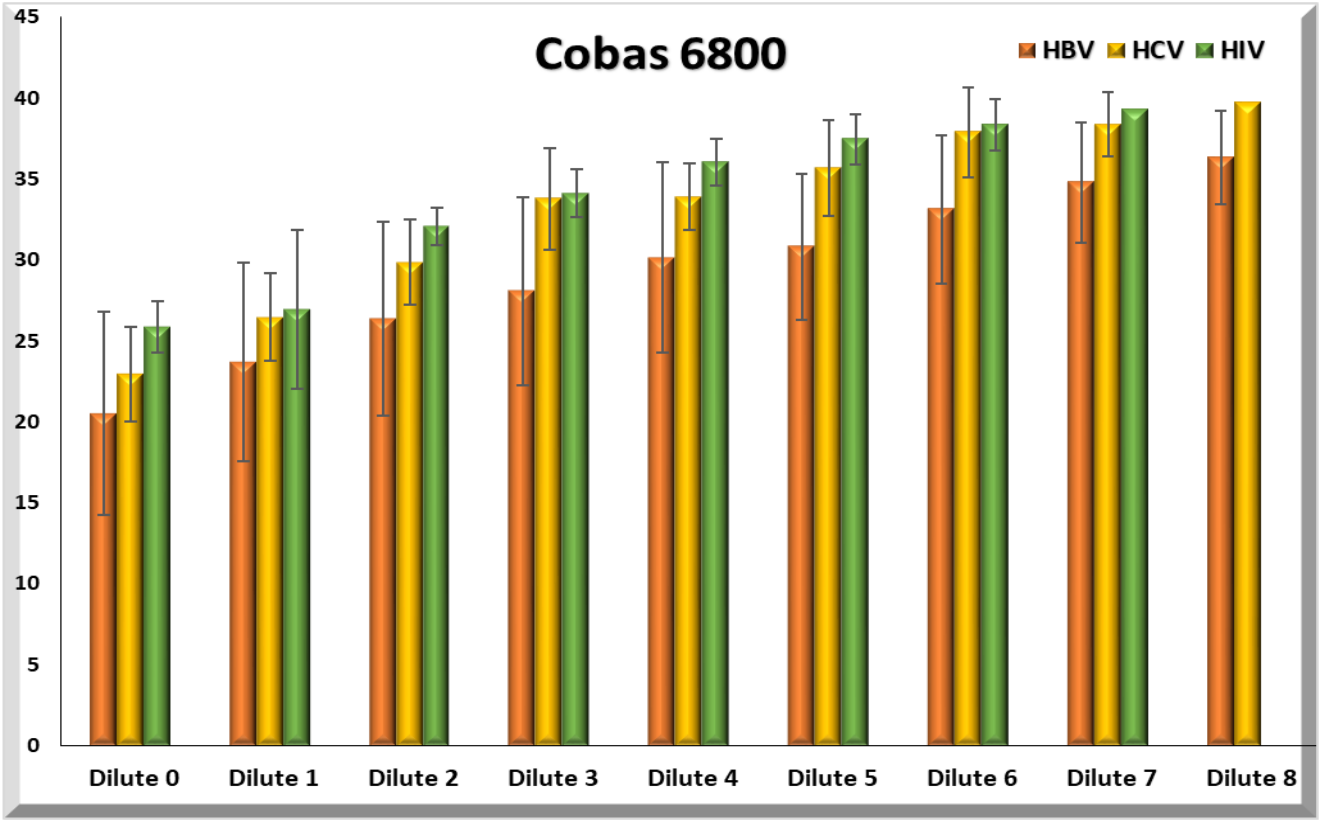

Figure 3: Bar chart of viral markers in dilutes.

