Detection of HSV in Skin and Genital Lesions: Comparison of Culture vs. AmpliVue HSV 1+2

Christian Renaud MD MSc FRCPC

Virology Laboratory

CHU Sainte-Justine, Montréal QC

November 19th 2014

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Université de Montréal

Disclosure

- For the current evaluation kits were supplied by Quidel Corporation [San Diego, CA, USA]

- No other financial support was received in order to carry out this evaluation

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Université de Montréal

Molecular detection of mucocutaneous HSV lesions

- Canadian and Québecois Guidelines on sexually transmitted infections mention superiority of molecular methods over culture due to higher sensitivity and excellent specificity
- Both culture and molecular methods are recommended as diagnostic tools for sexually transmitted infections
- Why go molecular?
  - Faster
  - Higher sensitivity
  - Easier to perform
  - Less hands on time
  - Easier sample transport
- Drawbacks?
  - Cost
  - Risk of contamination of CSF if performed simultaneously with high viral load lesions

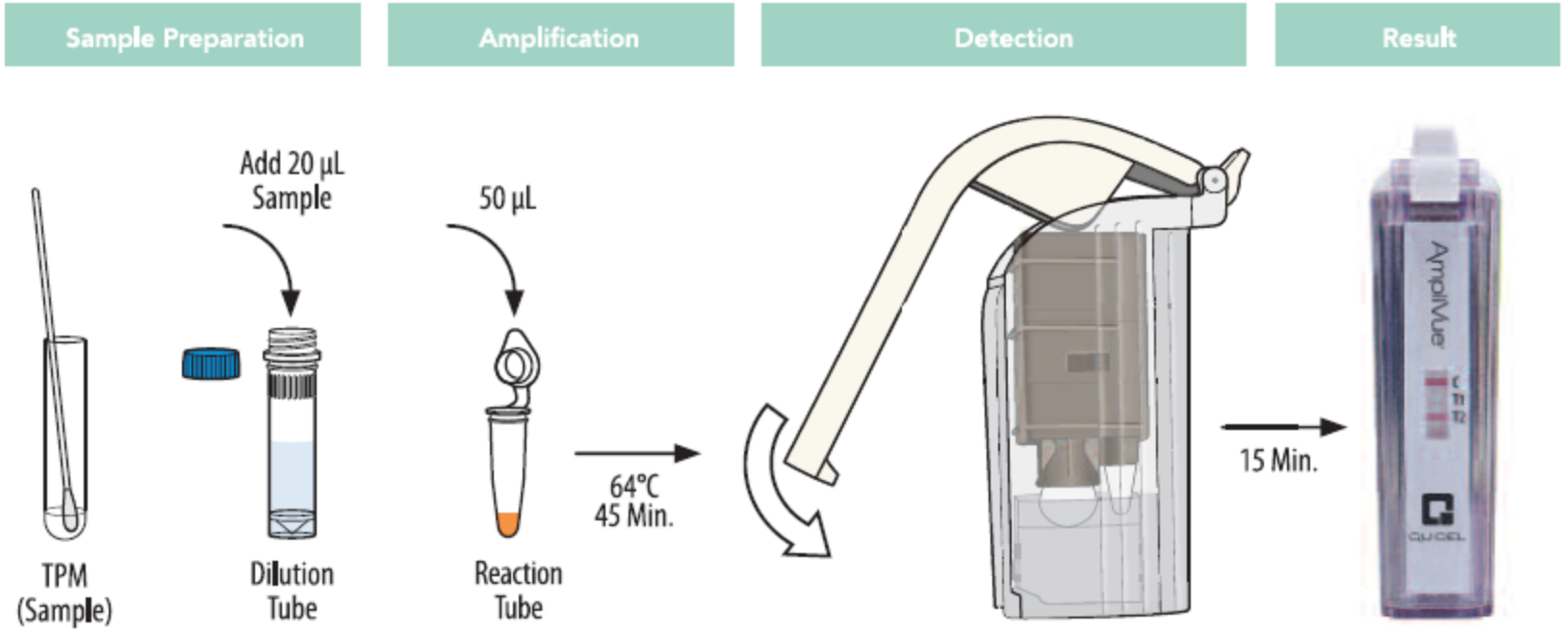
CHU Sainte-Justine

- CHU Sainte-Justine Virology Laboratory performs 8000 viral cultures/year including 3000 cultures for HSV
- 5 days/week HSV in-house PCR for CSF
- 2 ABI7500 thermocyclers used almost to full capacity
- Goals:
  - Faster turn around time
  - Reduced hands-on time
  - No contamination issues
  - No budget for new instrument

