Genital Zoster Infections - An Unexpected Finding Using a Molecular Assay

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Disclosures

Accelerate Diagnostics
Bruker Daltonics
Cepheid
EntericBio Diagnostics, LTD
Great Basin
iCubate
Meridian Bioscience

Micronics
Intelligent Molecular Diagnostics
Nanosphere
Phthisus
Quidel
Seegene
Objectives

• Characteristics of HSV and VZV infections
• Conventional diagnostic methods
• Molecular HSV/VZV detection assay
• Clinical trial study results
• The Laboratory Alliance VZV experience
• Evidence for VZV genital infection
• Impact on patient care
Biology

- Eight known human herpesviruses
- Divided into 3 major groups (alpha, beta, gamma)
- Alpha human herpes viruses include:
  - Herpes simplex type 1
  - Herpes simplex type 2
  - Varicella zoster virus

(Other HSVs: CMV, HHV-6, HHV-7, EBV, and HSV-8)
Characteristics of HSV and VZV Infections

- Cause cutaneous and mucocutaneous infections (VZV causes chickenpox)
- Highly contagious during symptomatic stage of disease
- Symptoms resolve resulting in dormant infection
Characteristics of HSV and VZV Infections

• Reactivation of infection
  - HSV at the same site of primary infection
  - VZV cutaneous lesions along dermatomes (varicella zoster, zoster, herpes zoster, shingles)

• Resolution of symptoms and return to dormancy
Types of HSV Infections

• Herpes genitalis (genital herpes)
• Herpes labialis (cold sores)
• Herpes gingivostomatitis
• Herpetic Whitlow
• Herpes keratitis
• Herpes encephalitis
• Herpes meningitis
Characteristics of Reactivation HSV Infection

• Generally reoccurs at the same or nearby anatomic site
• Reactivation HSV disease has the same clinical appearance as primary HSV infection
Characteristics of Reactivation Varicella Zoster Virus Infection

• VZV generally reactivates at an anatomic site along dermatomes

• Zoster typically has a markedly different clinical presentation than primary chicken pox and can be readily distinguished clinically by an experienced clinician

• Zoster typically has a markedly different clinical presentation than reactivation HSV
Comparison of HSV/VZV Cultural Methods

HSV ID and D3 Typing Test>
Shell Vial>Roll Tube
The doctor will see you when?

24 days
Average wait time to get a doctor’s appointment, a 30% increase since 2014.

NOTE: Includes cardiologists, dermatologists, ob/gyns, orthopedic surgeons, family practice doctors.
SOURCE: Merritt Hawkins survey of 1,414 medical offices in 15 major metro areas.
MICHAEL B. SMITH AND VERONICA BRAVO, USA TODAY.
PCR HSV 1+2/VZV Workflow

Kit Contents

12 tubes @ 1.8 ml/tube
- Process Buffer
12 vials
- 8x Master Mix
1 tube @ 1.9 ml/tube
- Rehydration Solution

Sample Preparation

1. Remove 100 µL liquid of specimen (user supplied).
2. Add 100 µL of specimen to a 1.5 mL centrifuge tube (user supplied).
3. Heat at 60°C for 5 min. (heat block user supplied).
4. Add 25 µL of Process Buffer, within 60 min. (contains process control material).

Amplification and Detection Procedure

5. Open Master Mix slowly. Add 135 µL of Rehydration Solution. Replace cap and allow it to sit for 1-2 minutes. Gently pipette rehydrated Master Mix up and down 2-3 x. Avoid creating bubbles.
6. Add 15 µL of Master Mix to each well.
7. Add 5 µL of sample with process control to plate well and seal the plate. Centrifuge plate for 15 seconds.
8. Load plate into PCR instrument.
Clinical Trial Sites

Nathan Ledeboer, Ph.D., DABMM, FAAM
Medical College of Wisconsin
Milwaukee, WI

Timothy S. Uphoff, Ph.D., D(ABMG)
Marshfield Laboratories
Marshfield, WI

Paul A. Granato, Ph.D., DABMM, FAAM
Laboratory Alliance of CNY
Syracuse, NY
Device Trial Protocol

Tested 924 freshly collected cutaneous and mucocutaneous specimens for the presence of HSV 1, HSV 2, and VZV using the Culture HSV ID and D$^3$ Typing Test.