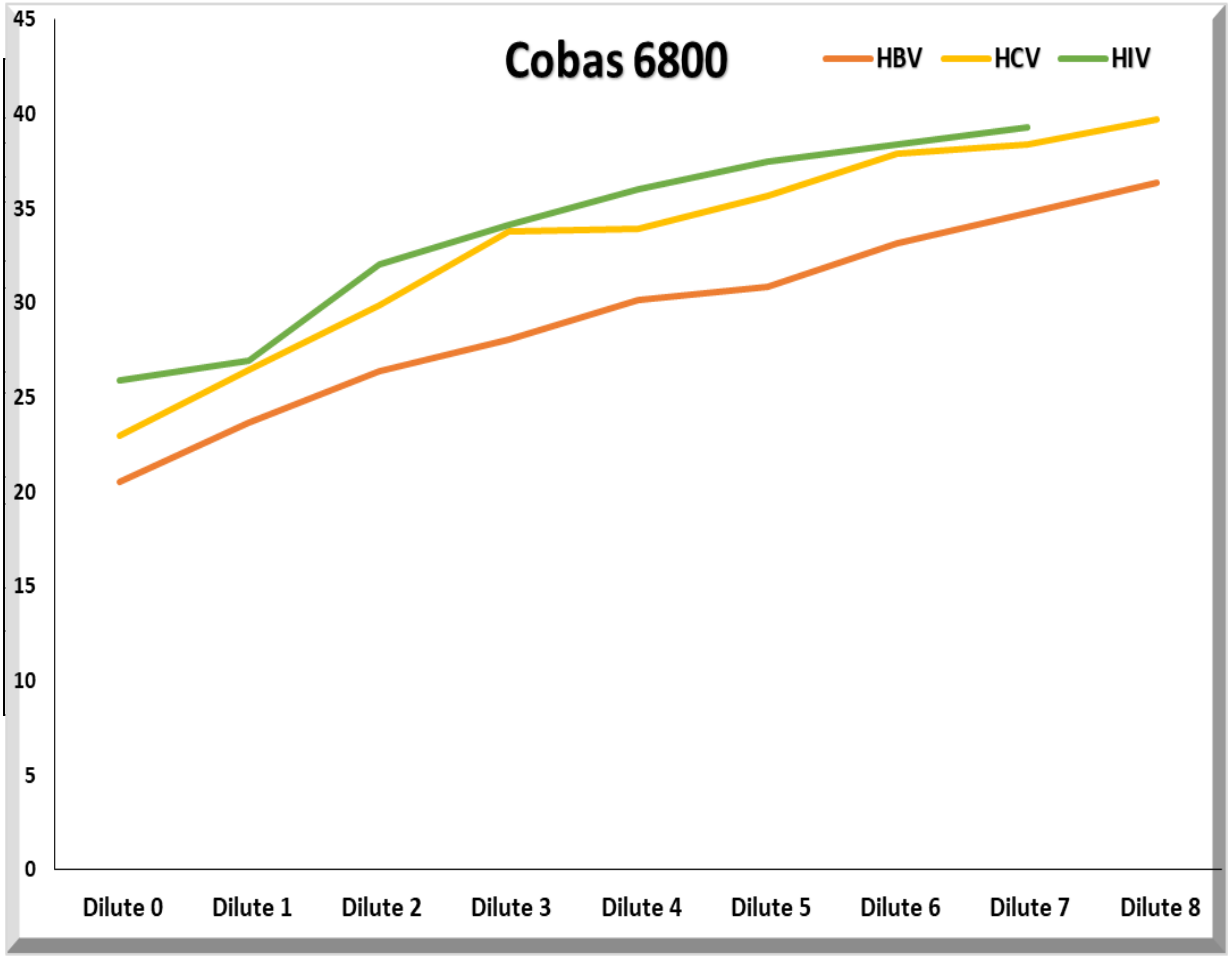

Figure 4: Line chart of viral markers in dilutes.

Table 7: Comparison between Cobas 6800 and Procleix Panther.