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Université de Montréal

Evaluation of AmpliVue HSV 1+2



- Molecular Isothermal Amplification
- No thermocycler required
- Closed system

QUIDEL

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Université de Montréal

AmpliVue – Anytime, Anywhere Molecular Assays

Assay Mechanism

- Multiplexing capabilities
- Asymmetrical amplification – generates single-stranded amplicons
- Detection probes bind to amplicons, the hybrid complex is captured and visualized on the test and control lines

Assay Features

- Internal process control included to ensure DNA amplification occurs
- Lyophilized Master Mix packaged in individual amplification tubes for ease of use and extended shelf life

QUIDEL

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Université de Montréal

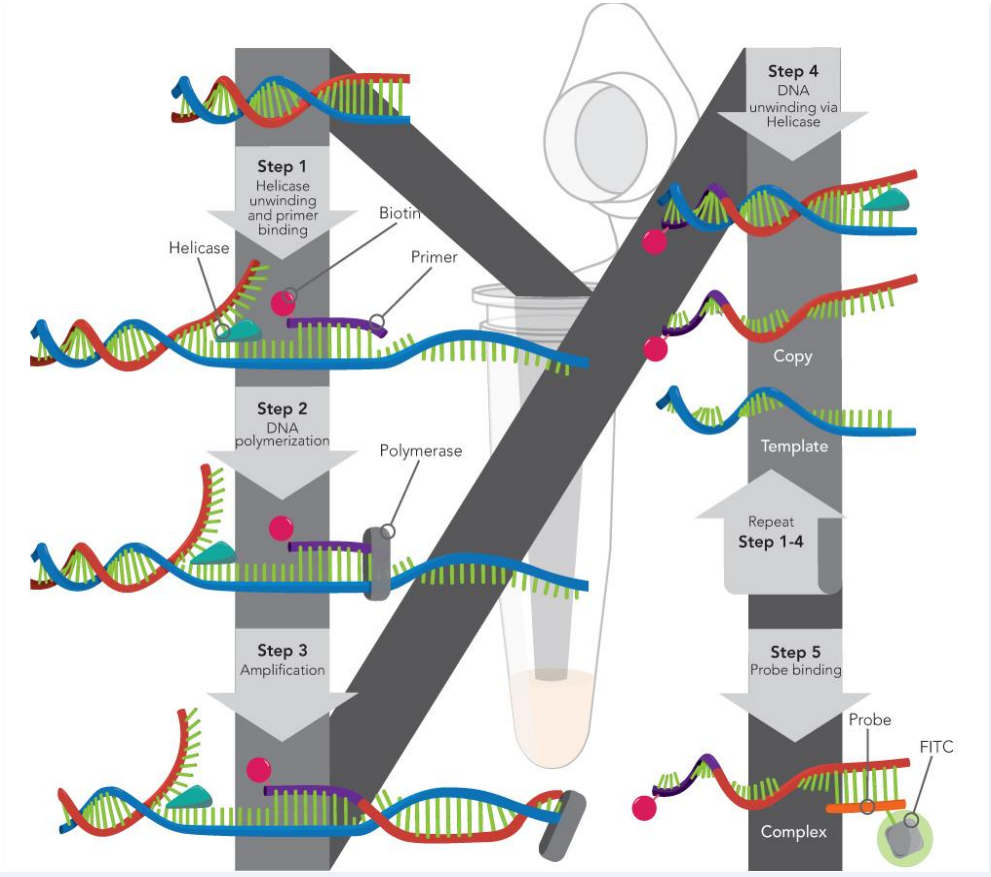
Helicase Dependent Amplification

Step 1:
The Helicase enzyme breaks the Hydrogen bonds between the nucleotide base pairs “unwinding” or “unzipping” the DNA at 64° C.