Performed the PCR HSV 1+2/VZV assay according to manufacturer’s instructions.

Arbitrated discordant results by an independent RT-PCR assay (ASR for HSV 1 or HSV 2 and a PCR assay for VZV).
### PCR HSV 1+2/VZV Performance

<table>
<thead>
<tr>
<th>Cutaneous and mucocutaneous swabs (N=924)</th>
<th>Comparator: Culture HSV ID and D³ Typing Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>124</td>
</tr>
<tr>
<td>Negative</td>
<td>3**</td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
</tr>
</tbody>
</table>

- **Sensitivity**: 124/127 = 97.6%
- **Specificity**: 777/797 = 97.5%

**Post-discordant analysis:**

- **Sensitivity**: 97.6%
- **Specificity**: 99.2%
- **PPV**: 95.8%
- **NPV**: 99.6%

- Fourteen (14) of the twenty (20) positives were positive by an additional RT-PCR assay.
- **Three (3) of the three (3) negatives were negative by an additional RT-PCR assay.**
## PCR HSV 1+2/VZV Performance

### HSV-2

<table>
<thead>
<tr>
<th>Cutaneous and mucocutaneous swabs (N=924)</th>
<th>Comparator: Culture HSV ID and D³ Typing Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>130</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
</tr>
</tbody>
</table>

- **Sensitivity**: $\frac{130}{130} = 100.0\%$
- **Specificity**: $\frac{764}{794} = 96.2\%$

### Post-discordant analysis:
- **Sensitivity**: 100%
- **Specificity**: 99.6%
- **PPV**: 98.1%
- **NPV**: 100%

*Twenty-seven (27) of the thirty (30) positives were positive by an additional RT-PCR assay.*
### PCR HSV 1+2/VZV Performance

<table>
<thead>
<tr>
<th>Cutaneous and mucocutaneous swabs (N=924)</th>
<th>Comparator: Culture HSV ID and D³ Typing Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>31</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
</tr>
</tbody>
</table>

| Sensitivity | 31/31 | 100% |
| Specificity | 610/623 | 97.9% |

* Ten (10) of the thirteen (13) positives were positive by an additional RT-PCR assay.

**Post-discordant:**
- **Sensitivity:** 100%
- **Specificity:** 99.5%
- **PPV:** 93.1%
- **NPV:** 100%
# Resolved Arbitration of Discordant Results

<table>
<thead>
<tr>
<th></th>
<th>HSV-1</th>
<th>HSV-2</th>
<th>VZV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCR</td>
<td>Culture</td>
<td>PCR</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>97.9%</td>
<td>89.9%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>99.6%</td>
<td>99.2%</td>
<td>96.2%</td>
</tr>
</tbody>
</table>
Conclusions

1. The PCR HSV 1+2/VZV performed markedly better than an established cultural method for the detection of HSV 1, HSV 2, and VZV in cutaneous and mucocutaneous specimens.

2. The time-to-result for the PCR assay was reduced compared to the culture method.
Assay Procedure - Heat Lysis

1. Setup the Workflow Tray with patient samples, Process Buffer Tubes and Reaction Tubes.
2. Specimen in transport media.
3. Add 20 μL to Process Buffer Tube.
4. Heat for 5 minutes at 95°C.

