Genital Zoster Infections - An Unexpected Finding Using a Molecular Assay

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of Central New York, LLC
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Disclosures

Accelerate Diagnostics
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iCubate
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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

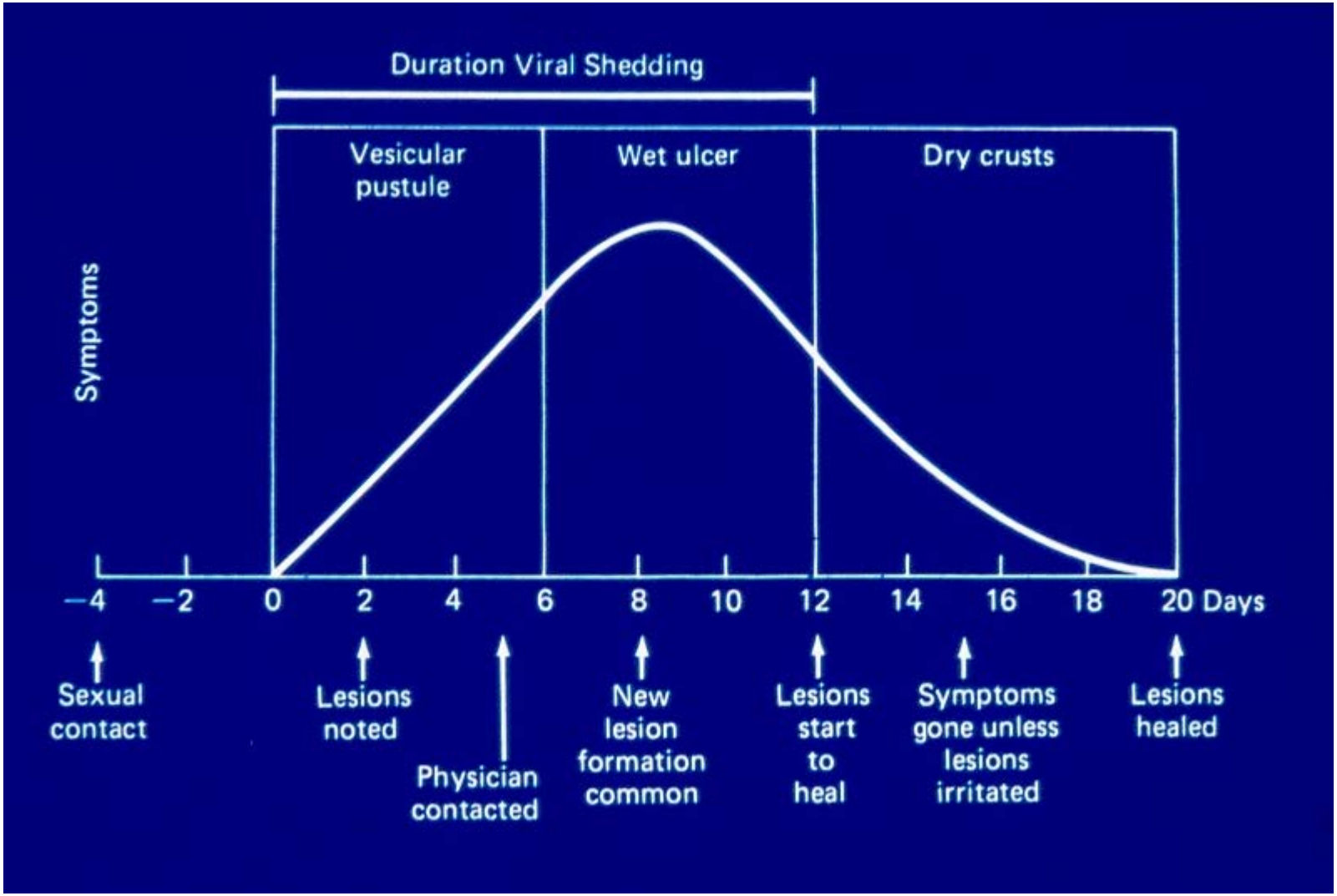
(Other HSVs: CMV, HHV-6, HHV-7, EBV, and HSV-8)

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

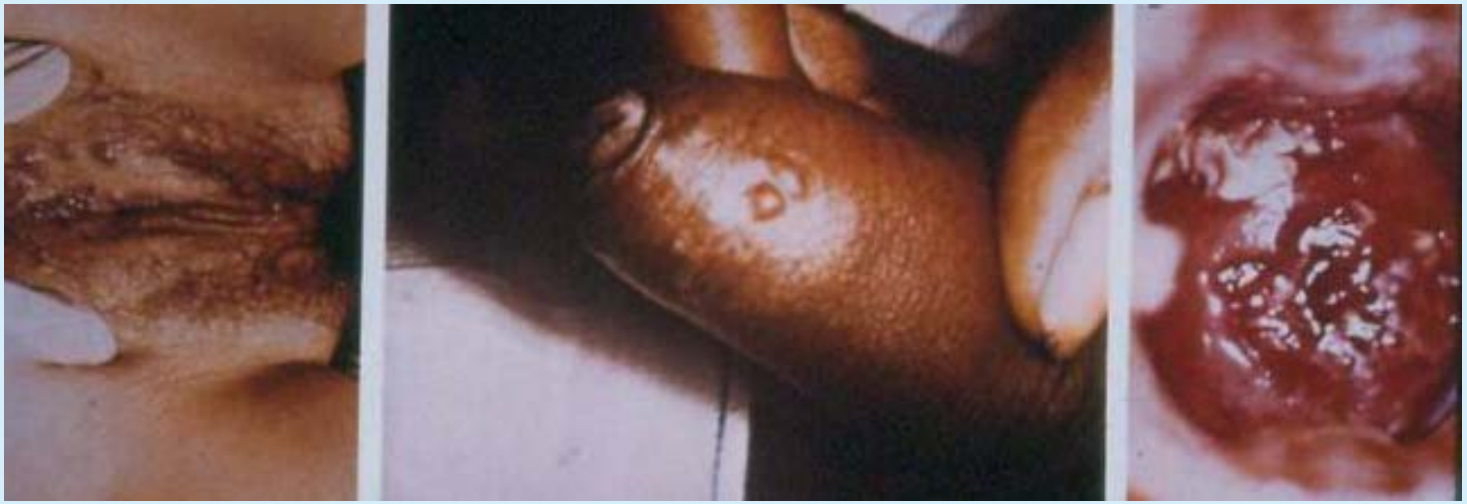
Characteristics of HSV and VZV Infections

