Multiplex PCR Detection of Herpesviruses and Varicella: Why and How?

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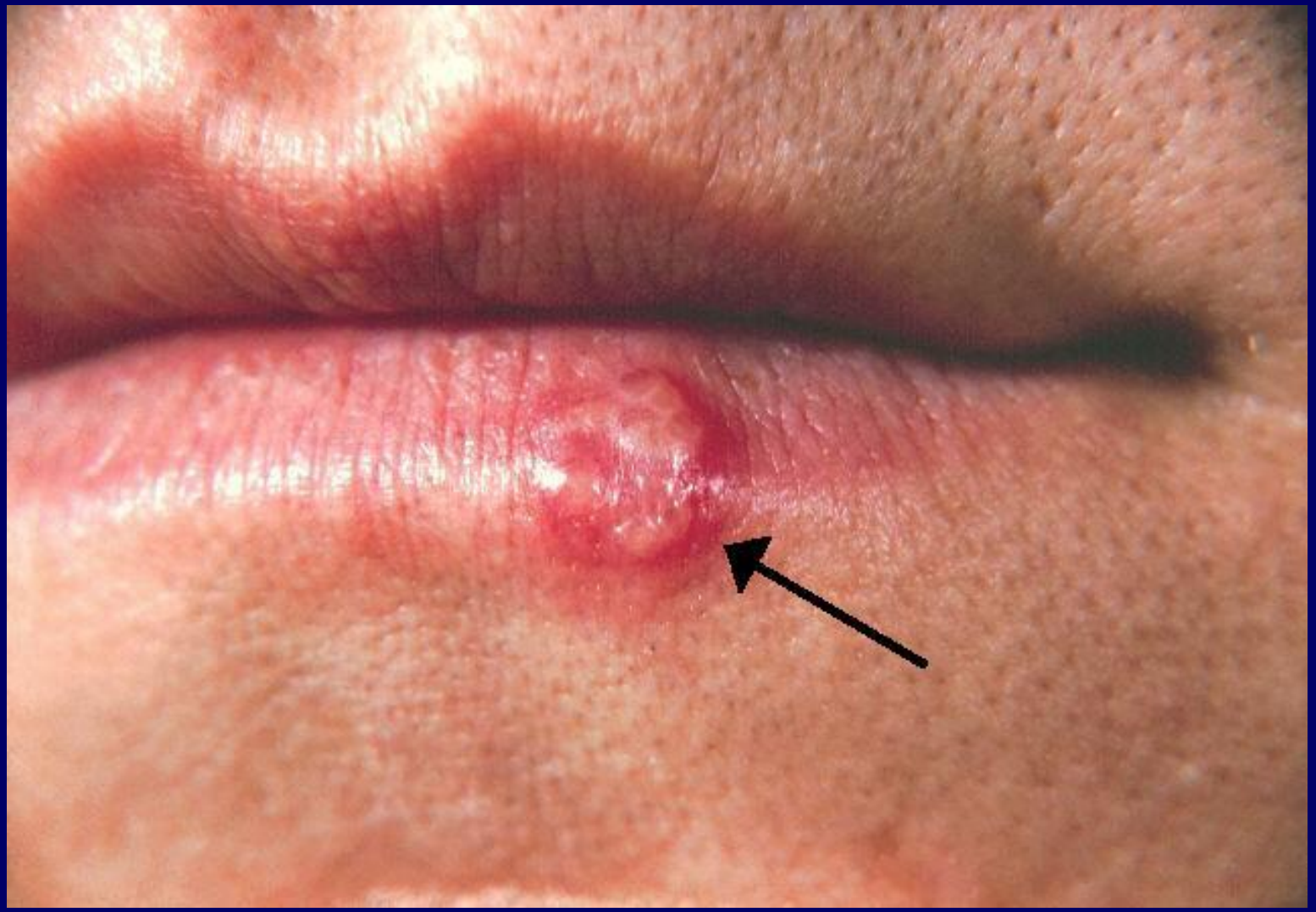
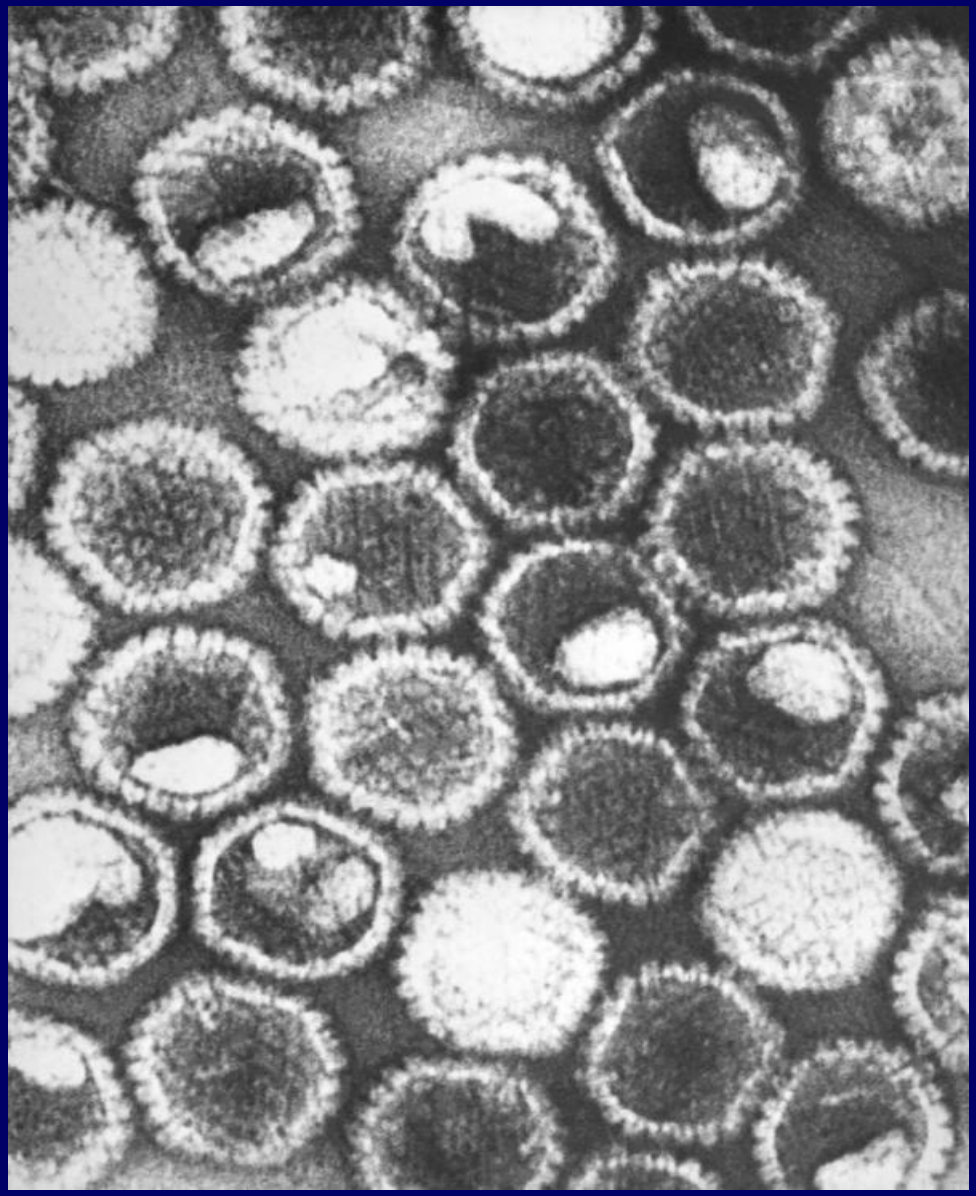
Objectives