Amplification and Detection

1. Transfer 50 μL to each Reaction Tube.
2. Rehydrate by pipetting up and down a minimum of 5 times.
3. Close lid tightly and proceed to next step.
4. Use the Transfer Rack to lift Reaction Tubes from the Workflow Tray.
5. Lower the Reaction Tubes
6. Release the Reaction Tubes from the Transfer Rack by pulling the Transfer Rack toward your body.
7. Close the lid and select appropriate assay protocol. Run complete in 50 minutes.
8. Review results.
Exceptions to the Rule

• Atypical clinical presentations of zoster

• Occurring at unusual anatomic sites

• At least 20% of atypical clinical presentations of zoster and/or reactivations that can be misdiagnosed by an inexperienced clinician

# HSV 1&2/VZV Assay - 2015

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Specimens</td>
<td>2,113</td>
</tr>
<tr>
<td>HSV 1</td>
<td>374</td>
</tr>
<tr>
<td>(17.7%)</td>
<td></td>
</tr>
<tr>
<td>HSV 2</td>
<td>362</td>
</tr>
<tr>
<td>(17.1%)</td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td>126</td>
</tr>
<tr>
<td>(6%)</td>
<td></td>
</tr>
</tbody>
</table>
## VZV Positive Specimens - 2015

<table>
<thead>
<tr>
<th>Total number</th>
<th>126</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genital Specimens</strong></td>
<td>14 (11.1%)</td>
</tr>
<tr>
<td>- 11 specimens (9 female, 2 male) available for confirmatory testing</td>
<td></td>
</tr>
<tr>
<td>- All confirmed by two alternative molecular methods</td>
<td></td>
</tr>
<tr>
<td>- Sanger sequencing</td>
<td></td>
</tr>
</tbody>
</table>
## HSV 1&2/VZV Assay 2016

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Specimens</strong></td>
<td>2,397</td>
</tr>
<tr>
<td><strong>HSV 1</strong></td>
<td>392</td>
</tr>
<tr>
<td></td>
<td>(16.4%)</td>
</tr>
<tr>
<td><strong>HSV 2</strong></td>
<td>372</td>
</tr>
<tr>
<td></td>
<td>(15.5%)</td>
</tr>
<tr>
<td><strong>VZV</strong></td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>(6.5%)</td>
</tr>
</tbody>
</table>
### VZV Positive Genital Specimens - 2016

<table>
<thead>
<tr>
<th>Total number</th>
<th>156</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number positive from genital site</td>
<td>14 (9%)</td>
</tr>
<tr>
<td>- 13 female patients</td>
<td></td>
</tr>
<tr>
<td>- 1 male patient</td>
<td></td>
</tr>
</tbody>
</table>
VZV Positives Genital Specimens
January 1 to June 30, 2017

VZV detected in 8 genital specimens collected from female (6) and male (2) patients.
Arbitration Testing of 18 VZV Genital Specimens from 2016 to 2017

