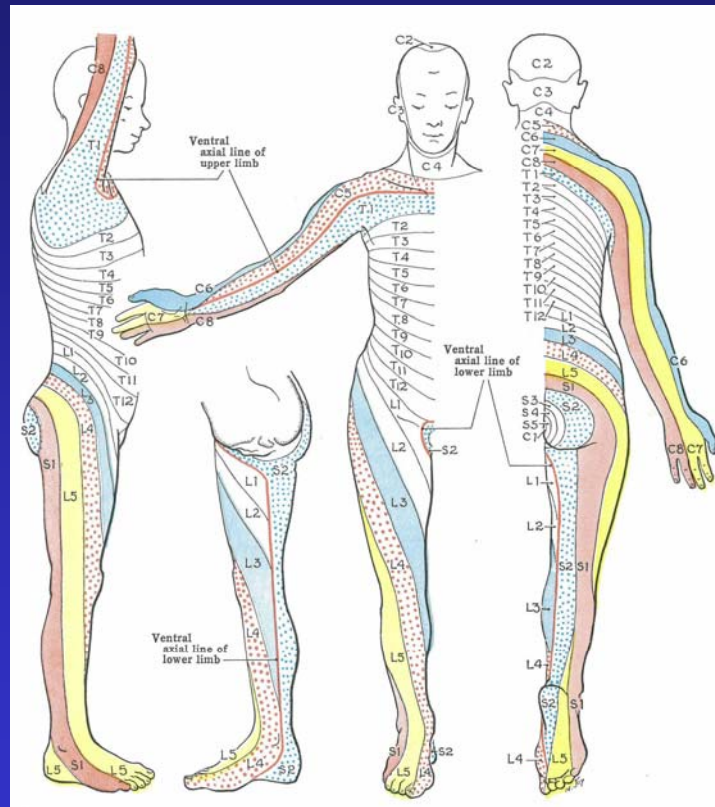
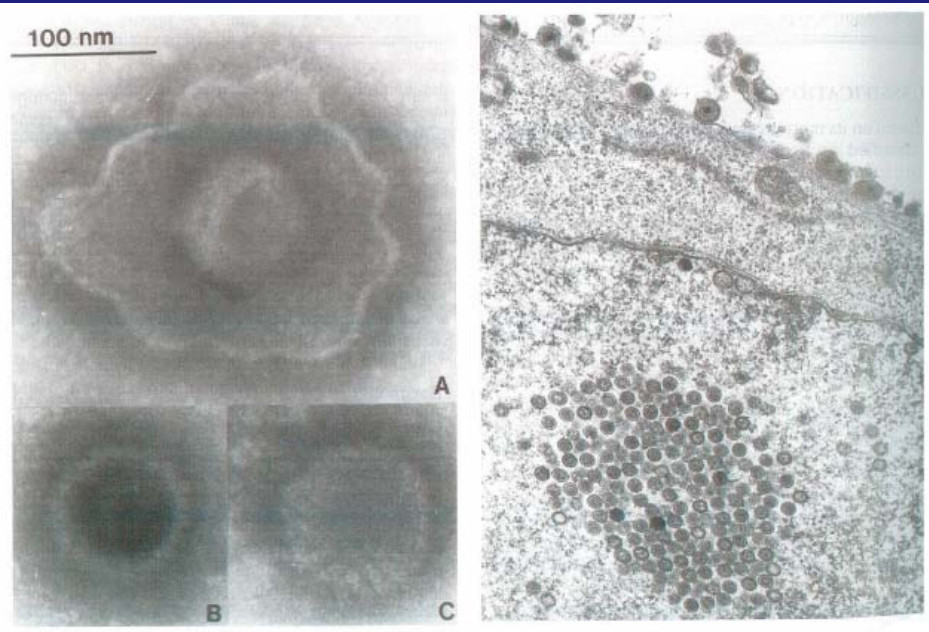