Step 2 and 3:
“Unwinding” exposes the single strand DNA to the Biotin-Labeled Primer and Polymerase that will extend the copy.

Step 4:
Once replication is complete, the Helicase performs the procedure again, leaving an original Template DNA and a Biotin-Labeled “copy.”

Step 5:
The Capture Probe attaches to the Biotin-Labeled DNA copy creating a Biotin/Capture Probe (FITC, DIG complex).



QUIDEL

How AmpliVue cartridge works – universal strip

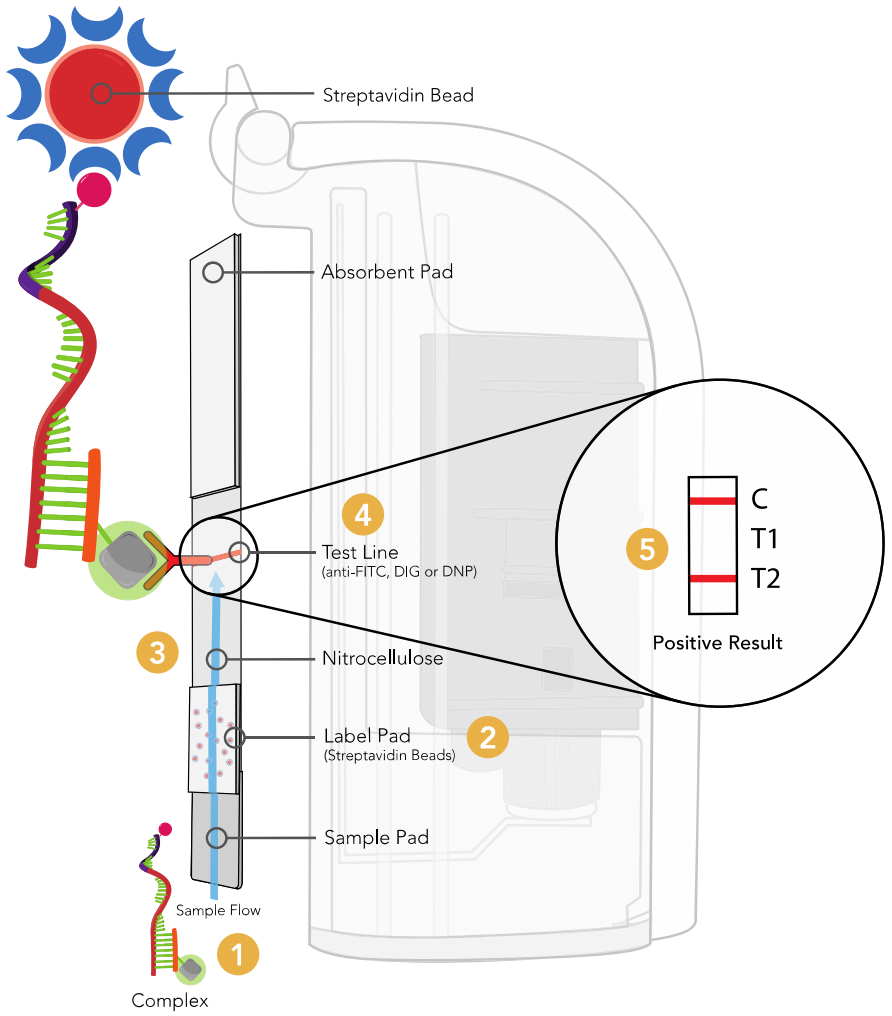
① 60 minutes of Iso-Thermal Amplification produces many Biotin/FITC Complexes in the amplification tube.

② With the cartridge closed, a buffer will carry the Biotin/Capture Probe Complexes across the Label Pad where Streptavidin coated Red beads adhere to the Biotin.

③ The Complex travels up the strip, being drawn by the Absorbent Pad with the Streptavidin coated Red beads to the Test Lines which contains Anti-FITC or Anti-DIG.