		HBV	HCV	HIV	Total
R-R	**N**	6	1	0	7
	%	75	25	0	43.75
R-NR	**N**	2	3	4	9
	%	25	75	100	56.25
Total	**N**	8	4	4	16
	%	100	100	100	100
Chi-square	**X^2**	8.434			
	P-value	0.015*			

Four of the nine discordant samples were both MP- and ID-NAT–reactive by the Cobas 6800 test but nonreactive by the Procleix assay. 2 of 5 (HK2, HK4, and HK6; Table 4 and Figure 5) were positive on the HBV UltraQual assay at NGI and detected by the Roche viral load assay and therefore confirmed as HBV DNA–positive. Because the fourth sample (HK3) did not display reactivity by an independent NAT assay (either the UltraQual HBV or the Roche HBV viral load assay) and was nonreactive for the presence of HIV-2 RNA, it was classified as unresolved. Two of the three samples that were confirmed HBV DNA–positive were HBsAg-negative and therefore classified as HBV NAT yields (HK2 and HK6), despite being nonreactive on the Ampliscreen assays used for discriminatory testing (Table 5).

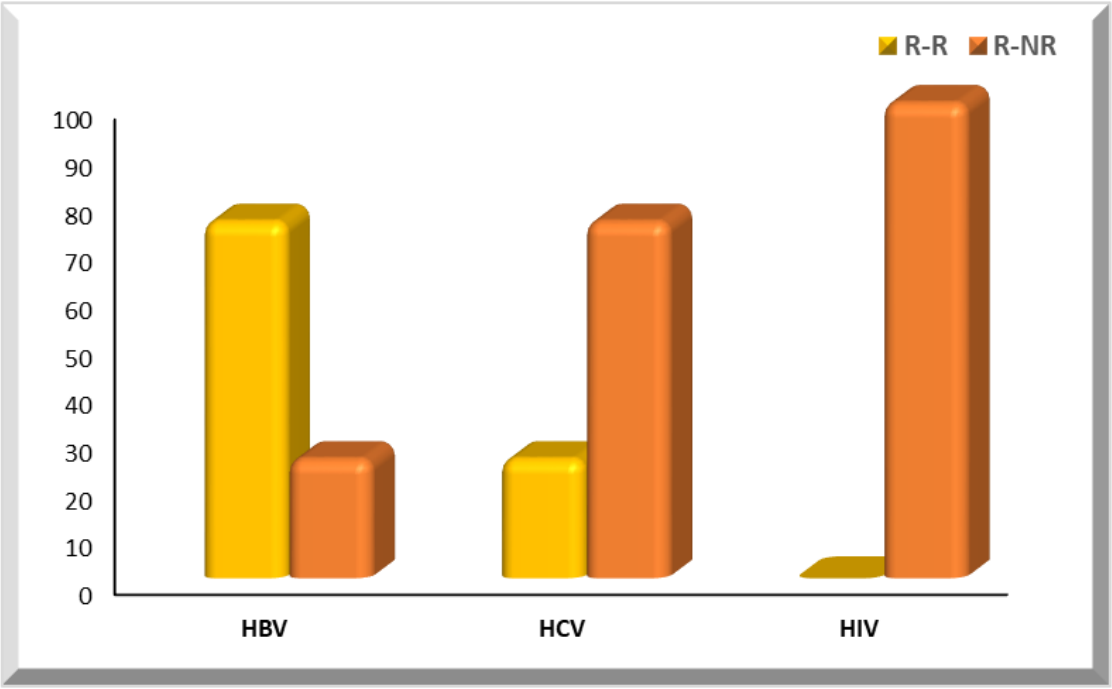

Figure 5: Bar chart showing Comparison between Cobas 6800 and Procleix Panther.

Table 8: Matching of two methods per viral marker.