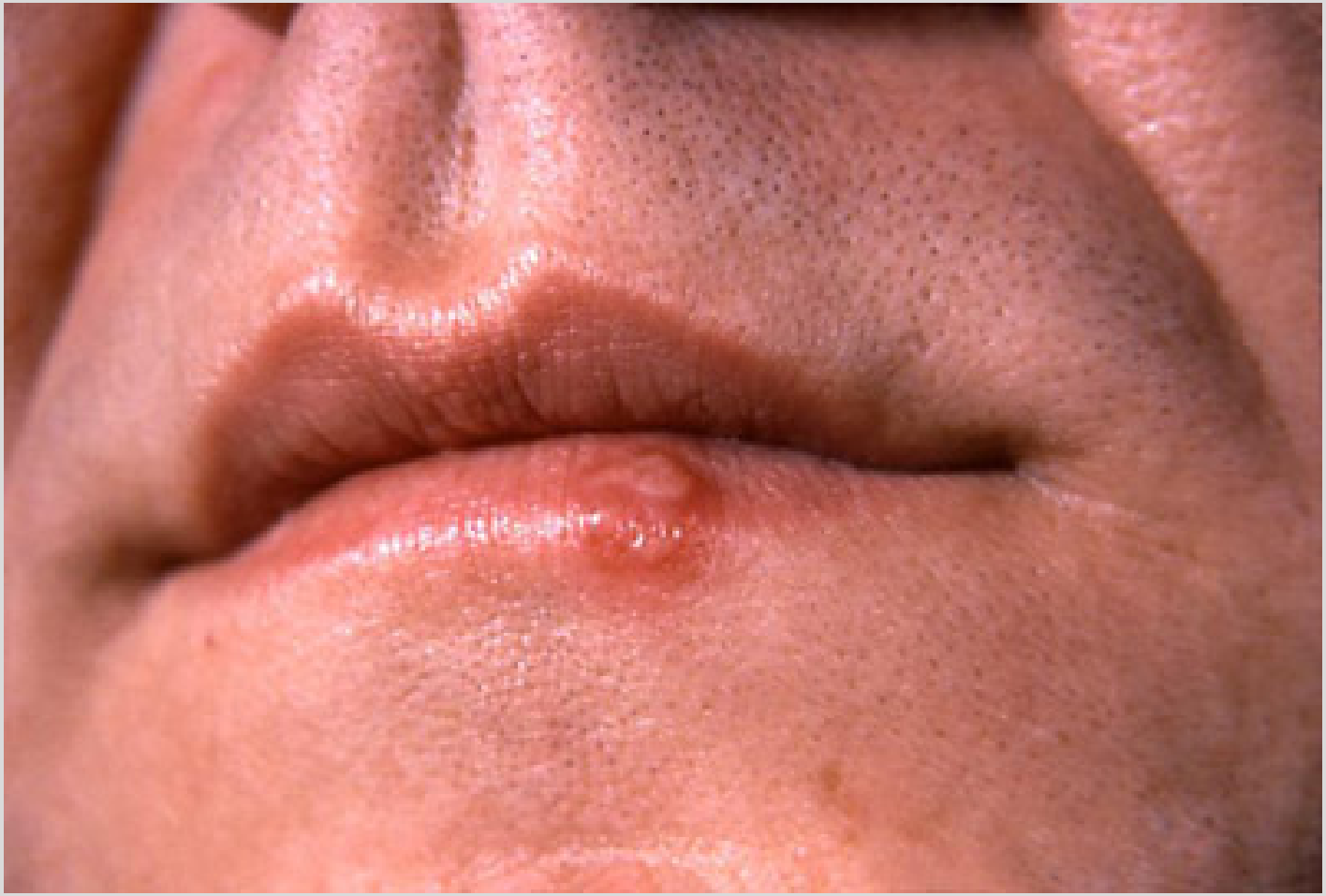
- Reactivation of infection
  - HSV at the same site of primary infection
  - VZV cutaneous lesions along dermatomes (varicella zoster, zoster, herpes zoster, shingles)
- Resolution of symptoms and return to dormancy