④ Test lines will "Capture" the Complex and Biotin/Streptavidin beads adhering to the Complex at the FITC-Labeled Probes and DIG-Labeled Probes.

⑤ This process will result in a Red Line appearing at the T2 and/or T1 (Test) line and indicate a Positive result.



QUIDEL

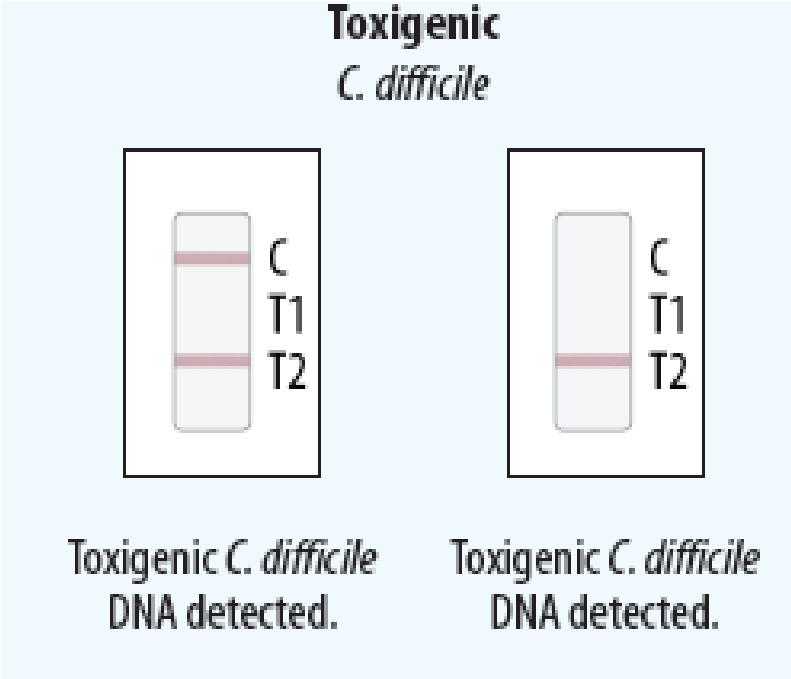
Lines are clearly visible near the limit of detection