Items		Group				Total		Chi-square		% of Match
		Cobas 6800		Procleix panther						
		N	%	N	%	N	%	X^2	P-value	
HBV	N/R-N/R	1	11.1%	1	11.1%	2	11.1%	0.292	0.864	89%
	R-R	6	66.7%	5	55.6%	11	61.1%			
	R-N/R	2	22.2%	3	33.3%	5	27.8%			
HCV	N/R-N/R	5	55.6%	5	55.6%	10	55.6%	0.000	1.000	100%
	R-R	1	11.1%	1	11.1%	2	11.1%			
	R-N/R	3	33.3%	3	33.3%	6	33.3%			
HIV	N/R-N/R	5	55.6%	5	55.6%	10	55.6%	0.000	1.000	100%
	R-N/R	4	44.4%	4	44.4%	8	44.4%			
Total	N/R-N/R	11	40.7%	11	40.7%	22	40.7%	0.130	0.937	96%
	R-R	7	25.9%	6	22.2%	13	24.1%			
	R-N/R	9	33.3%	10	37.0%	19	35.2%			

Of the 7 samples' RR for HBV DNA by both systems, all (100%) remained Procleix reactive when retested in pools of 4 but (94.4%) were Ultrioreactive when tested in a pool size of 8 (Table 6). One of the HBV NAT yield samples (HK5) was reactive in pools of both 4 and 8, but the other yield sample (HK1) was nonreactive in both pool sizes.
Of these 9 samples, 8 were nonreactive upon discriminatory testing on the Ampliscreen assays (HIV-1, HCV, and HBV) and then nonreactive when retested in duplicate on the Cobas 6800 (ID-NAT) and therefore confirmed as NAT-negative. The remaining sample was reactive on both the HBV Ampliscreen assay and the Procleix assay (LOD, 1.6 IU/mL).

DISCUSSION:

A high-throughput and high-sensitivity automated multiplex assay is needed to meet the testing requirements of large blood testing centers. In this report, we compare Cobas 6800 and Procleix panther, which simultaneously detects HBV DNA, HCV RNA, and HIV-1 RNA. Although one of the two assays Cobas 6800 has been evaluated previously, this study presents novel performance data for the second assay Procleix panther as well as the first "head-to-head" performance evaluation of these assays and their respective testing platforms.
The evaluation of the analytical sensitivity of the two assays in this study demonstrated comparable 95 percent detection limits for HIV-1 and HBV when testing by ID-NAT. However, the Cobas 6800 showed significantly better analytical sensitivity for the detection of HCV RNA (2 IU/mL vs. 6 IU/mL). Nevertheless, the results indicate that both assays are highly sensitive and when used in the ID-NAT format, or at the manufacturer's supported pool sizes of 4 and 8 (Procleix) or 6 (Cobas 6800), they would both meet the regulatory standard for NAT blood screening assays.

It is important to note, however, that the analytical sensitivity figures reported here are based on ID-NAT data from both systems and the manufacturer's recommended testing format for the Cobas 6800 in this study was pools of 6. The use of this pooled testing format would be expected to result in a proportionate decrease in the assay's analytical sensitivity, although this may not necessarily result in an equivalent decrease in its clinical sensitivity.

When comparing our results to other published data, the 95 percent detection limits reported here for Procleix assay are consistent with the results of the analytical sensitivity studies reported by the manufacturer for TIGRIS.20 In addition, our calculated 95 percent detection limits compare favorably with two other recently published studies (8, 18). In the first by Koppelman and colleagues,(8) the 95 percent detection limits reported for the Ultrio assay were 11.0 IU per mL for HBV, 4.6 IU per mL for HCV, and 26 IU per mL for HIV-1, compared with 12.2, 2.0, and 42.2 IU per mL, respectively, in this study. A similar level of agreement is evident in comparison with the second study by McCormick and coworkers (18). Caution is required, however, when comparing these estimates as different testing platforms and international standards for HIV-1 and HCV were used (19). In addition, the HIV-1 estimates reported in these two studies were based on the HIV-1 discriminatory assay, not the multiplex assay as used here (20, 21). In conclusion, efficient function of both techniques were elaborated, however, Procleix Ultrio was slightly superior to Cobas 6800 in few situations, although, new Cobas version is much updated and sensitive from old releases.

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Conflict of interest
There is no conflict of interest.

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