AAD Annual Meeting 2017:

Varicella-Zoster Virus: Diagnosis

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Varicella Zoster

Diagnosis

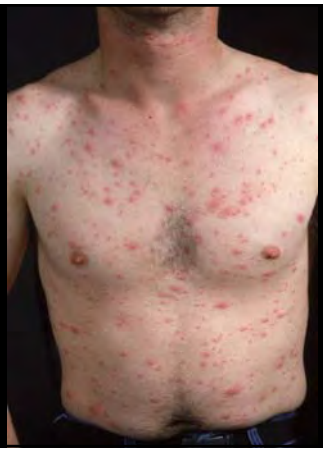
www.giantmicrobe.com

Varicella Zoster

- Primary infection = "chickenpox"
  - crops of vesicular lesions
  - centripetal predominance
- Reactivation from ganglia = "shingles"
  - painful, unilateral vesicular eruption
  - most often dermatomal
- Most often both conditions are often diagnosed on clinical grounds, and this is ok

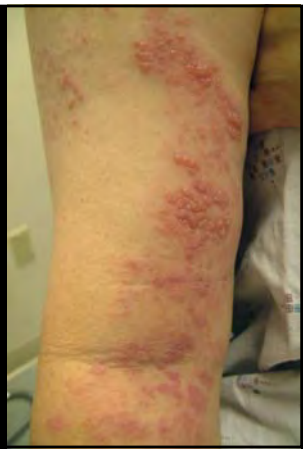
"Chickenpox"

truncal predominance (centripetal)



"Shingles"

extending down arm



VZV

Ancillary Diagnostic Techniques

Biopsy – cytopathic effect under H&E examination

Tzanck – sensitive in experienced hands

DFA – rapid, allows for diff. of VZV from HSV

Culture – fastidious technique, ≥ 5 days for results

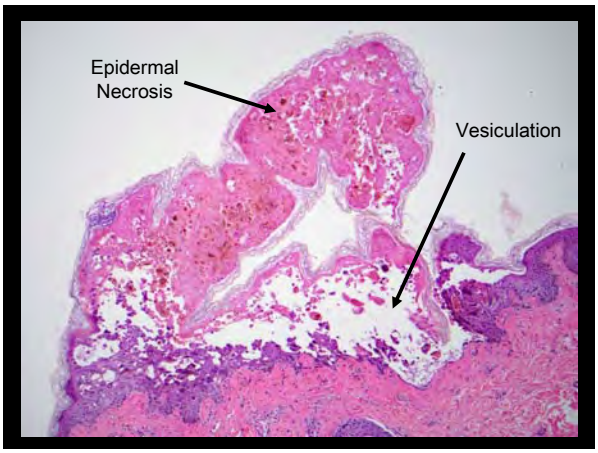
Antibodies – IgM rises first, IgG is sign of immunity

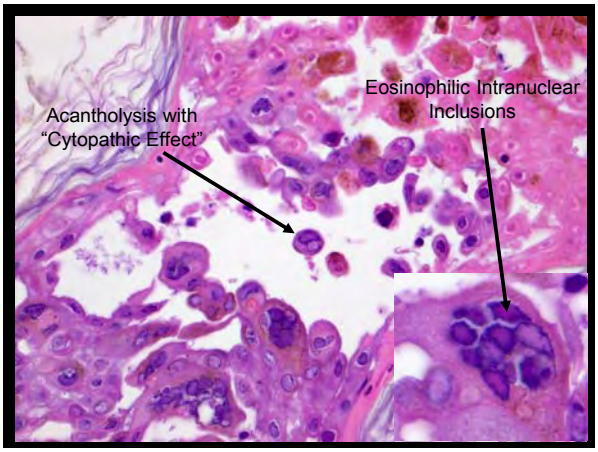
Slit-lamp – performed by specialists in VZO

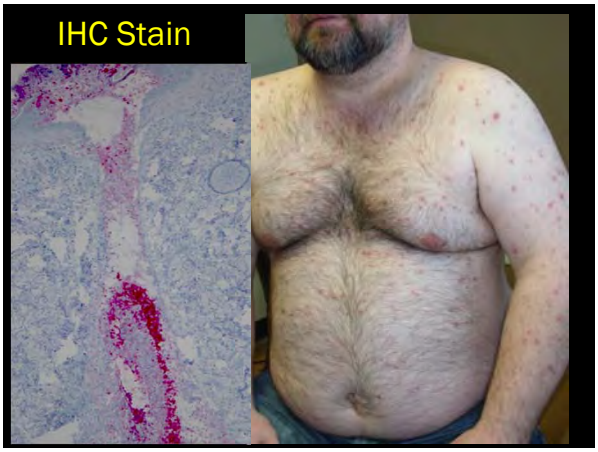
PCR – 4x more sensitive, increasing in popularity