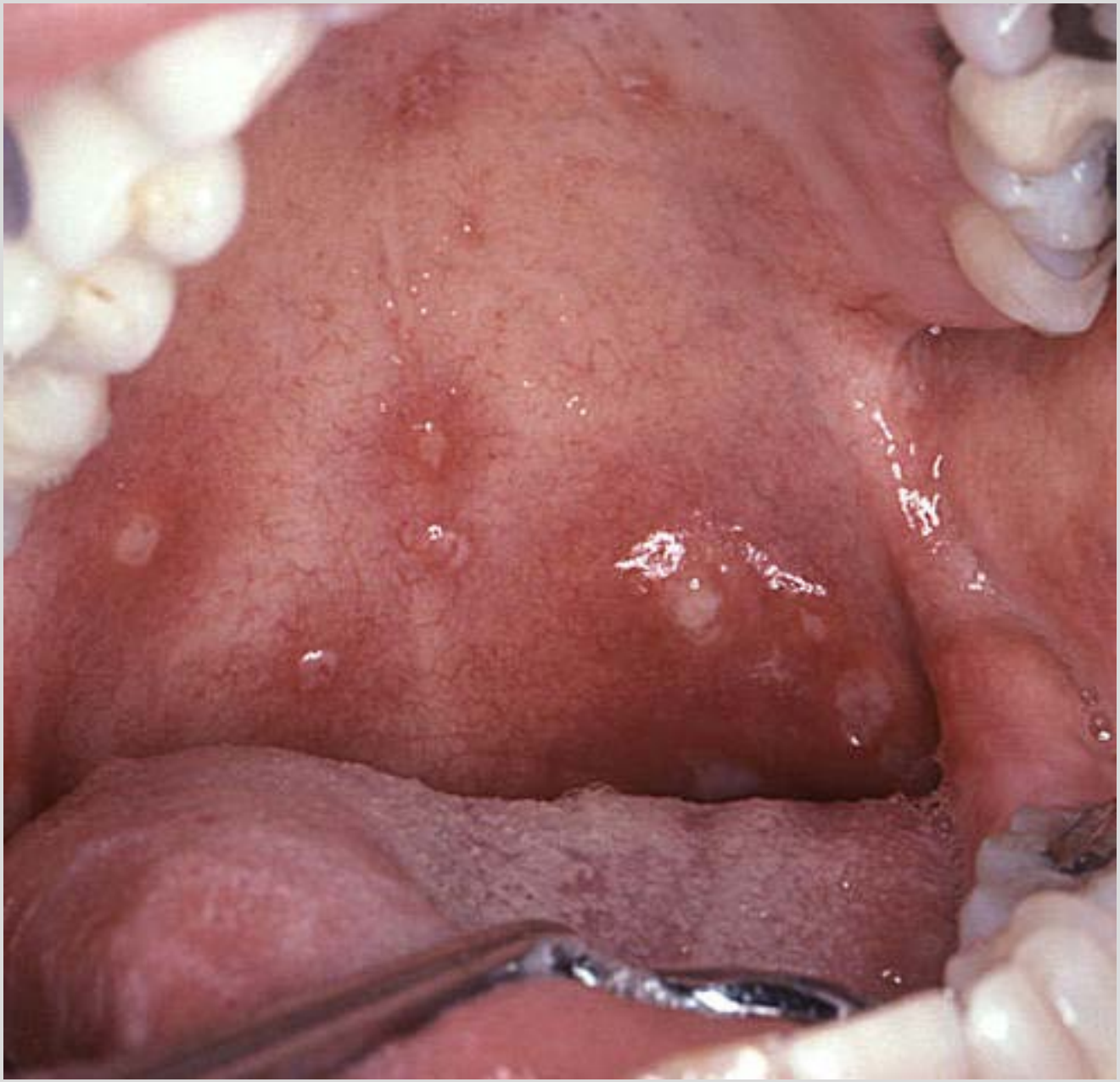

Duration Viral Shedding
Vesicular pustule
Wet ulcer
Dry crusts
Symptoms
−4
−2
0
2
4
6
8
10
12
14
16
18
20 Days
Sexual contact
Lesions noted
Physician contacted
New lesion formation common
Lesions start to heal
Symptoms gone unless lesions irritated
Lesions healed

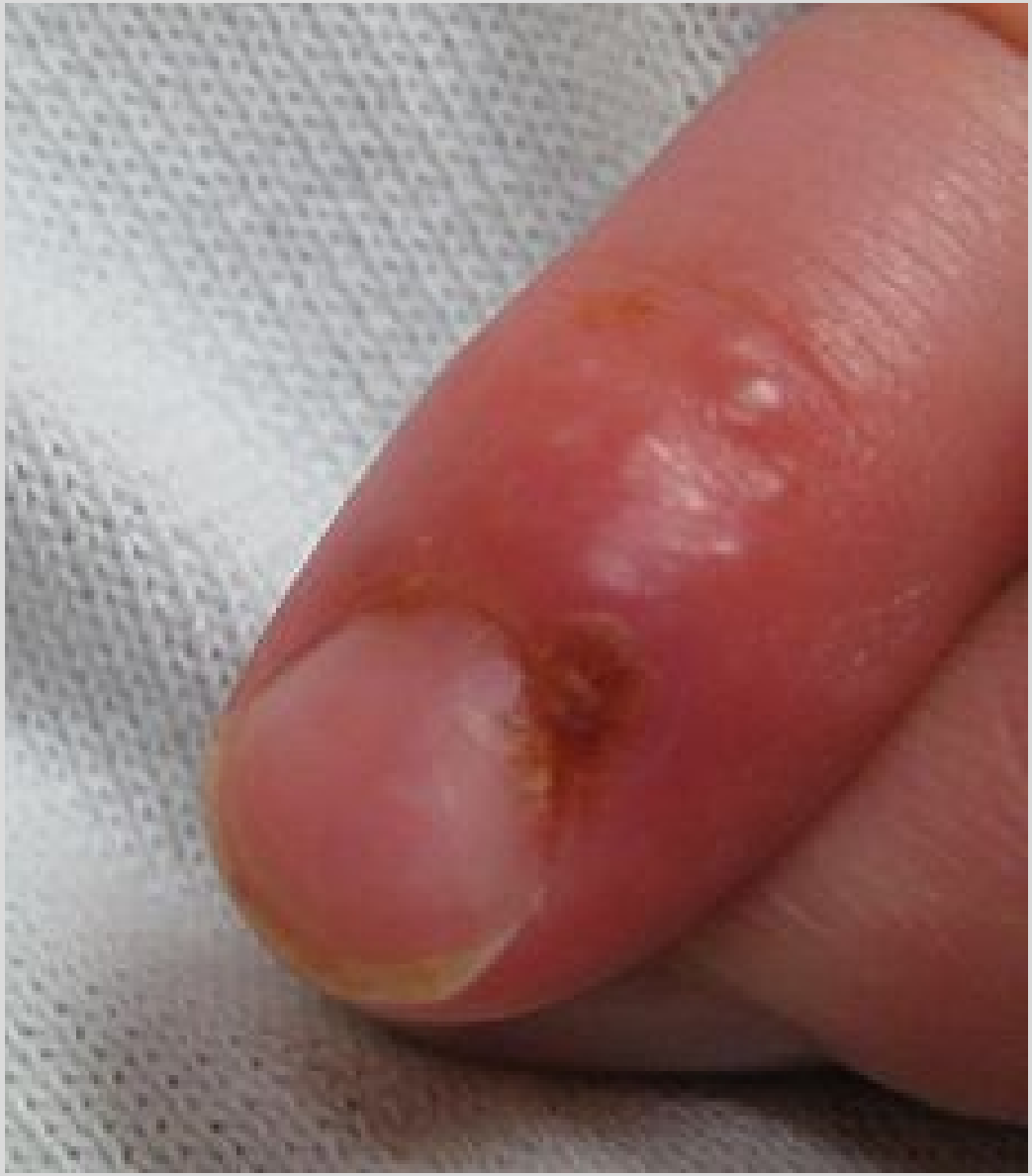
Types of HSV Infections

- Herpes genitalis (genital herpes)
- Herpes labialis (cold sores)
- Herpes gingivostomatitis
- Herpetic Whitlow
- Herpes keratitis
- Herpes encephalitis
- Herpes meningitis



