Varicella-Zoster Virus: Diagnosis

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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

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)

- Epidermal Necrosis
- Vesiculation

Acantholysis with "Cytopathic Effect"

Eosinophilic Intranuclear Inclusions

Varicella Zoster

IHC Stain
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

- 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

Findings

- 80% sensitivity
- 90% specificity

DFA Results

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

Allows for discrimination of: HSV1, HSV2 and VZV

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.”

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

• 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

Thank you.