1. To describe the clinical importance of lab diagnosis for herpes and varicella infections
2. To evaluate the altona RealStar *alpha* Herpesvirus multiplex PCR for diagnosing skin and mucous membrane lesions
3. To evaluate PCR testing with MSwab rapid nucleic acid extraction from skin and mucous membrane lesions

1. To describe the clinical importance of lab diagnosis for herpes and varicella infections



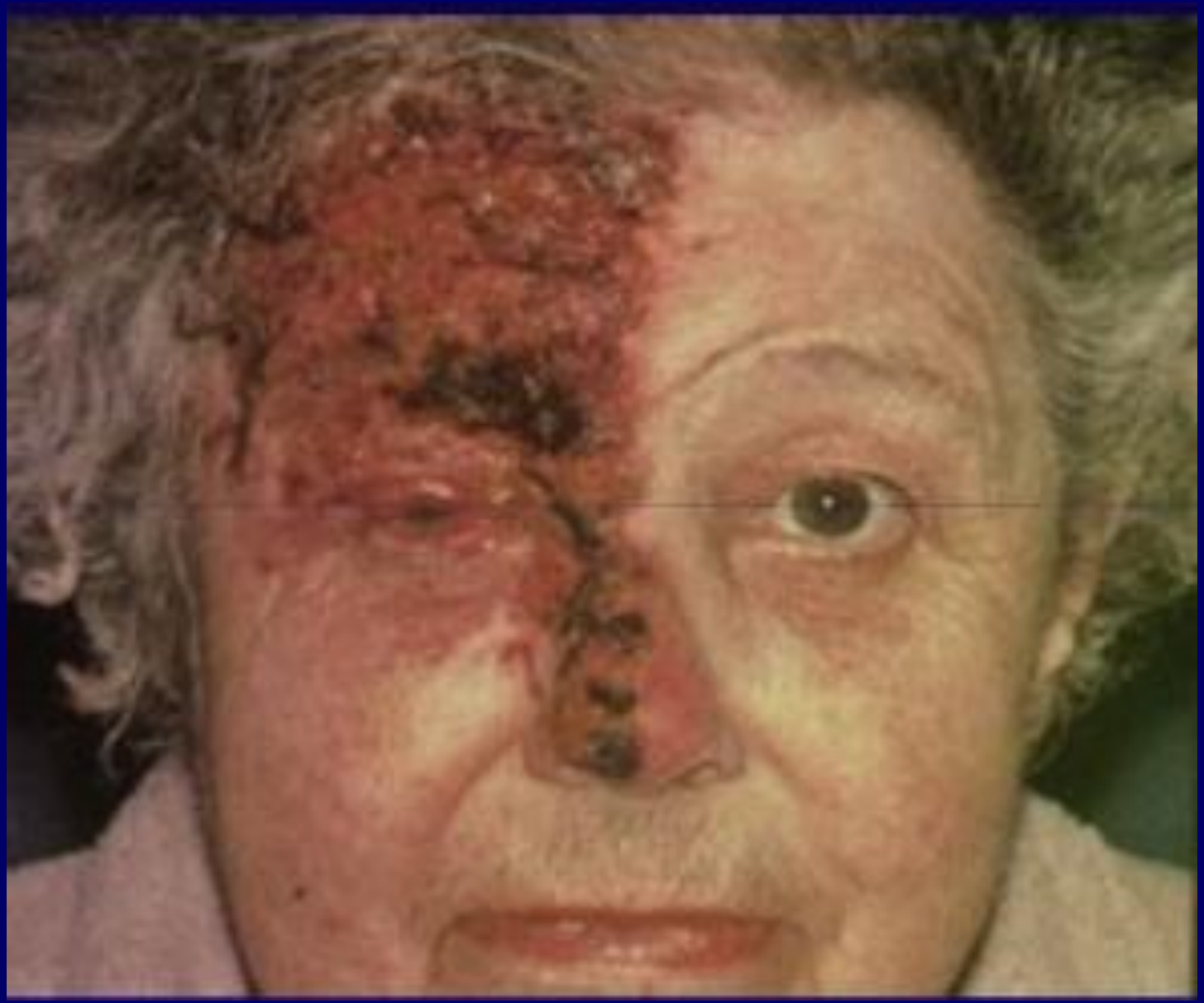
CDC/Dr. Herrmann



CDC/ Dr. Fred Murphy; Sylvia Whitfield

Herpes Simplex Figure 40. Neonatal herpes skin lesions of the face.

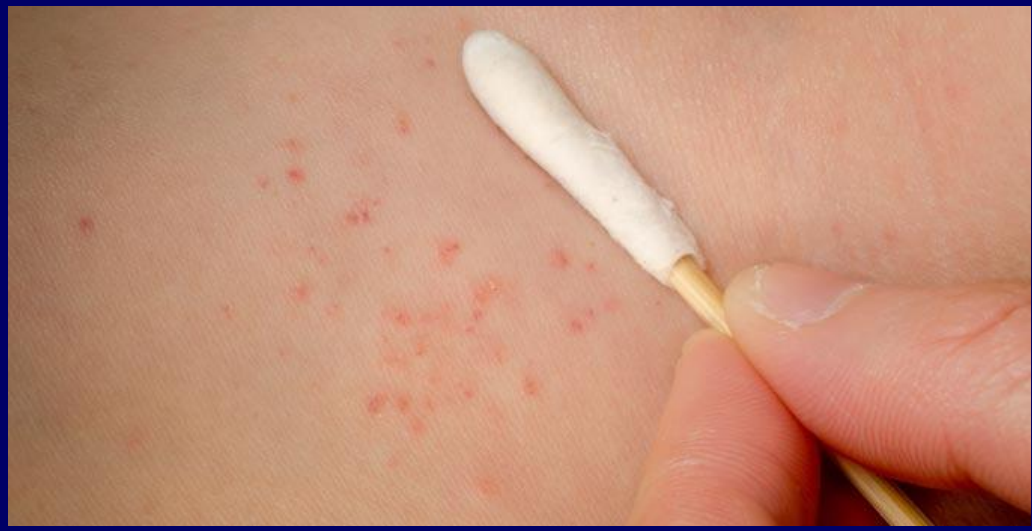
Committee on Infectious Diseases et al. Red Book Online
398-408



Photo/MN Oxman, University of California San Diego

Diagnosis of Herpes and Varicella

- Clinical pattern recognition
- Electron microscopy
- Tzanck smears
- DFA/ Culture
- Serology
- PCR



Why Diagnose Herpes Simplex?

- Initial episode severe—treat
- Immunocompromised
- Dangerous site: eyes
- Pregnant woman

- Recurrences
- Suppressive treatment

- Lab test if atypical, severe, drug coverage

Why Diagnose Varicella Zoster?

- Initial episode severe—treat in adults
- Immunocompromised
- Diagnose for treatment, infection control

- Recurrences—shingles
- Post-herpetic neuralgia
- Early treatment most effective
- Shingles vaccine