Replicate 1 Replicate 2 Replicate 3

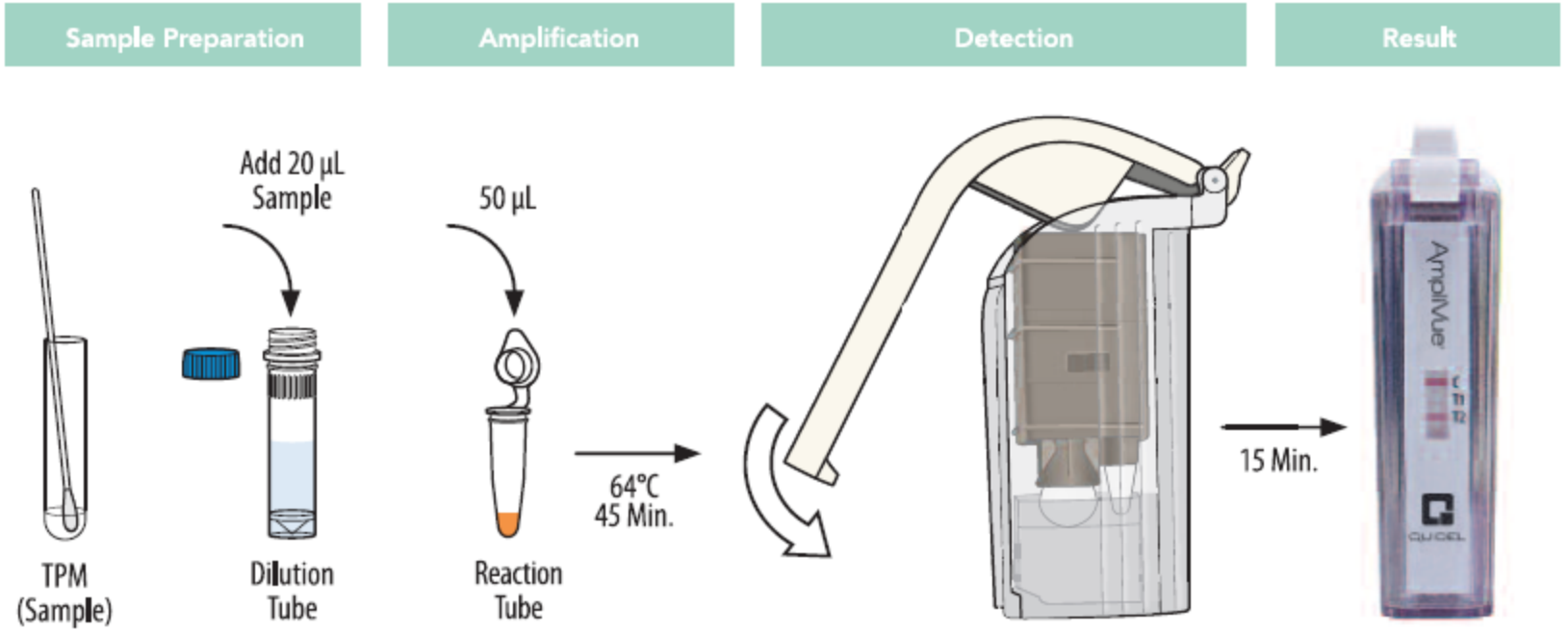
QUIDEL

Internal control

- The internal control reflects if amplification and detection occurred.
- Generating the test line but not the control can be read as positive because the target material was amplified and detected.
  - This may occur because the sample itself has an overabundance of target DNA which creates competition for the reagents in the master mix.
- Thus the T2 line is well established and not enough reagent was available to amplify and develop a Control line.
- The internal control is a process control and indicates the process of HDA was successful.



Fast and easy – results in 1 hour



- Lysis tube and Lysis heat block is not needed
- Amplification is only 45 minutes, not 1 hour

QUIDEL

AmpliVue HSV 1+2

HSV-1 Cutaneous Lesions			
Combines Sites - Cutaneous Lesion (N=340)	ELVIS viral culture **Reference Method**		
AmpliVue HSV 1+2	POS	NEG	Total
POS	30	9	39
NEG	0	301	301
Total	30	310	340
		95% CI	
Sensitivity	100%	88.60%	100%
Specificity	97.10%	94.60%	98.50%

HSV-1 Mucocutaneous Lesions			
Combined Sites – Mucocutaneous Lesions (N=785)	ELVIS viral culture **Reference Method**		
AmpliVue HSV 1+2 Assay	POS	NEG	Total
POS	149	22	171
NEG	8	606	614
Total	157	628	785
		95% CI	
Sensitivity	94.90%	90.30%	97.40%
Specificity	96.50%	94.80%	97.70%

*One thousand three hundred fifty-five (1355) specimens from symptomatic male and female patients were tested. The swab specimens have been categorized as cutaneous (skin lesion, genital – penis), or mucocutaneous (anorectal, genital – vaginal/cervical, nares, ocular, oral lesion and urethral). Nineteen (19) tests were considered invalid and were removed from the performance analysis.

**The reference ELVIS viral culture used in this study was unable to detect co-infected specimens. As a result, if a specimen was positive for HSV-2 it was removed from the calculation of HSV-1 clinical performance. Two hundred eleven (211) specimens identified as HSV-2 positive by ELVIS have been removed from the initial one thousand three hundred thirty-six (1336) specimens. The data below is for the remaining one thousand one hundred twenty-five (1125) specimens.

