COMPARISON OF 3 MOLECULAR ASSAYS FOR THE DETECTION OF HSV 1 AND 2 FROM GENITAL AND NON-GENITAL LESION SPECIMENS

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Abstract (revised)

Background: Herpes simplex virus types I and II (HSV-1, HSV-2) are enveloped DNA viruses belonging to the *Herpesviridae* family. HSV-1 is generally associated with non-genital contact such as oral secretions and causes eye, oropharynx and CNS infections. HSV-2 is most commonly associated with genital contact with lesions and can produce asymptomatic or symptomatic infections. Detection of HSV Types 1 and 2 has traditionally been performed by cell culture with immunofluorescent typing of HSV-1 and 2. To reduce turnaround time, culture is being replaced by molecular methods in clinical laboratories. Several FDA-approved tests are available that may differ in analytic performance. This study compared the performance characteristics of 3 molecular assays (Focus Simplexa™ HSV 1&2 Direct, Roche cobas ® HSV1&2 and the BD ProbeTec™ HSV 1&2 Qx) using clinical samples from genital and non-genital (eye, oral and skin) sites submitted in M4 VTM.

Methods: 141 specimens (70 genital and 71 non-genital) for herpes virus molecular testing were split and tested on all three platforms within a 24 hour period. Accuracy was assessed compared to a patient infected standard based on consensus between two or more assay results. Discrepant analysis was performed by Focus Diagnostics R&D using alternate target PCR and sequencing. Analytic sensitivity was assessed using serial dilutions of quantified stock organisms tested daily in triplicate for 3 days on all platforms. Precision of Ct values for positive controls (including internal control) was assessed. A time comparison of the 3 assays was performed as well.

Results: Pairwise comparison of all assays demonstrated >95% agreement (weighted kappa values >0.89) which was characterized as 'very good' (Prism 6, GraphPad). Focus detected 5 additional positive samples compared to the patient infected standard (4 HSV-1, 1 HSV-2) and BD had 1 negative sample compared to the patient infected standard (HSV-1). Discrepant analysis confirmed 4/5 Focus positives (3 HSV-1, 1 HSV-2) and agreed with the BD negative. LOD values, calculated by Probit analysis (SPSS 23, IBM), ranged from 2-105 cps/mL for HSV-1 and 5-27 cps/mL for HSV-2. Analytical sensitivity was greatest for the Roche assay for both HSV-1 and HSV-2. BD had a lower HSV-1 LOD, but higher HSV-2 LOD than Focus. Precision based on between run coefficients of variance were all <2.25%. Time comparison results (based on a run of 8 specimens/controls) demonstrated BD (215 min), Roche (185 min) and Focus (72 min).

Conclusions: Accuracy comparisons demonstrated good agreement between the 3 assays. All assays had excellent analytical sensitivity and precision, although Focus detected HSV in additional patient samples. The BD Viper had the longest time to result, followed by Roche and Focus. Choice of assay for a clinical laboratory can be made based on space, cost, and specimen volume with the expectation of similar analytical performance using genital and non-genital specimens.

Methods

Both genital (N=70) and non-genital (N=71; eye-28, oral-5 and skin-38) specimens submitted for HSV-1 and HSV-2 testing with the BD ProbeTec™ assay on the Viper platform were aliquotted and tested with the Focus Simplexa™ HSV 1&2 Direct and Cobas HSV 1&2 assays within 24 hours of receipt. After vortexing, the following M4 volumes were pipetted: 500µL into a BD Qx Swab Diluent tube (1.5mL) for testing on the Viper system, 400µL into a Copan Mswab preservation media tube (1.6mL) for Cobas testing and 50µL into an empty 1.5mL micro-tube for Focus testing. Agreement was assessed using Cohen's kappa (GraphPad Prism 6.0). Serial dilutions of stock HSV-1 and HSV-2 (ZeptoMetrix, Buffalo, NY) were prepared in M4 to assess limit of detection. Probit analysis was performed (IBM SPSS23 Statistics) to determine LOD concentrations at 95% confidence. Control data from patient and serial dilution runs were used to compute precision. Because the BD Viper does not provide a result that is at a consistently quantifiable level, its precision was not calculated.