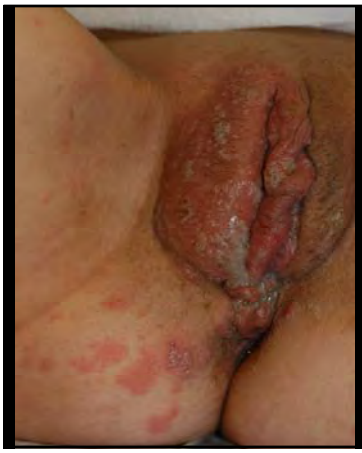
Typical Biopsy Findings

H&E stain alone
(cytopathic effect indicative of *Herpes* family infection)

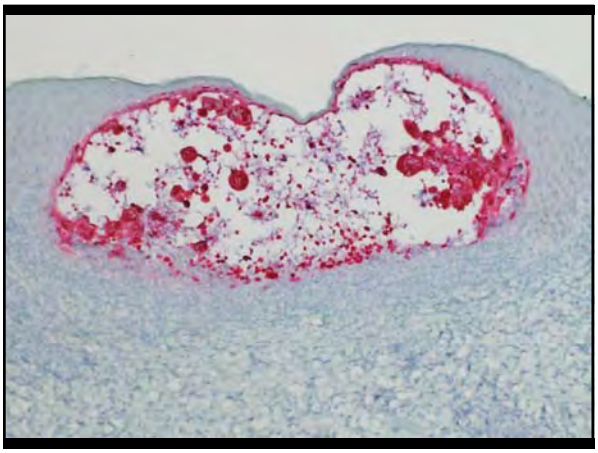


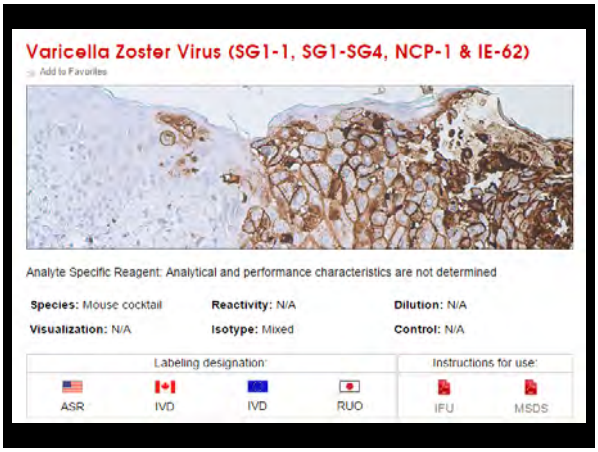


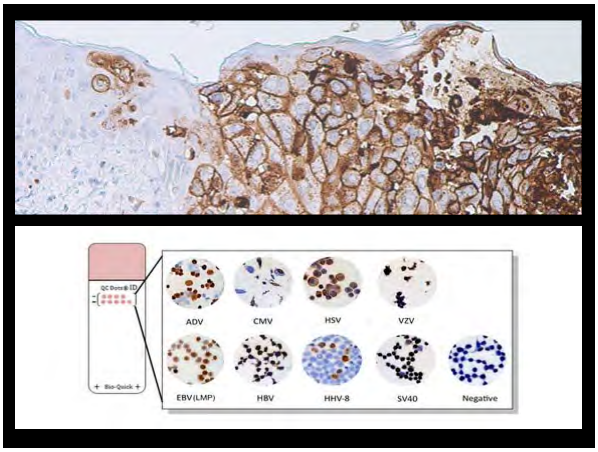




Varicella Zoster







What is an "analyte specific reagent" (ASR)?

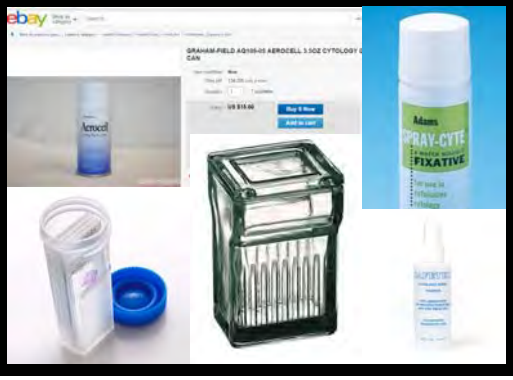
- FDA defines ASR as "antibodies… intended for use in a diagnostic application… in biological specimens."
- An ASR is the active ingredient of an in-house test, and ***must be labeled as such*** in a report
- It requires a validation and disclaimer