- Performed specimen extraction
- Eluates were tested on the VZV r-gene ASR
- Eluates were also tested in duplicate using a PCR HSV 1+2/VZV assay.
- The PCR amplified duplicate samples were pooled and sent for Sanger sequencing using forward and reverse primers.
- All discernible sequences were used to do a BLAST search in the NCBI database.
Table 1. Two PCR results along with the corresponding sequencing data.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>HSV-1</th>
<th>HSV-2</th>
<th>VZV</th>
<th>VZV</th>
<th>Sequencing Result</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neg</td>
<td>Neg</td>
<td>23.7</td>
<td>23.6</td>
<td>VZV</td>
<td>7.00E-46</td>
</tr>
<tr>
<td>2</td>
<td>Neg</td>
<td>Neg</td>
<td>22.5</td>
<td>22.1</td>
<td>VZV</td>
<td>1.00E-42</td>
</tr>
<tr>
<td>3</td>
<td>Neg</td>
<td>Neg</td>
<td>16.7</td>
<td>15.7</td>
<td>VZV</td>
<td>1.00E-42</td>
</tr>
<tr>
<td>4</td>
<td>Neg</td>
<td>Neg</td>
<td>23</td>
<td>22.3</td>
<td>VZV</td>
<td>3.00E-45</td>
</tr>
<tr>
<td>5</td>
<td>Neg</td>
<td>Neg</td>
<td>28.4</td>
<td>28.4</td>
<td>VZV</td>
<td>2.00E-46</td>
</tr>
<tr>
<td>6</td>
<td>Neg</td>
<td>Neg</td>
<td>28.1</td>
<td>27.8</td>
<td>VZV</td>
<td>3.00E-44</td>
</tr>
<tr>
<td>7</td>
<td>Neg</td>
<td>Neg</td>
<td>25.8</td>
<td>25.3</td>
<td>VZV</td>
<td>3.00E-45</td>
</tr>
<tr>
<td>8</td>
<td>Neg</td>
<td>Neg</td>
<td>24.3</td>
<td>23.3</td>
<td>VZV</td>
<td>7.00E-46</td>
</tr>
<tr>
<td>9</td>
<td>Neg</td>
<td>Neg</td>
<td>28.7</td>
<td>28.1</td>
<td>VZV</td>
<td>7.00E-46</td>
</tr>
<tr>
<td>10</td>
<td>Neg</td>
<td>Neg</td>
<td>18.8</td>
<td>18.1</td>
<td>VZV</td>
<td>3.00E-44</td>
</tr>
<tr>
<td>11</td>
<td>Neg</td>
<td>Neg</td>
<td>20.4</td>
<td>19.8</td>
<td>VZV</td>
<td>1.00E-43</td>
</tr>
<tr>
<td>12</td>
<td>Neg</td>
<td>Neg</td>
<td>21.6</td>
<td>21</td>
<td>VZV</td>
<td>7.00E-46</td>
</tr>
<tr>
<td>13</td>
<td>Neg</td>
<td>Neg</td>
<td>28.3</td>
<td>27.8</td>
<td>VZV</td>
<td>3.00E-44</td>
</tr>
<tr>
<td>14</td>
<td>Neg</td>
<td>Neg</td>
<td>19.3</td>
<td>18.5</td>
<td>VZV</td>
<td>7.00E-46</td>
</tr>
<tr>
<td>15</td>
<td>Neg</td>
<td>Neg</td>
<td>19.3</td>
<td>18.3</td>
<td>VZV</td>
<td>2.00E-47</td>
</tr>
<tr>
<td>16</td>
<td>Neg</td>
<td>Neg</td>
<td>28.6</td>
<td>27.8</td>
<td>VZV</td>
<td>3.00E-45</td>
</tr>
<tr>
<td>17</td>
<td>Neg</td>
<td>Neg</td>
<td>24</td>
<td>22.8</td>
<td>VZV</td>
<td>3.00E-45</td>
</tr>
<tr>
<td>18</td>
<td>Neg</td>
<td>Neg</td>
<td>20.9</td>
<td>20.2</td>
<td>VZV</td>
<td>7.00E-46</td>
</tr>
</tbody>
</table>

Conclusion: All 18 vaginal samples were positive for VZV according to both PCR assays and Sanger sequencing.
Importance of Distinguishing HSV vs VZV Infection

Treatment:
- VZV less susceptible to acyclovir, valacyclovir and famciclovir

* Patient counseling:
- Likelihood of reoccurrence
- Impact on patient’s emotional and psychological health and well-being
- Reactivation zoster lesions contain viable virus that can be transmitted by direct contact
- VZV could be an STD adding an entirely new and previously unrecognized component to the public health significance of this disease
Summary

- HSV and VZV are common causes of cutaneous and mucocutaneous infections
- Typical HSV and VZV lesions are distinguished based upon appearance and anatomic location
- Atypical presentations of zoster can occur in unusual anatomic sites
Summary

• Over 10% of VZV positive specimens at Laboratory Alliance were from male and female urogenital sites

• The HSV 1+2 & VZV assay allows for the improved detection of HSV 1, HSV 2, and VZV from cutaneous and mucocutaneous specimens

• The assay also allows for the unexpected detection of VZV from atypical anatomic sites
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