# Diagnosis of Varicella Zoster Virus Infection



Dr Mike Catton  
Director, VIDRL

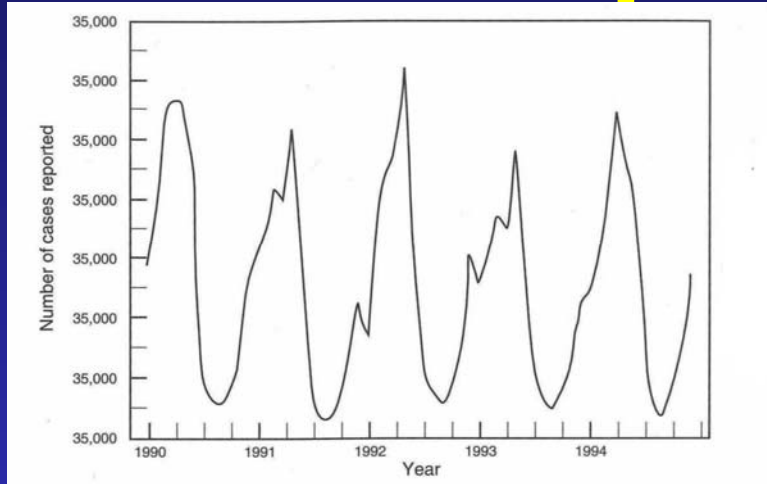
# Varicella Zoster Virus: Virology



- 150-200nm typical herpes particle
- Icosahedral capsid 162 capsomeres
- Ds linear DNA 125 kb, 68 ORFs
- Lipid envelope with glycoprotein spikes
- Single serotype
- Replication largely restricted to primates
- Labile, slow growing *in vitro*

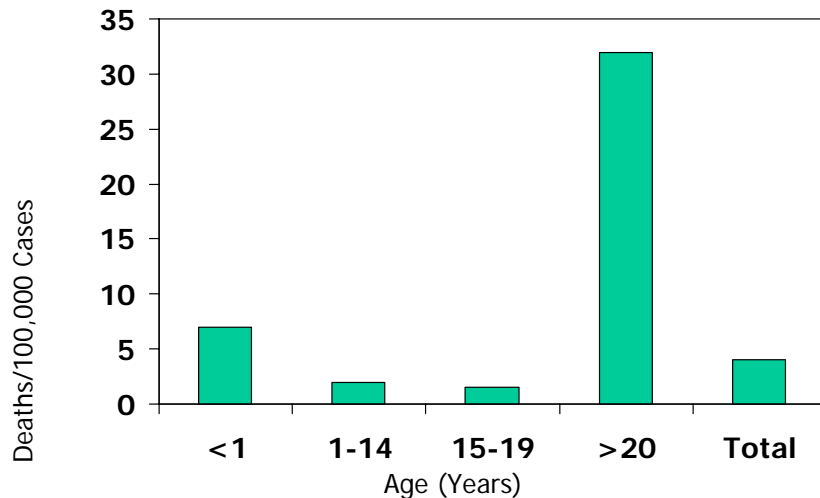
Cohen JI, Straus SE. In: Fields Virology, 4<sup>th</sup> Ed, 2001.