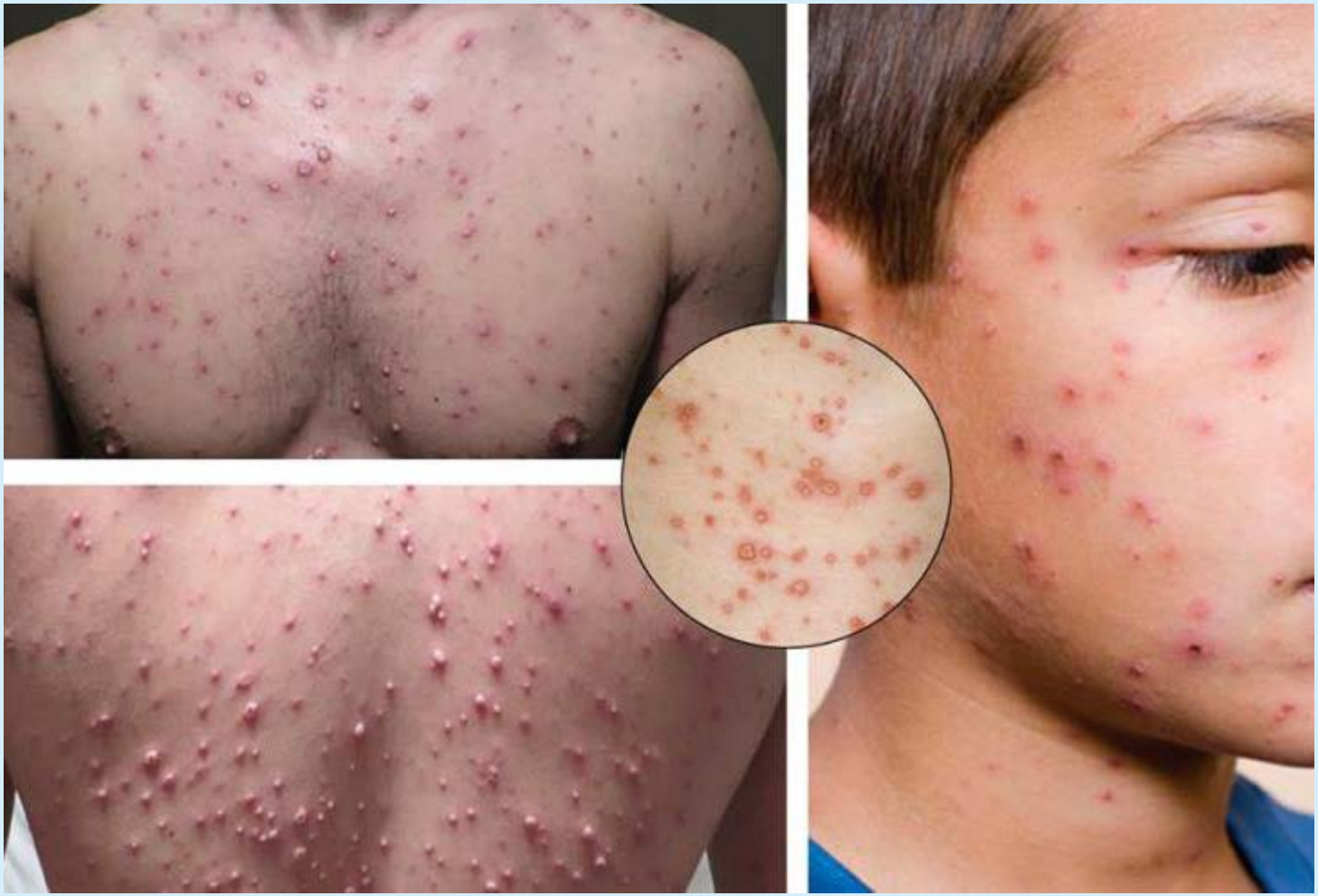


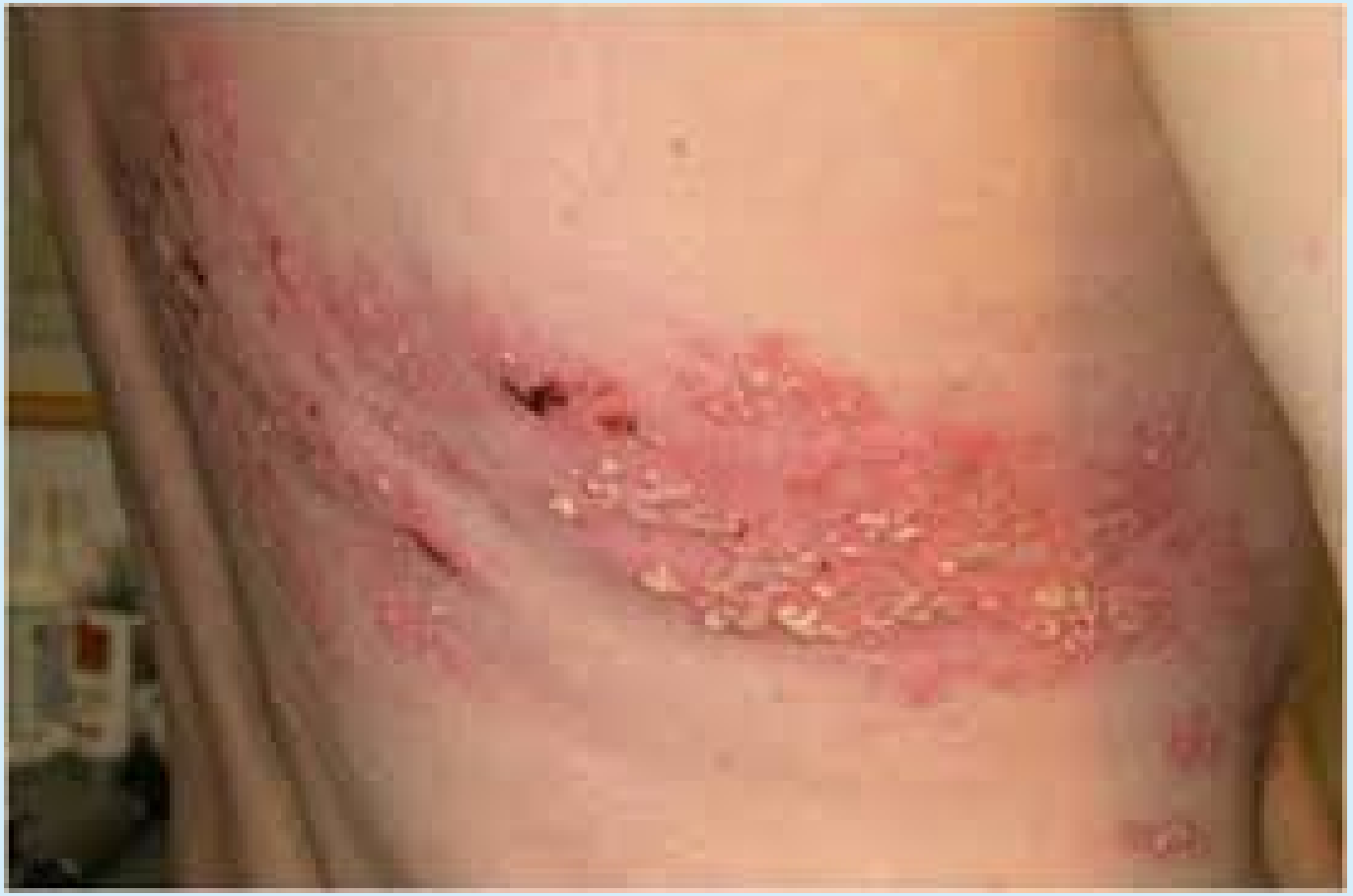




Characteristics of Reactivation HSV Infection

- Generally reoccurs at the same or nearby anatomic site
- Reactivation HSV disease has the same clinical appearance as primary HSV infection





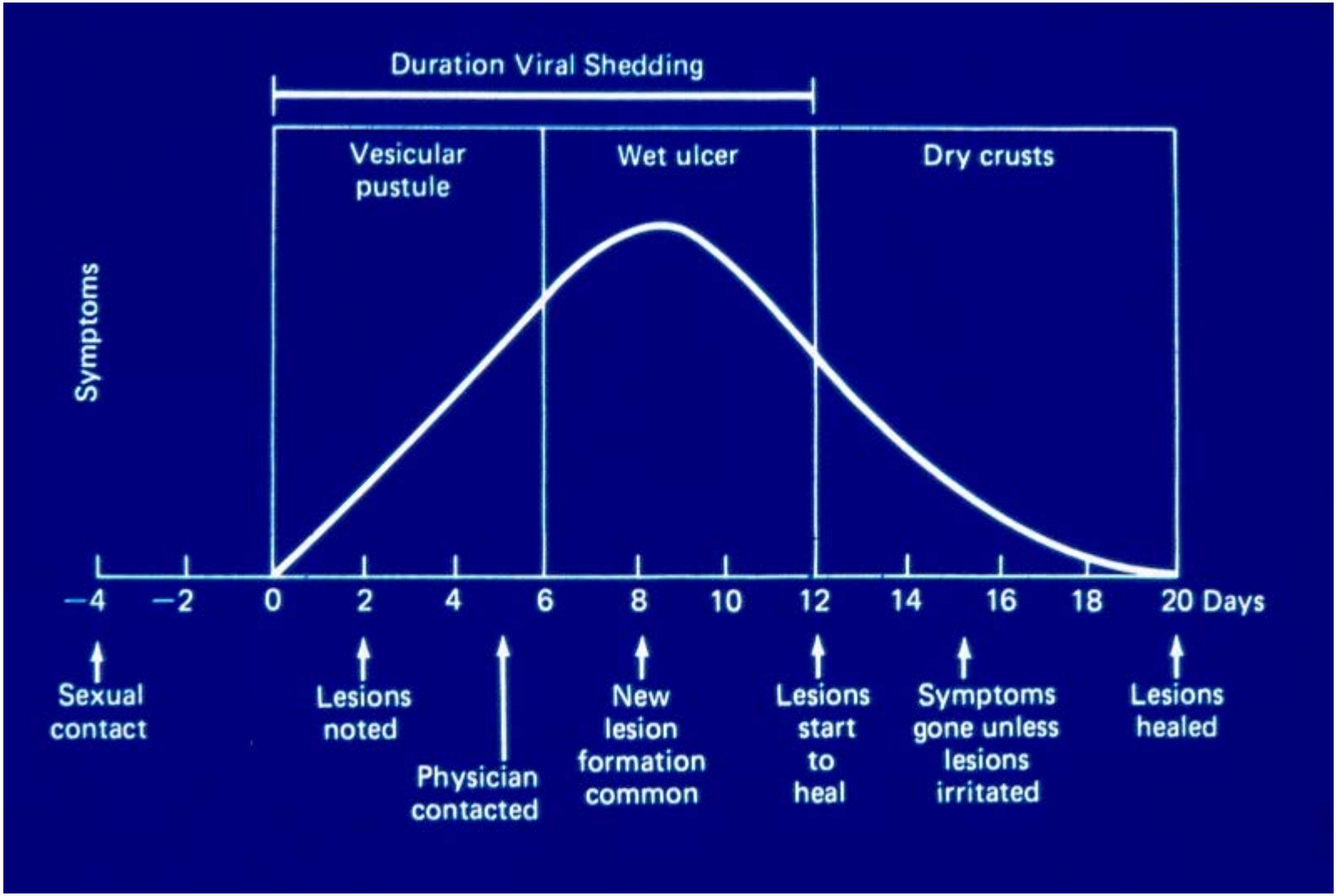
Characteristics of Reactivation Varicella Zoster Virus Infection

- 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


Duration Viral Shedding
Vesicular pustule
Wet ulcer
Dry crusts
Symptoms
−4
−2
0
2
4
6
8
10
12
14
16
18
20 Days
Sexual contact
Lesions noted
Physician contacted
New lesion formation common
Lesions start to heal
Symptoms gone unless lesions irritated
Lesions healed

USA SNAPSHOTS©

The doctor will see you when?

