COMPARATIVE EVALUATION OF THREE AUTOMATED PLATFORMS FOR THE DETECTION OF HERPES SIMPLEX VIRUS IN CLINICAL SPECIMENS

345

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Abstract

Introduction: The use of real-time PCR is a sensitive and specific method for the detection of herpes simplex virus types 1 and 2 (HSV-1/2) in clinical specimens. Specimens are first processed to extract nucleic acids, followed by amplification and analysis. Historically, this has involved separate steps with manual intervention by laboratory technologists. Recently, automated platforms have been developed that can perform all of these functions on a single instrument, improving workflow and potentially reducing turn-around time. In this study, we compared three commercially-available systems; APTIMA HSV on the Panther (Hologic, San Diego, CA), Cobas® 4800 (Roche Molecular Systems Inc, Pleasanton, CA), and ARIES® (Luminex Corporation, Austin, TX) for the qualitative detection of HSV-1/2 in clinical samples.

Methods: Two hundred ninety-eight clinical specimens (genital [n=194], dermal [n=76], oral [n=18], respiratory [n=8], and ocular [n=2]) in viral transport media (VTM) were submitted for routine HSV-1/2 real-time PCR by a laboratory developed test (LDT). Testing by the LDT consists of 200 µL of sample being extracted on the MagNA Pure (Roche Diagnostics) followed by analysis of 5 µL using the Roche HSV-1/2 analyte specific reagents (ASR) on the LightCycler 2.0 (Roche). Following routine testing, samples in VTM were divided into aliquots of 200 µL, 500µL, and 1000 µL, which were tested for the presence of HSV-1/2 per manufacturer's instructions by the ARIES, APTIMA HSV, and Cobas HSV, respectively. Results were compared to a "consensus finding" defined as the result obtained from ≥ 3 of the 4 assays.

Results: Following testing of 298 specimens, the Cobas 4800 and ARIES assays demonstrated a sensitivity of 100% for HSV-1 (66/66) and HSV-2 (60/60). The APTIMA assays showed a sensitivity of 91% (60/66) for HSV-1 and 92% (55/60) for HSV-2. Specificities ranged from 95% (221/232) by the Cobas 4800 to 100% (232/232) by APTIMA for HSV-1, and 97% (230/238) by the Cobas 4800 to 100% (238/238) by APTIMA for HSV-2. Upon initial testing, a "consensus finding" was not observed for seven specimens, which were positive by the Cobas and ARIES assays but negative by the APTIMA and LDT. These seven discordant samples were tested further by the Simplexa HSV-1/2 assay (Focus Diagnostics, Cypress, CA) with 4 resulting as negative and 3 as positive (HSV-1 [n=2], HSV-2 [n=1]). Interestingly, 6 specimens were positive for both HSV-1 and HSV-2 by the Cobas. Among these six samples, 4 were positive for only HSV-2, 1 was positive for only HSV- 1, and 1 was completely negative by the other three assays.

Conclusions: The Cobas and ARIES assays showed 100% sensitivity for the detection of HSV-1/2 in clinical specimens. The APTIMA assays demonstrated a sensitivity of 91% for HSV-1 and 92% for HSV-2. Specificity was ≥ 95% by all 3 automated platforms. Turnaround time ranged from ~2 h (ARIES) to 2.5 h (APTIMA) with the least amount of hands-on time (~10 min) being required by the ARIES. The three commercial systems can perform all current functions on a single platform, thereby improving workflow and reducing turnaround time in the clinical laboratory.