QUIDEL

AmpliVue HSV 1+2

HSV-2 Cutaneous Lesions			
Combines Sites - Cutaneous Lesion (N=399)	ELVIS viral culture **Reference Method**		
AmpliVue HSV 1+2	POS	NEG	Total
POS	58	15	73
NEG	1	325	326
Total	59	340	399
		95% CI	
Sensitivity	98.30%	91.00%	99.70%
Specificity	95.60%	92.80%	97.30%

HSV-2 Mucocutaneous Lesions			
Combines Sites - Mucocutaneous Lesion (N=937)	ELVIS viral culture **Reference Method**		
AmpliVue HSV 1+2	POS	NEG	Total
POS	148	33	181
NEG	4	752	756
Total	152	785	937
		95% CI	
Sensitivity	97.40%	93.40%	99.00%
Specificity	95.80%	94.20%	97.00%

*One thousand three hundred fifty-five (1355) specimens from symptomatic male and female patients were tested. The swab specimens have been categorized as cutaneous (skin lesion, genital – penis), or mucocutaneous (anorectal, genital – vaginal/cervical, nares, ocular, oral lesion and urethral). Nineteen (19) tests were considered invalid and were removed from the performance analysis.

**The reference ELVIS viral culture used in this study was unable to detect co-infected specimens. As a result, if a specimen was positive for HSV-2 it was removed from the calculation of HSV-1 clinical performance. Two hundred eleven (211) specimens identified as HSV-2 positive by ELVIS have been removed from the initial one thousand three hundred thirty-six (1336) specimens. The data below is for the remaining one thousand one hundred twenty-five (1125) specimens.

QUIDEL

Evaluation of AmpliVue HSV 1+2 in comparison to viral culture

- Testing of 323 consecutive mucocutaneous samples with viral culture and AmpliVue (reflex testing performed with an in-house PCR)
- Samples were received in 3 mL UTM
- Viral Culture set up (everyday of the week: 7/7)
  - VERO cell in-house
  - MRHF commercial (Diagnostic Hybrids/Quidel Corporation)
  - Kept for 5 days
  - DFA confirmation (PathoDx) via CPE
- Aliquot for Amplivue (4°C) performed 5/7
- Aliquot for in-house PCR (-20°C) and batch testing

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant
Université de Montréal

Sample Types

113	lesions (lips, buttocks, labia, tight, cutaneous, scrotum, foreskin…)
91	vulva
27	penis
25	throat/mouth
24	vagina
12	ano-rectal
10	unspecified
6	genital (unspecified)
5	eye/conjonctiva
4	tongue
4	cervix
2	urethra

HSV-1 Calculations

Sensitivity	$\frac{a}{a+c}$	= **98.65 %**	95% CI: **92.67 %** to **99.77 %**
Specificity	$\frac{d}{b+d}$	= **96.67 %**	95% CI: **93.54 %** to **98.55 %**
Positive Likelihood Ratio	$\frac{\text{Sensitivity}}{100 - \text{Specificity}}$	= **29.59**	95% CI: **14.97** to **58.52**
Negative Likelihood Ratio	$\frac{100 - \text{Sensitivity}}{\text{Specificity}}$	= **0.01**	95% CI: **0.00** to **0.10**
Disease prevalence	$\frac{a+c}{a+b+c+d}$	= **23.57 % (*)**	95% CI: **18.98 %** to **28.66 %**
Positive Predictive Value	$\frac{a}{a+b}$	= **90.12 % (*)**	95% CI: **81.46 %** to **95.63 %**
Negative Predictive Value	$\frac{d}{c+d}$	= **99.57 % (*)**	95% CI: **97.62 %** to **99.93 %**

*9 total invalids (2.8%): All calculations excluding invalid results. Invalid results most likely due to user processing error or assay inhibition.

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant
Université de Montréal

HSV-1

		Culture: Pos	Culture: Neg
Amplivue	Pos	73	8
	Neg/invalid	3	239*

9 invalid (2.8%)*

Kappa= 0.923 SE of kappa = 0.025; 95% confidence interval: From 0.873 to 0.972 The strength of agreement is considered to be « very good. »

OPA (overall percentage agreement) = 97.1
PPA (positive percentage agreement) = 98.6
NPA (negative percentage agreement)=96.6

*9 total invalids (2.8%): All calculations excluding invalid results. Invalid results most likely due to user processing error or assay inhibition.