# Varicella Zoster Virus: Epidemiology



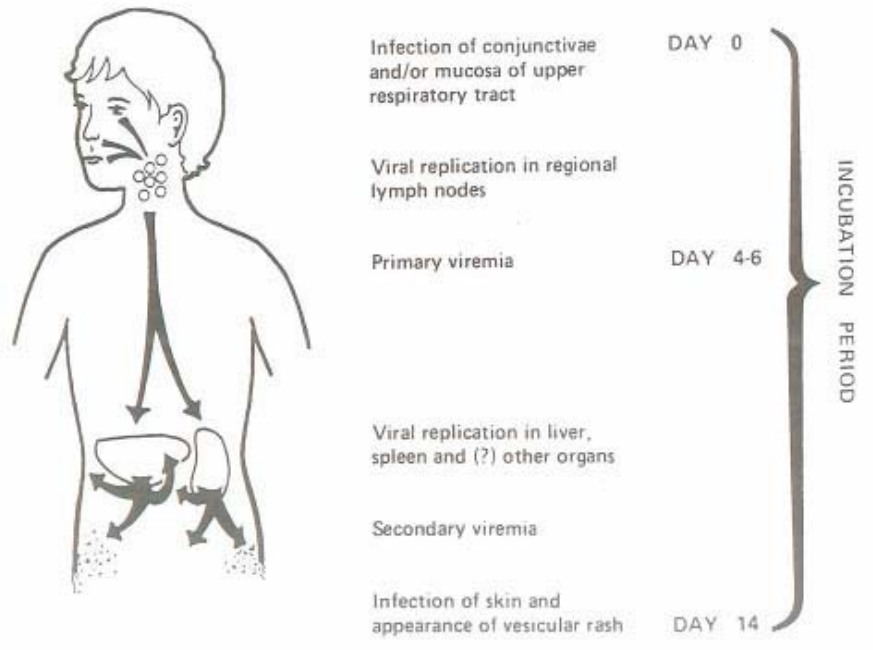
- Endemic
- Winter/early spring seasonality
- Primary infection mostly children < 10 years
- 95% adults seropositive, (75% no VZ history immune)
- 2° household attack rate 60-90%

From Wharton M. Infect Dis Clin North Am 10:571-581, 1996.



From Preblud SR. Pediatrics 1986; 78:728-35.

# Varicella: Pathogenesis



- Respiratory transmission
- Transmission 4 days before rash to 5 days after
- 10-21 day incubation period
- Fenner's mousepox model
- Less cell destructive than HSV
- CMI important to immunity
- Latency in sensory nerve ganglia

Gershon AA, Silverstein SJ. In: Clinical Virology 2<sup>nd</sup> Ed, 2002.

# Varicella: Clinical Presentation



American Society of Microbiology

- Several days flu-like prodrome in older children/adults
- Cropping rash trunk/scalp to periphery
- Evolves macules-vesicles-crusts over 8-12 hr
- Mild fever, headache malaise
- Increased severity in adults/immunosuppressed
- Complications: skin infection, CNS involvement, pneumonitis
- Severe neonatal disease if mother infected 2 days before – 4 days after birth



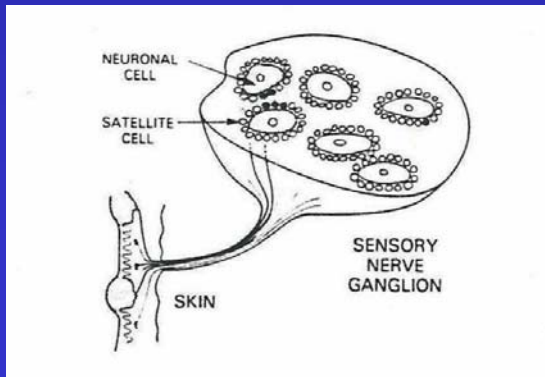
Hong Kong Department of Health

# Zoster: Pathogenesis and Clinical Presentation



Whitley RJ. In: Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases 6<sup>th</sup> Ed, 2005.

- Incidence 10-20%
- Declining CMI and humoral immunity increase risk
- Virions travel down sensory neuron to skin
- Prodrome of pain/paraesthesia 1-4 days
- Unilateral vesicular rash confined to a sensory dermatome usually thoracic or trigeminal nerve (ophthalmic)
- Complications
  - \* dissemination in immunosuppressed
  - \* motor neuropathy
  - \* pneumonitis
  - \* post-herpetic neuralgia





# Varicella Zoster Complications

## Skin infection

- Secondary bacterial infection of vesicles
- Most common VZV complication

## CNS infection

- Acute cerebellar ataxia 1/4000 cases < 15 years
- Encephalitis 0-2% cases

## Pneumonitis

- Potentially life threatening specially in pregnant women
- 0.25% cases

## Past-herpetic neuralgia

- Chronic pain occurring in 25-50% patients over 50 years

## Congenital Varicella Syndrome

- Occurs in <5% infants born to mothers with primary varicella, usually 1<sup>st</sup> trimester
- Cicatricial skin lesions, limb deformities, ocular abnormalities, CNS disease