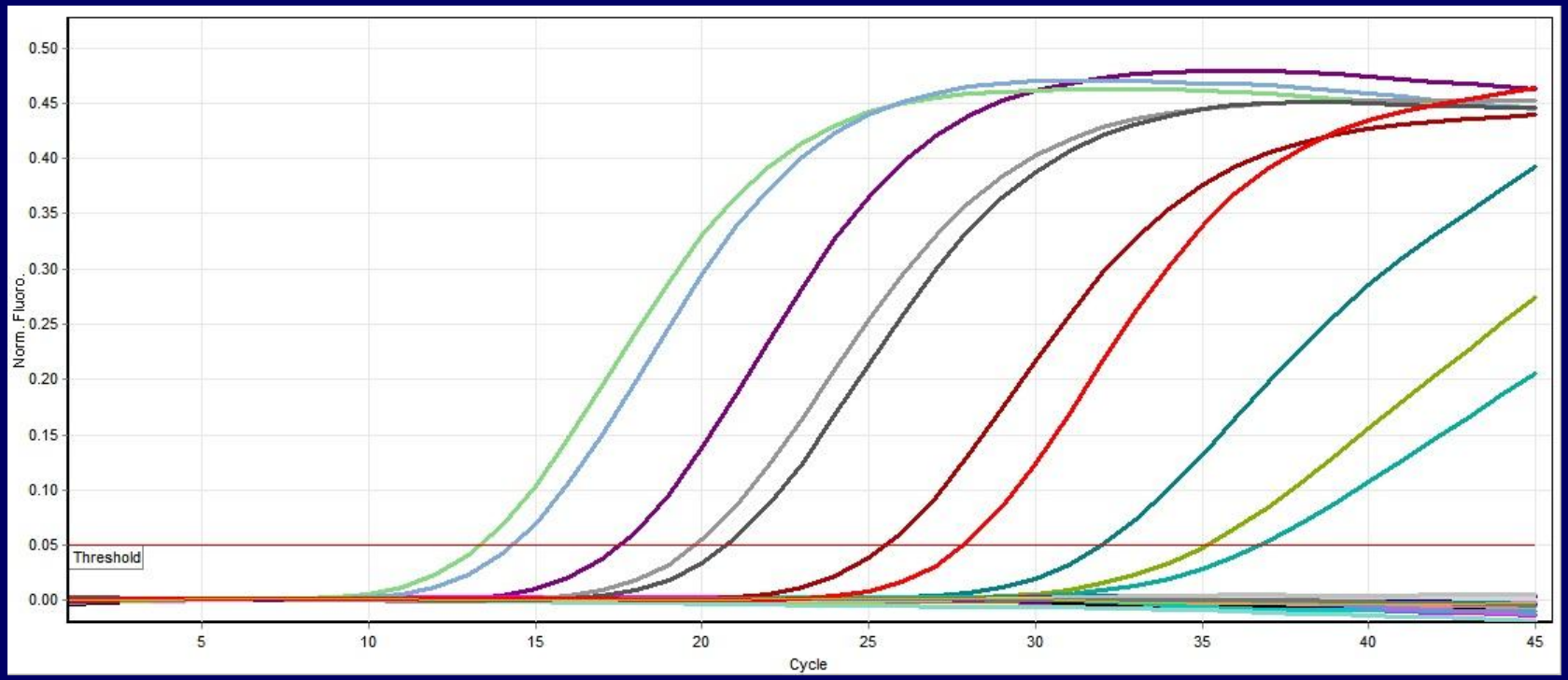
2. To evaluate the altona RealStar *alpha* Herpesvirus multiplex PCR for diagnosing skin and mucous membrane lesions

Study to Evaluate Culture vs. PCR

- Collected HSV1, HSV2 and VZV positives from culture for 1 year (n=74)
- Randomly 61 sample negatives (13%)
- Culture and DFA methods:
  - DFA for VZV
  - Incubate for 24-72 hours in H-V cells
  - Stain with monoclonal antibodies against HSV-1,-2 or VZV antigen
- PCR Methods: altona Diagnostics *alpha* Herpes virus RealStar on Rotor-Gene

RealStar® (RT-) PCR Kits

altona
DIAGNOSTICS


0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Norm. Fluoro.
Threshold
5
10
15
20
25
30
35
40
45
Cycle

Results: Culture and DFA

- **74 Culture positives:**
  - **44 HSV-1**
  - **19 HSV-2**
  - **2 HSV untypeable**
  - **9 VZV (DFA or culture)**