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Université de Montréal

HSV-2 Calculations

Sensitivity	$\frac{a}{a+c}$	= **100.00 %**	95% CI: **90.17 %** to **100.00 %**
Specificity	$\frac{d}{b+d}$	= **98.20 %**	95% CI: **95.85 %** to **99.41 %**
Positive Likelihood Ratio	$\frac{\text{Sensitivity}}{100 - \text{Specificity}}$	= **55.60**	95% CI: **23.33** to **132.53**
Negative Likelihood Ratio	$\frac{100 - \text{Sensitivity}}{\text{Specificity}}$	= **0.00**	
Disease prevalence	$\frac{a+c}{a+b+c+d}$	= **11.46 % (*)**	95% CI: **8.16 %** to **15.52 %**
Positive Predictive Value	$\frac{a}{a+b}$	= **87.80 % (*)**	95% CI: **73.78 %** to **95.87 %**
Negative Predictive Value	$\frac{d}{c+d}$	= **100.00 % (*)**	95% CI: **98.64 %** to **100.00 %**

*9 total invalids (2.8%): All calculations excluding invalid results. Invalid results most likely due to user processing error or assay inhibition.

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant
Université de Montréal

HSV-2

AmpliVue \ Culture	Pos	Neg
Pos	36	5
Neg/Invalid	0	282

9 invalids (2.8%)*

Kappa= 0.926 SE of kappa = 0.033; 95% confidence interval: from 0.862 to 0.990. The strength of agreement is considered to be « very good. »

OPA (overall percentage agreement) = 98.4
PPA (positive percentage agreement) = 100
NPA (negative percentage agreement) = 98.3

*9 total invalids (2.8%): All calculations excluding invalid results. Invalid results most likely due to user processing error or assay inhibition.

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Université de Montréal

HSV 1+2 Calculations

Sensitivity	$\frac{a}{a+c}$	= **99.09 %**	95% CI: **95.02 %** to **99.85 %**
Specificity	$\frac{d}{b+d}$	= **93.63 %**	95% CI: **89.35 %** to **96.56 %**
Positive Likelihood Ratio	$\frac{\text{Sensitivity}}{100 - \text{Specificity}}$	= **15.55**	95% CI: **9.19** to **26.32**
Negative Likelihood Ratio	$\frac{100 - \text{Sensitivity}}{\text{Specificity}}$	= **0.01**	95% CI: **0.00** to **0.07**
Disease prevalence	$\frac{a+c}{a+b+c+d}$	= **35.03 % (*)**	95% CI: **29.76 %** to **40.59 %**
Positive Predictive Value	$\frac{a}{a+b}$	= **89.34 % (*)**	95% CI: **82.46 %** to **94.20 %**
Negative Predictive Value	$\frac{d}{c+d}$	= **99.48 % (*)**	95% CI: **97.12 %** to **99.91 %**

*9 total invalids (2.8%): All calculations excluding invalid results. Invalid results most likely due to user processing error or assay inhibition.

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Université de Montréal

HSV 1 + 2

AmpliVue \ Culture	Pos	Neg
Pos	109	13
Neg/Invalid	3	198

Potentially 4% more positives

9 invalids (2.8%)*

Kappa= 0.904 SE of kappa = 0.025 ; 95% confidence interval: From 0.856 to 0.953 The strength of agreement is considered to be « very good. »

OPA (overall percentage agreement) = 95.5
PPA (positive percentage agreement) = 99.1
NPA (negative percentage agreeement) = 93.6

*9 total invalids (2.8%): All calculations excluding invalid results. Invalid results most likely due to user processing error or assay inhibition.

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant
Université de Montréal

Conclusion

- Amplivue HSV 1+2 has « very good » agreement with viral culture *(VERO+MRHF for 5 days)* for each target separately and both combined targets
- Sensitivity/Specificity is :
  - 96%/96% for HSV-1
  - 100%/98.2% for HSV-2
  - 97.3%/93.8% for HSV 1+2
- 2.8% of invalid results (not any specific sample types, mostlikely processing errors)
- Comparable sensitivity is expected; potentially 4% more positives
- Very easy to perform (batch twice a day)

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant
Université de Montréal