# Varicella zoster in the immunosuppressed

- Impaired CMI increases risk of severe disease
- HIV, malignancies esp haematological, esp. BMT
- Varicella may disseminate and/or recur
  - Extensive/prolonged skin lesions
  - Pneumonia
  - Hepatitis
  - Encephalitis
- Zoster may disseminate and/or recur
  - Extensive/prolonged skin lesions
  - Pneumonia
  - Encephalitis
  - Necrotizing retinopathy



# Perinatal Varicella

- Maternal chickenpox around delivery may be life threatening for baby
- Baby's illness severity relates to timing of maternal illness
- Worst outcome if baby infected transplacentally, then born before maternal IgG develops
- Baby IM ZIG candidate if:  
maternal chickenpox 5 days before to 2 days after delivery

Timing of chickenpox in mother and baby in relation to severity				
Onset of mother's rash in relation to delivery	Onset of baby's rash in relation to delivery (days)	No died	No survived	Neonatal mortality (%)
5-21 days before	0-4	0	27	0
4 days before to 2 days after	5-10	7	16	30

# Varicella Zoster: Diagnosis

- Clinical diagnosis suffices for most varicella and zoster cases.
- Laboratory diagnosis appropriate
  - for patient management:
    - Immunosuppressed patient
    - Varicella in a neonate
    - Zoster in a young person
    - Atypical/unusual clinical presentations
    - Significant clinical complications
  - for infection control/public health:
    - Index case in a nosocomial exposure event
    - Differentiation from smallpox in very selected cases

# Varicella Zoster: Specimen Collection and Transport

## Detection of virus:

- Vesicle fluid/infected cells from lesion base
- Suitable for IFA, EM, PCR, viral culture
- Deroofing fresh lesion and firm swabbing
- Viral transport medium
- Labile in transport
- Variable recovery from CSF, plasma, urine, t/s

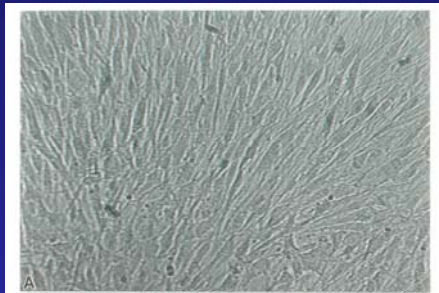
## Serology:

- Acute phase clotted blood

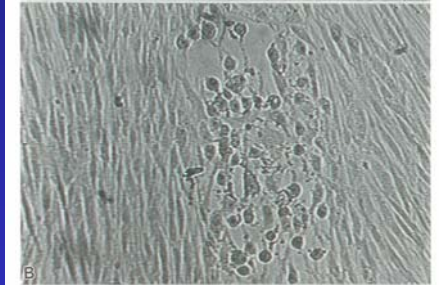
# Varicella Zoster: Viral Culture

## Cytopathic effect (CPE) in HDF Cell Culture

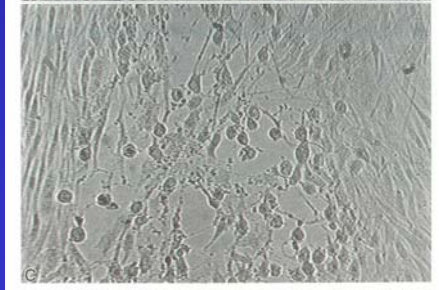
Uninfected



3 days post inoculation



6 days post inoculation



### TUBE CULTURE

- Labile virus, transport delay further compromises recovery
- Grows only in human diploid and monkey kidney cell lines
- Approximately 1 week to positivity
- Characteristic CPE
- Confirmation usually by scraping and IFA

### RAPID CULTURE

- Fibroblast cultures on coverslips or in multiwell trays
- Acetone fixation several days after inoculation
- Staining with FITC conjugated anti-VZV glycoprotein mab
- More sensitive/rapid than conventional tube culture

Landry ML, Hsiung GD. In: Hsiung's Diagnostic Virology, 1994.