Each assay was performed according to the manufacturer's instrument protocol. BD Viper requires an off-board heating step of 15 minutes, followed by a 15 minute cooling before loading the instrument to run. The Cobas x480 was loaded with all reagents and specimens to begin extraction and subsequent amplification plate preparation. When complete, the amplification plate was transferred to the x480z analyzer for real-time PCR. The Simplexa assay was set up by adding 50µL of thawed master mix to the appropriate well of a wedge on the 8-well Direct Amplification Disc (DAD), followed by addition of 50µL of M4 specimen. The DAD was loaded onto the 3M Integrated Cycler and analysis started.

Results

Tables 1-3: Agreement with Patient Infected Standard for Genital Specimens

Focus Simplexa™

Table 1: Genital	HSV-1 Positive	HSV-2 Positive	HSV Negative	Total
Simplexa™ HSV-1 Positive	9	0	3	12
Simplexa™ HSV-2 Positive	0	14	1	15
Simplexa™ HSV Negative	0	0	43	43
Total	9	14	47	70

Agreement 93.4%
Kappa=0.891; SE of kappa=0.053
95% CI=0.787-0.994
Strength of agreement 'very good'
Weighted kappa=0.860, 'very good'

Roche Cobas®

Table 2: Genital	HSV-1 Positive	HSV-2 Positive	HSV Negative	Total
Cobas® HSV-1 Positive	8	0	0	8
Cobas® HSV-2 Positive	0	14	0	14
Cobas® HSV Negative	0	0	48	48
Total	8	14	48	70

Agreement 100%
Kappa=1.000; SE of kappa=0.000
95% CI=1.000-1.000
Strength of agreement 'perfect'
Weighted kappa=1.000, 'perfect'

BD ProbeTec™

Table 3: Genital	HSV-1 Positive	HSV-2 Positive	HSV Negative	Total
ProbeTec™ HSV-1 Positive	8	0	0	8
ProbeTec™ HSV-2 Positive	0	14	0	14
ProbeTec™ HSV Negative	0	0	48	48
Total	8	14	48	70

Agreement 100%
Kappa=1.000; SE of kappa=0.000
95% CI=1.000-1.000
Strength of agreement 'perfect'
Weighted kappa=1.000, 'perfect'

Tables 4-6: Agreement with Patient Infected Status for Non-Genital Specimens

Table 4: Non-Genital	HSV-1 Positive	HSV-2 Positive	HSV Negative	Total
Simplexa™ HSV-1 Positive	18	0	1	19
Simplexa™ HSV-2 Positive	0	2	0	2
Simplexa™ HSV Negative	0	0	50	50
Total	18	2	51	71

Agreement 98.6%
Kappa=0.967; SE of kappa=0.033
95% CI=0.902-1.000
Strength of agreement 'very good'
Weighted kappa=0.965, 'very good'

Table 5: Non-Genital	HSV-1 Positive	HSV-2 Positive	HSV Negative	Total
Cobas® HSV-1 Positive	18	0	0	18
Cobas® HSV-2 Positive	0	2	0	2
Cobas® HSV Negative	0	0	51	51
Total	18	2	51	71

Agreement 100%
Kappa=1.000; SE of kappa=0.000
95% CI=1.000-1.000
Strength of agreement 'perfect'
Weighted kappa=1.000, 'perfect'

Table 6: Non-Genital	HSV-1 Positive	HSV-2 Positive	HSV Negative	Total
ProbeTec™ HSV-1 Positive	16	0	0	16
ProbeTec™ HSV-2 Positive	0	2	0	2
ProbeTec™ HSV Negative	1	0	52	53
Total	17	2	52	71