Tzanck Prep
Bedside Cytology

Dr. Arnauld Tzanck, 1886-1954

- Locate a fresh lesion and clean the area gently
- Unroof blister - scrape roof/base with #15 blade
- Transfer material to glass slide
- "Fix" with couple sprays of cytofix (preferred)
  - or with 95% EtOH, or gentle heat, or air dry
- Stain with Wright/Giemsa for about 40-60 sec
- Rinse gently and carefully dry slide by blotting
- Add drop of immersion oil and cover slip

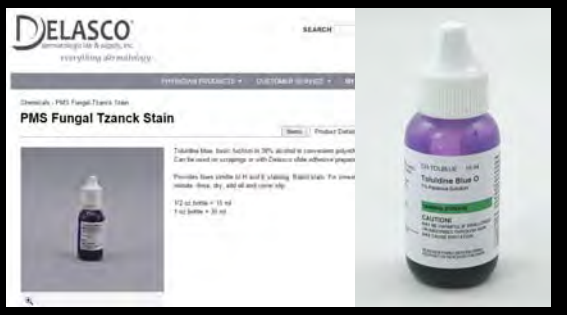
Methods of Fixation



When nothing else is around?

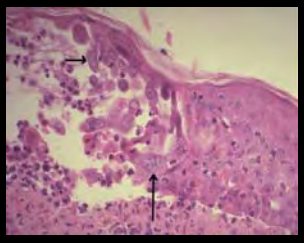
- My "Kit" – #15 blade, glass slide, alcohol pads (kind used for injections), and shoes

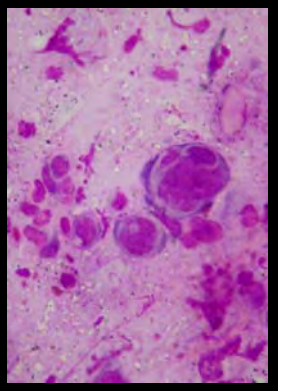
Tzanck stains

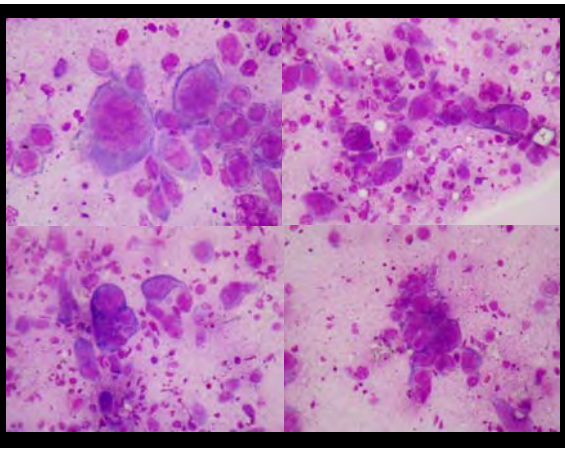


Tzanck Findings

- 80% sensitvity
- 90% specificity



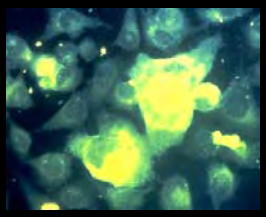




DFA Results

Allows for discrimination of: HSV1, HSV2 and VZV

- Lower cost (relative to culture)
- Rapid turn around (90 minutes)



Antibody Tests - IgM

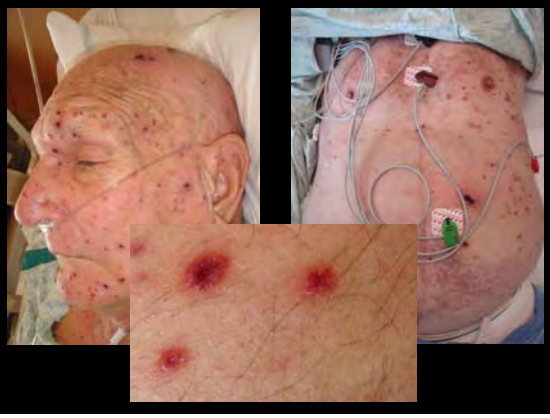
- IgM rises in acute infection (and in zoster)
- IgM testing is ***LESS*** sensitive than PCR
- Commercial IgM test can give false negative
- A positive IgM ELISA result does not exclude re-infection or reactivation (zoster)
- A positive IgM result from a person with characteristic rash is usually interpreted as primary varicella (but it doesn't have to be)

CDC Admonishment

- "Patients with zoster may mount an IgM response and are expected to mount a memory IgG response."
- Positive IgM by ELISA can be indicative of:
  - primary VZV infection
  - or reactivation (zoster)
- It is difficult to detect an increase in IgG for laboratory diagnosis of zoster as patients may have a high baseline antibody titers