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

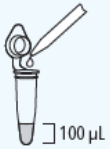
12 tubes @ 1.8 mL/tube	12 vials	1 tube @ 1.9 mL/tube
		
Process Buffer	8x Master Mix	Rehydration Solution

Sample Preparation

1

Remove 100 µL aliquot 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

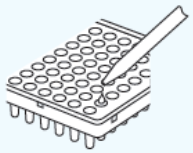
Add 25 µL of Process Buffer, within 60 min. (contains process control material).

Amplification and Detection Procedure

5

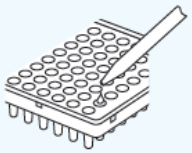
Open Master Mix slowly. Add 135 µL of Rehydration Solution. Replace cap and allow it to sit for 1-2 minutes. Gently pipette rehydrated Master Mix up and down 2-3 times. Avoid creating bubbles.

6



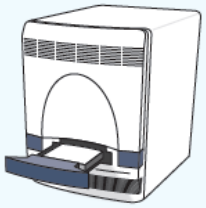
Add 15 µL of Master Mix to each well.

7



Add 5 µL of sample with process control to plate well and seal the plate. Centrifuge plate for 15 seconds.

8



Insert plate into 7500 Fast Dx or

Clinical Trial Sites

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Milwaukee, WI

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

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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

HSV-1			
Cutaneous and mucocutaneous swabs (N=924)	Comparator: Culture HSV ID and D³ Typing Test		
	Positive	Negative	Total
Positive	124	20*	144
Negative	3**	777	780
Total	127	797	924
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			
Cutaneous and mucocutaneous swabs (N=924)	Comparator: Culture HSV ID and D^3 Typing Test		
	Positive	Negative	Total
Positive	130	30*	160
Negative	0	764	764
Total	130	794	924
Sensitivity	130/130	100.0%	
Specificity	**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

VZV			
Cutaneous and mucocutaneous swabs (N=924)	Comparator: Culture HSV ID and D³ Typing Test		
	Positive	Negative	Total
Positive	31	13*	44
Negative	0	610	610
Total	31	623	654
Sensitivity	31/31	100%	
Specificity	610/623	97.9%	

Post-discordant:

- **Sensitivity:** 100%
- **Specificity:** 99.5%
- **PPV:** 93.1%
- **NPV:** 100%

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

Resolved Arbitration of Discordant Results

	HSV-1		HSV-2		VZV	
	PCR	Culture	PCR	Culture	PCR	Culture
Sensitivity	97.9%	89.9%	100%	82.8%	100%	75.6%
Specificity	99.6%	99.2%	96.2%	99.6%	99.5%	100%

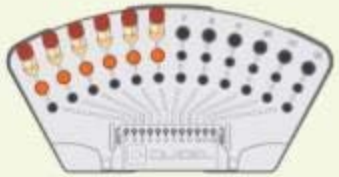
Conclusions

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

VORTEX
5 seconds

4

Add 20 µL to Process Buffer Tube.

5

VORTEX
5 seconds

6

Heat for 5 minutes at 95°C.

7

VORTEX
5 seconds

Amplification and Detection

1

Transfer 50 µL to each Reaction Tube.

2

Rehydrate by pipetting up and down a minimum of 5 times.

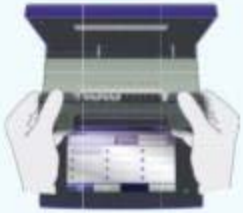
3

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.

PCM302000ENO0 (08/16)