Results: Culture

- **<u>74 Culture positives:</u>**
  - **44 HSV-1** — **29 Head and neck, 10 genital**
  - **19 HSV-2** — **2 lip, hand 14 genital**
  - **9 VZV** — **eye, skin, trunk, buttocks**

Results: Culture and PCR

- **74 Culture positives:**
  - **44 HSV-1**
  - **19 HSV-2**
  - **2 HSV untypeable**
  - **9 VZV**

73 PCR positives:

- **43 HSV1, 1 HSV1/VZV**
- **17 HSV2, 1 HSV2/VZV, 1 HSV1 (back)**
- **1 HSV1, 1 VZV**
- **8 VZV**
- **1 neg X 2**

Results: Culture and PCR

- **61 Culture negatives:**

13 PCR positives:

7 HSV1

4 HSV2

2 VZV

inc. VZV/HSV2

Results: Culture and PCR

- **Source:** **13 PCR positives:**

4 mouth, 3 genital	7 HSV1
genital	4 HSV2
throat	2 VZV
buttocks	inc. VZV/HSV2

Summary Sensitivity

	Culture or PCRX2	Negative	Per Cent
PCR	83	1	99%
Culture	74	10	88%

Sensitivity = 83/84 = 99% (95% CI: 94-100)
Specificity = 48/51 = 94% (95% CI: 84-98)
Positive Predictive Value = 97%
Negative Predictive Value = 98%

Sampling-Adjusted Sensitivity

	Culture or PCRX2	Negative	Per Cent
PCR of positives	73	1	99%
PCR of 61 Neg	10	51	16%
Out of 500 Neg	82	418	16%
Culture	74	82	47%

Alpha Herpes Virus multiplex PCR

- Same day results for HSV-1, HSV-2, VZV
- Despite suboptimal sample, PCR was highly sensitive:
  - 99% versus 47-88% for culture
  - Resolves HSV-1, HSV-2
- Detected dual infections with HSV/VZV

3. To evaluate PCR testing with MSwab rapid nucleic acid extraction from skin and mucous membrane lesions

Methods: Sample Processing

- Swabs in UTM collected from clinical lab weekly
- UTM poured off and stored at -80^{o}C.
- Swab without media stored at -80^{o}C.
- Swabs thawed at RT
- Transferred to 1.0 mL of M swab medium.
- Vortexed well for 30 seconds
- Transferred 200µl to microfuge tube
- Heated for 3 minutes at 97-98^{o}C.
- Quick vortex
- Spin 2 min at 14,000 rpm.

MSwab Nucleic Acid Extraction

1. Swab in UTM: 200 µl into easyMag extraction (bioMérieux) on board lysis

2. Swab head in MSwab medium, 200 µl boiled X 3 minutes

3. Each amplified with RealStar alpha Herpesvirus PCR Kit

easyMag vs. MSwab Method:

- Culture positive (n=74)
- UTM/easyMag: 73/74=98%, total 76 viruses
  - One negative VZV (non-repeatable)
  - One dual VZV/H1
  - Two extra VZV/H2
- MSwab: 73/74=98%, total 75 viruses
  - One negative VZV (non-repeatable)
  - Missed VZV in dual VZV/H1
  - Two extra VZV/H2 (concordant with easyMag)

easyMag vs. MSwab Method:

- **Culture negative (n=61)**
- **UTM/easyMag: 13 positive (21%), 14 viruses**
  - **One extra VZV**
  - **Two extra HSV1**
- **Mswab: 11 positive (18%), 12 viruses**
  - **One extra HSV1**

Summary of MSwab Extraction Method

- Rapid and inexpensive method
- High agreement between easyMag and MSwab
- Detected 86 (easyMag) vs. 84 (MSwab) positive patients
- Detected 90 (easyMag) viruses positive vs. 87 (MSwab)

Acknowledgements:
altona Diagnostics
Copan Italia


always a drop ahead.
altona
DIAGNOSTICS