Antibody Tests - IgG

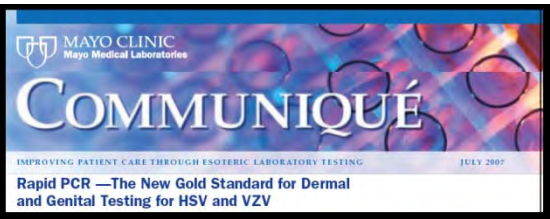
- A positive IgG ELISA indicates antibodies from past wild-type infection or vaccination
- No commercial assay can detect immunity in all vaccinated persons (lacks sensitivity)
- IgG – positive result indicates immunity
- Paired acute and convalescent sera w/ 4x rise in IgG has excellent specificity for varicella, but is not as sensitive as PCR



More from the CDC…

"In people with compromised immune systems, it may be difficult to distinguish between varicella and disseminated herpes zoster by serological testing or even by physical examination.

In these instances, a history of VZV exposure or of a rash that began in a dermatomal pattern, with results of VZV antibody testing at/before the time of onset may help guide the diagnosis."



"… discontinued culture for these viruses…"

- PCR on swabs from lesions
- Same day results

More about PCR…

- PCR genotyping – distinguishes wild-type VZV from vaccine type (Oka)-strain
- May be performed on blood, cerebrospinal fluid, or biopsy specimens
- Fast, cheap, and has the highest sensitivity of ***ALL*** diagnostic techniques

Methods of Skin Sampling

- Polyester Swab Method
  - a sterile needle is used to un-roof the vesicle
  - vigorously swab the base of the lesion, without causing bleeding
  - collect epithelial cells from the base of the lesion (highest concentration of virus)
  - avoid cotton and wooden swabs as these absorb extraction buffer and inhibit PCR

Methods of Skin Sampling

- Glass Slide Method
  - rake the edge of the slide over the lesion, abrading the lesion
  - ensure that skin cells are gathered
  - swab the abraded lesion and collect the material also
  - dried maculopapular lesion material is stable for several weeks at ambient temperature

Methods of Skin Sampling

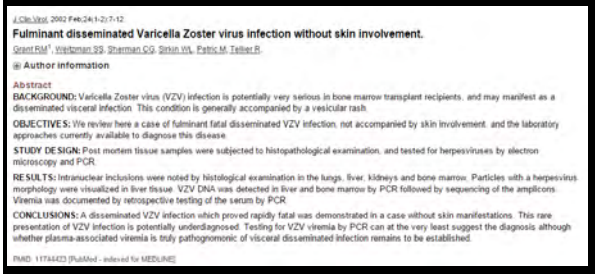
- Collecting Crusts (Scabs)
  - crusts are also excellent for PCR detection
  - crusts can be lifted off with glass slide
  - transferred directly into break-resistant, snap-cap or screw-top tubes
  - shipped per “shipping instructions” to CDC

CDC Video available at:
http://www.cdc.gov/shingles/lab-testing/collecting-specimens.html

IMAGES IN CLINICAL MEDICINE

Disseminated Zoster

PCR does not have to be done only on skin…



J Clin Virol. 2002 Feb;24(1-2):7-12.

Fulminant disseminated Varicella Zoster virus infection without skin involvement.

Grant RM[1], Weitzman SS, Sherman CG, Sirkin WL, Petric M, Tellier R.

Author information

Abstract

BACKGROUND: Varicella Zoster virus (VZV) infection is potentially very serious in bone marrow transplant recipients, and may manifest as a disseminated visceral infection. This condition is generally accompanied by a vesicular rash.

OBJECTIVES: We review here a case of fulminant fatal disseminated VZV infection, not accompanied by skin involvement, and the laboratory approaches currently available to diagnose this disease.

STUDY DESIGN: Post mortem tissue samples were subjected to histopathological examination, and tested for herpesviruses by electron microscopy and PCR.

RESULTS: Intranuclear inclusions were noted by histological examination in the lungs, liver, kidneys and bone marrow. Particles with a herpesvirus morphology were visualized in liver tissue. VZV DNA was detected in liver and bone marrow by PCR followed by sequencing of the amplicons. Viremia was documented by retrospective testing of the serum by PCR.

CONCLUSIONS: A disseminated VZV infection which proved rapidly fatal was demonstrated in a case without skin manifestations. This rare presentation of VZV infection is potentially underdiagnosed. Testing for VZV viremia by PCR can at the very least suggest the diagnosis although whether plasma-associated viremia is truly pathognomonic of visceral disseminated infection remains to be established.

PMID: 11744423 [PubMed - indexed for MEDLINE]

Thank you.