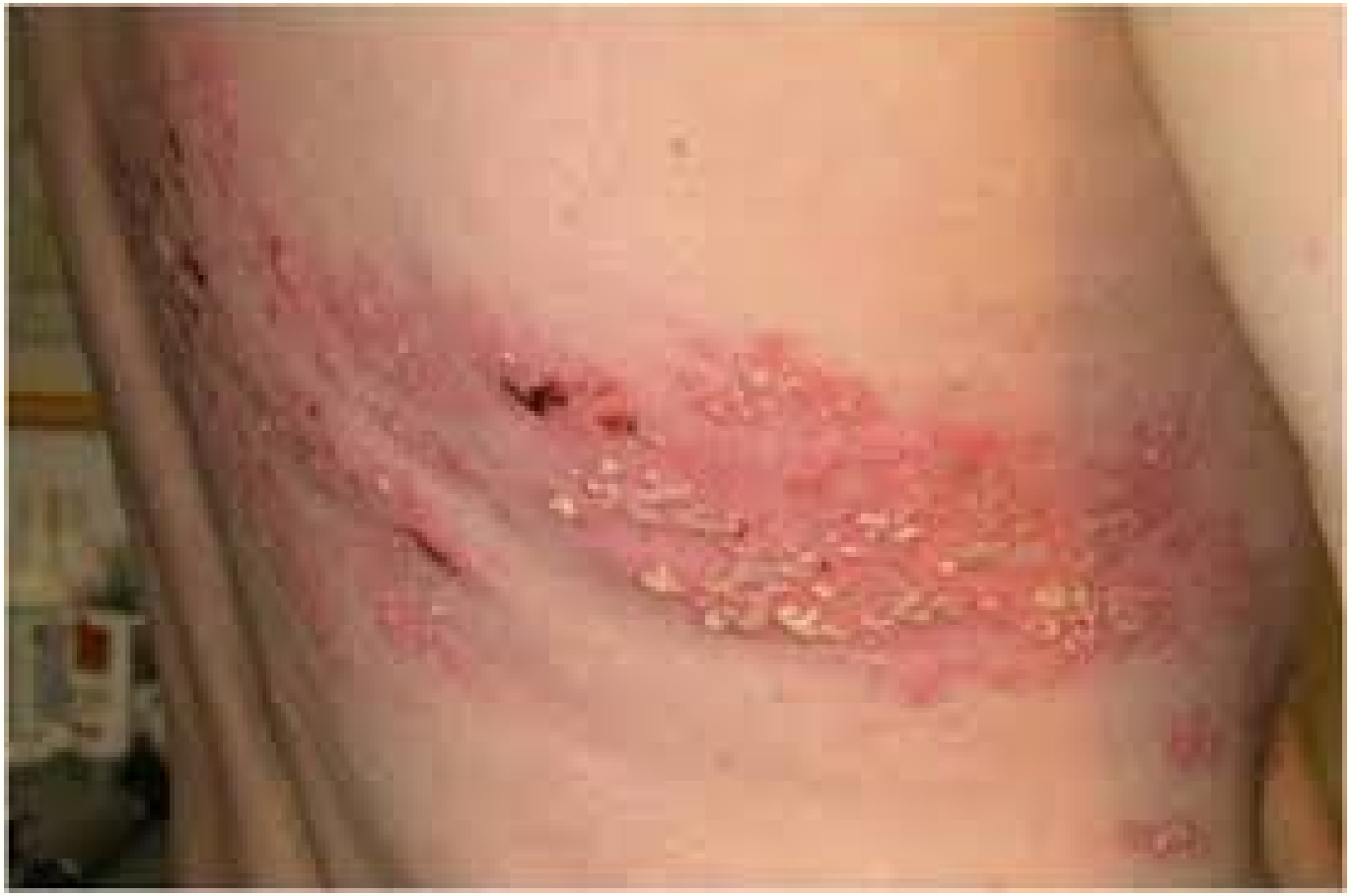
The Laboratory Alliance

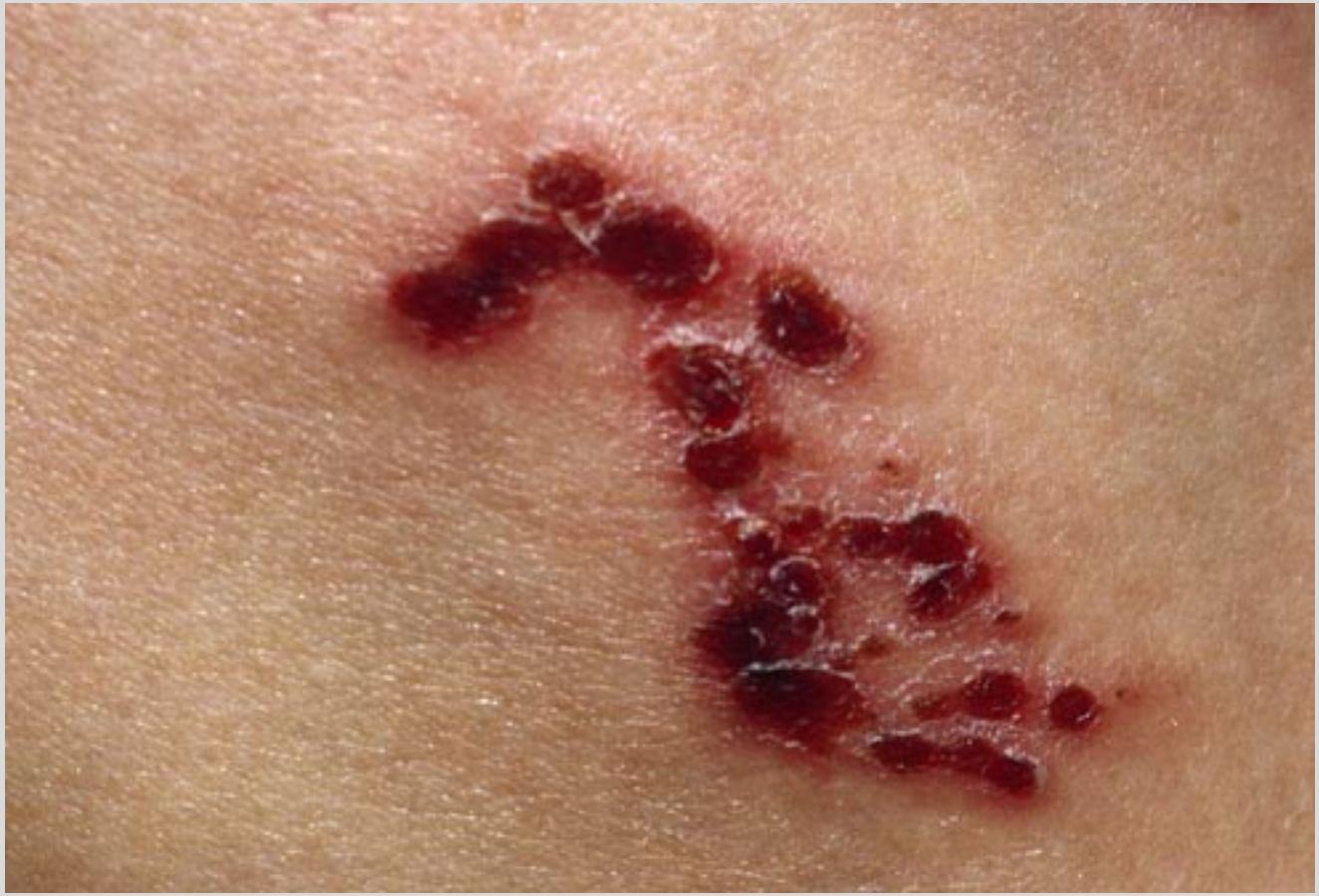
VZV Experience

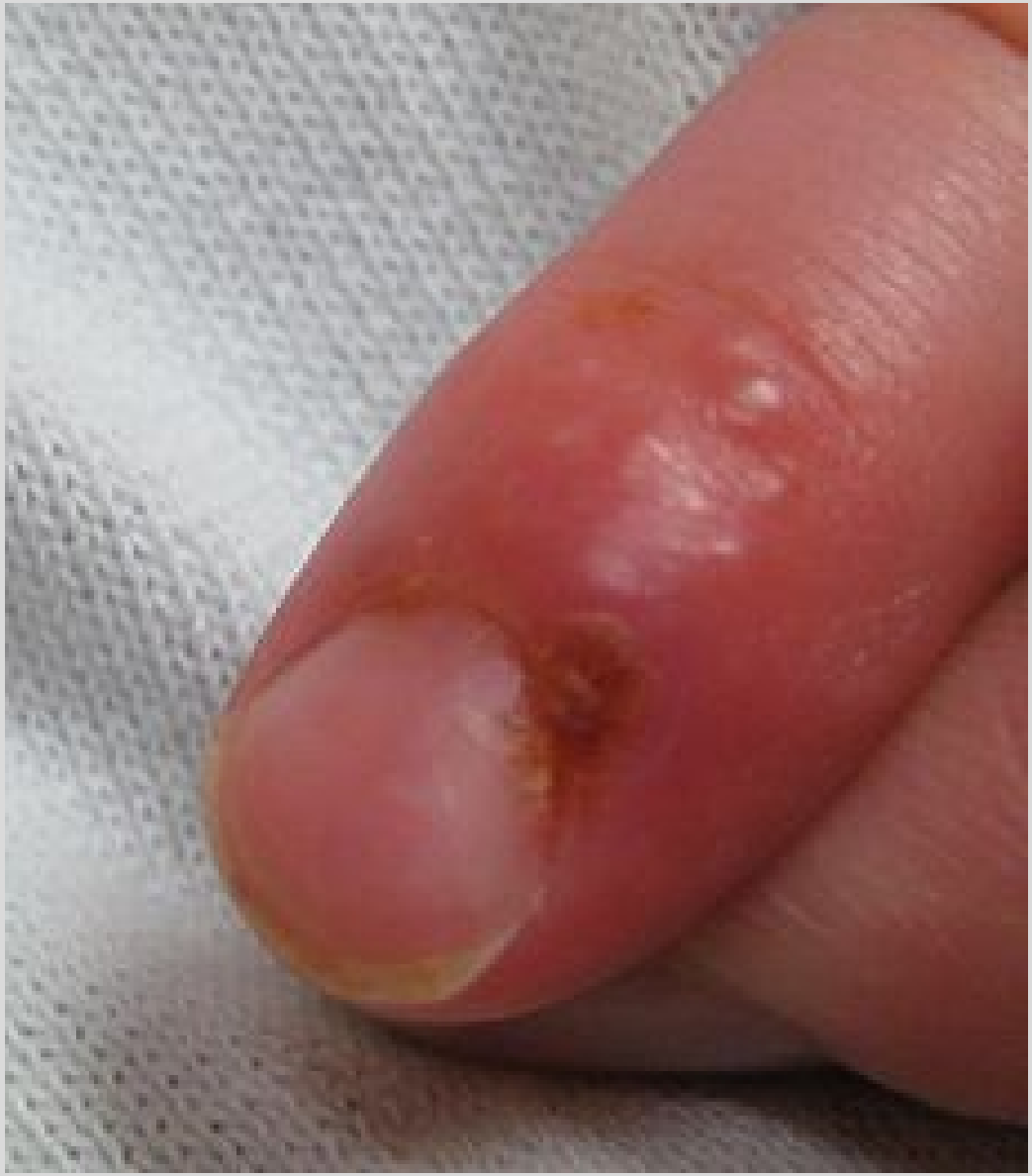
Exceptions to the Rule

- 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[1]

[1]Ruben et al. 1997. Routine detection of herpes simplex virus and varicella zoster virus by polymerase chain reaction reveals that initial zoster is frequently misdiagnosed as herpes simplex virus. Brit. J. Dermatol. 137:259-261.







HSV 1&2/VZV Assay - 2015

Total Specimens	2,113
HSV 1	374 (17.7%)
HSV 2	362 (17.1%)
VZV	126 (6%)

VZV Positive Specimens - 2015

Total number	126
Genital Specimens - **11 specimens (9 female, 2 male) available for confirmatory testing** - **All confirmed by two alternative molecular methods** - **Sanger sequencing**	14 (11.1%)

P.A. Granato, M.A. DeGilio, and E.A. Wilson. 2016. The unexpected detection of varicella-zoster virus in genital specimens using the Lyra Direct HSV 1+2/VZV Assay. *Journal of Clinical Virology*. 84: 87-89.

HSV 1&2/VZV Assay 2016

Total Specimens	2,397
HSV 1	392 (16.4%)
HSV 2	372 (15.5%)