Materials and Methods

- Specimens: 298 clinical specimens (genital [n=194], dermal [n=76], oral [n=18], respiratory [n=8], and ocular [n=2]) were submitted for routine HSV-1/2 real-time PCR by a laboratory developed test (LDT) (Figure 1 and 2 A)
- Following routine testing, samples were divided into aliquots of 200 µL, 500 µL and 1000 µL, which were tested for the presence of HSV-1/2 per manufacturer's instructions by the ARIES, Aptima, and Cobas systems, respectively (Figure 1 and 2 B, C, D)
- Results were compared to a "consensus finding" defined as the result obtained from ≥ 3 of the 4 assays

Figure 1. Study Design

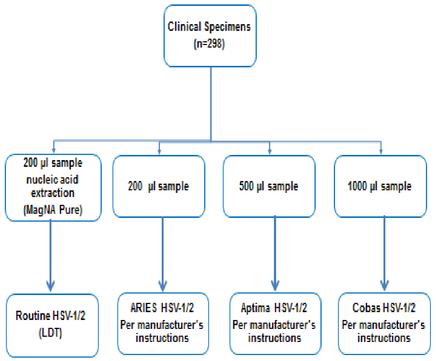

Figure 2. Assay Platforms

A. Roche LightCycler

B. Luminex ARIES

C. Hologic Panther

D. Roche Cobas 4800

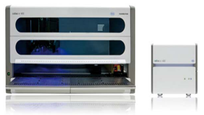

Table 1: Comparison of HSV-1 Assays

	No. of samples showing a consensus result of:				
Analyte and Assay	Positive	Negative	Non-typeable	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
LDT HSV-1					
Positive	61	1	10	100 (92.9-100)	99.6 (97.3-100)
Negative	0	226			
ARIES HSV-1					
Positive	66	3		100 (93.4-100)	98.7 (96.1-100)
Negative	0	229			
Aptima HSV-1					
Positive	60	0		90.9 (81.2-96.1)	100 (98.0-100)
Negative	6	232			
Cobas HSV-1					
Positive	66	11		100 (93.4-100)	95.3 (91.6-97.4)
Negative	0	221			

Table 2: Comparison of HSV-2 Assays

	No. of samples showing a consensus result of:				
Analyte and Assay	Positive	Negative	Non-typeable	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
LDT HSV-2					
Positive	54	0	10	98.2 (89.5-100)	100 (98.1-100)
Negative	1	233			
ARIES HSV-2					
Positive	60	5		100 (92.8-100)	97.9 (95.0-99.2)
Negative	0	233			
Aptima HSV-2					
Positive	55	0		91.7 (81.5-96.8)	100 (98.1-100)
Negative	5	238			
Cobas HSV-2					
Positive	60	8		100 (92.8-100)	96.6 (93.4-98.4)
Negative	0	230			

Table 3: Time Comparison of the Three Commercial HSV Assays

	Aptima	Cobas	ARIES
# Samples/Run	22[a]	22[a]	12[b]
Daily Maintenance	10-15 Min	10-15 Min	4.17 Min
Sample Prep	8 Min	8 Min	0 Min
Reagent Prep	40 Min	30 Min	1.33 Min
Run Loading	5 Min	15 Min	4.33 Min
Run Time	180 Min	180 Min	114 Min
Total Time	248 Min	248 Min	123.8 Min

[a] Timing data were based on a run of 22 samples; The maximum throughput is 96 samples/run.
[b] Timing data were based on a run of 12 samples; The maximum throughput is 12 samples/run.

Conclusions

1. The Cobas and ARIES assays showed 100% sensitivity for the detection of HSV-1/2 in clinical specimens.
2. The APTIMA demonstrated lower sensitivities for HSV-1 (90.9%) and HSV-2 (91.7%).
3. Specificities ranged from 95% by the Cobas to 100% by the APTIMA assays.
4. Turnaround time ranged from ~2 h (ARIES) to 2.5h (APTIMA) with the least amount of hands-on time (~10 min) being required by the ARIES.

References

1. Cui M, et al. 2014. Clinical performance of Roche cobas 4800 HPV test. *J Clin Microbiol*. June; 52 (6):2210-2211.
2. Ratnam S, et al. 2014. Workflow and maintenance characteristics of five automated laboratory instruments for the diagnosis of sexually transmitted infections. *J Clin Microbiol*. July; 52 (7):2299-2304.