Agreement 98.6%
Kappa=0.965; SE of kappa=0.035
95% CI=0.896-1.000
Strength of agreement 'very good'
Weighted kappa=0.962, 'very good'

Tables 7-9: Agreement with Patient Infected Standard for Overall (Genital and Non-Genital) Specimens

Table 7: Genital/Non-Genital	HSV-1 Positive	HSV-2 Positive	HSV Negative	Total
Simplexa™ HSV-1 Positive	27	0	4	31
Simplexa™ HSV-2 Positive	0	16	1	17
Simplexa™ HSV Negative	0	0	93	93
Total	27	16	98	141

Agreement 96.5%
Kappa=0.927; SE of kappa=0.032
95% CI=0.864-0.990
Strength of agreement 'very good'
Weighted kappa=0.917, 'very good'

Table 8: Genital/Non-Genital	HSV-1 Positive	HSV-2 Positive	HSV Negative	Total
Cobas® HSV-1 Positive	26	0	0	26
Cobas® HSV-2 Positive	0	16	0	16
Cobas® HSV Negative	0	0	99	99
Total	26	16	99	141

Agreement 100%
Kappa=1.000; SE of kappa=0.000
95% CI=1.000-1.000
Strength of agreement 'perfect'
Weighted kappa=1.000, 'perfect'

Table 9: Genital/Non-Genital	HSV-1 Positive	HSV-2 Positive	HSV Negative	Total
ProbeTec™ HSV-1 Positive	24	0	0	24
ProbeTec™ HSV-2 Positive	0	16	0	16
ProbeTec™ HSV Negative	1	0	100	101
Total	25	16	100	141

Agreement 99.3%
Kappa=0.984; SE of kappa=0.016
95% CI=0.953-1.000
Strength of agreement 'very good'
Weighted kappa=0.980, 'very good'

Discrepant Analysis Results

Specimen	Site	Viper Result	Simplexa Result	Cobas Result	PCR with bi-directional sequencing	Interpretation
6551	Vaginal	Negative	HSV-1	Negative	Human HSV-1	HSV-1
5475	Mouth	Negative	HSV-1	HSV-1	Not Detected	Negative
0456	Vaginal	Negative	HSV-1	Negative	Not Detected	Negative
0435	Mouth	Negative	HSV-1	Negative	Human HSV-1	HSV-1
6145	Genital lesion	Negative	HSV-1	Negative	Human HSV-1	HSV-1
0312	Genital lesion	Negative	HSV-2	Negative	Human HSV-2	HSV-2

Limit of Detection

LOD	HSV-1 (cps/mL)	95% CI	HSV-2 (cps/mL)	95% CI
Simplexa™ HSV	105.8	76.97-208.23	24.45	18.41-50.97
Cobas® HSV	1.75	1.31-2.82	4.42	3.28-8.49
ProbeTec™ HSV	15.14	11.10-26.31	26.71	17.03-88.41

Precision

	HSV-1 Positive Control	HSV-2 Positive Control	IC
Cobas®	1.35	1.42	1.18
Simplexa™	1.09	0.71	2.21

Instrument Comparison

Turnaround Time: 215 minutes

Turnaround Time: 185 minutes

Turnaround Time: 72 minutes

# Samples	BD ProbeTec on Viper	Roche Cobas 4800	Focus Integrated Cycler-1 cycler	Focus Integrated Cycler-2 cyclers	Focus Integrated Cycler-3 cyclers
6+ controls	215 min	185 min	72 min	72 min	72 min
14+controls	215 min	185 min	144 min	72 min	72 min
22+ controls	215 min	185 min	216 min	144 min	72 min

Conclusions

- Good agreement between all 3 assays
- Both genital (IVD) and non-genital specimens perform well
- Excellent sensitivity and precision
- Viper longest time to first result, followed by Cobas and Simplexa; time to last result depends on test volume
- Comparable clinical performance allows space, cost and specimen volume to factor into platform decision