VZV	156 (6.5%)

VZV Positive Genital Specimens - 2016

Total number	156
Number positive from genital site - **13 female patients** - **1 male patient**	14 (9%)

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.

	PCR HSV1+2/VZV Result			VZV ASR Ct Results		
Sample ID	HSV-1	HSV-2	VZV	VZV	Sequencing Result	E-value
1	Neg	Neg	23.7	23.6	VZV	7.00E-46
2	Neg	Neg	22.5	22.1	VZV	1.00E-42
3	Neg	Neg	16.7	15.7	VZV	1.00E-42
4	Neg	Neg	23	22.3	VZV	3.00E-45
5	Neg	Neg	28.4	28.4	VZV	2.00E-46
6	Neg	Neg	28.1	27.8	VZV	3.00E-44
7	Neg	Neg	25.8	25.3	VZV	3.00E-45
8	Neg	Neg	24.3	23.3	VZV	7.00E-46
9	Neg	Neg	28.7	28.1	VZV	7.00E-46
10	Neg	Neg	18.8	18.1	VZV	3.00E-44
11	Neg	Neg	20.4	19.8	VZV	1.00E-43
12	Neg	Neg	21.6	21	VZV	7.00E-46
13	Neg	Neg	28.3	27.8	VZV	3.00E-44
14	Neg	Neg	19.3	18.5	VZV	7.00E-46
15	Neg	Neg	19.3	18.3	VZV	2.00E-47
16	Neg	Neg	28.6	27.8	VZV	3.00E-45
17	Neg	Neg	24	22.8	VZV	3.00E-45
18	Neg	Neg	20.9	20.2	VZV	7.00E-46

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

Acknowledgements

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Thank you! Questions?

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