# Varicella Zoster: Immunofluorescent (IFA) Antigen Detection



- Direct staining of vesicular cell smears with FITC mabs to gp98-gp62
- Rapid, result available in hours
- More sensitive than conventional tube cell culture (97.5 vs 49.4%)
- Critically depends on specimen quality (numbers of infected cells)
- Low throughput
- Subjective: requires skilled staff

# Varicella Zoster: Electron Microscopy



- Typical herpes virions readily visualised from vesicle fluid
- Negative staining with PTA or UA
- Rapid but labour-intensive
- Role limited to differential diagnosis of smallpox in highly selected cases



# Varicella Zoster: Nucleic Acid Testing

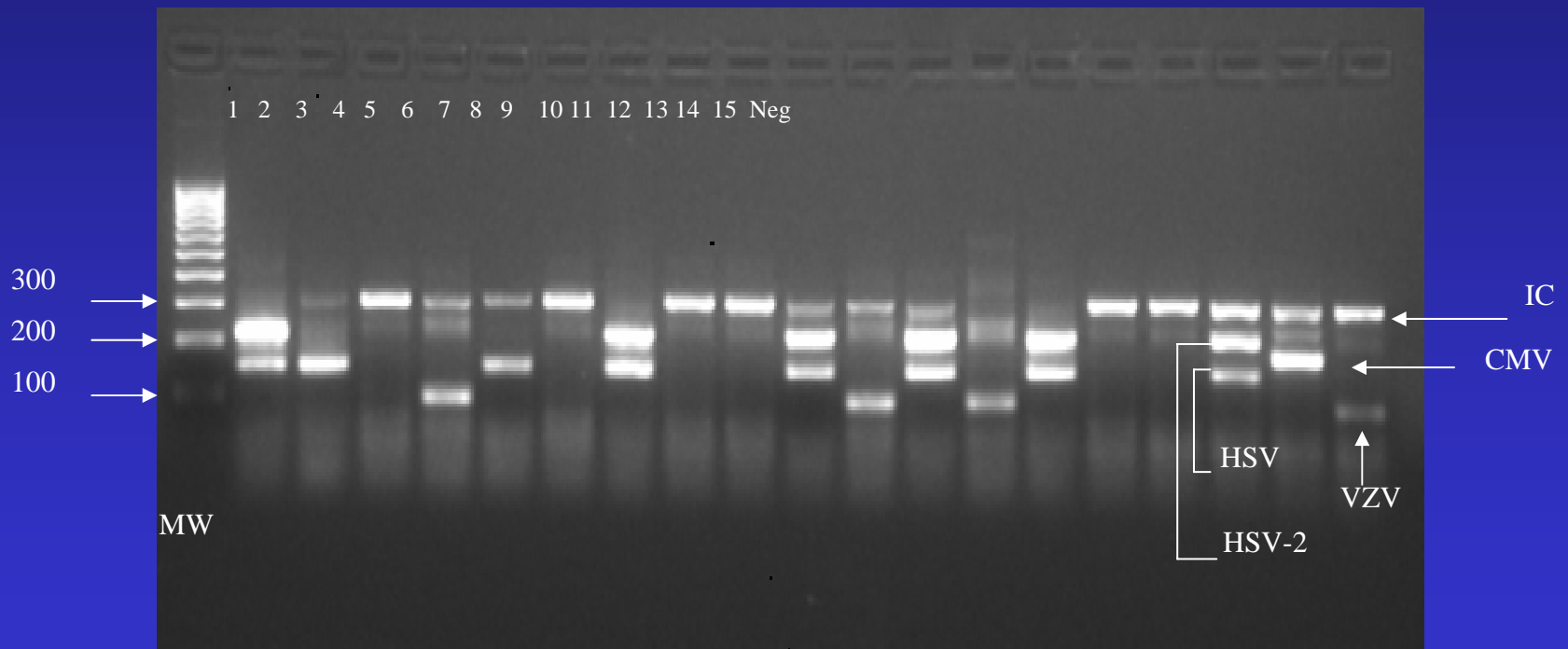


- Assay of choice for detection of VZV
- Many gene targets used for PCR: pol, IE etc
- Analytical sensitivity of 1-10 target copies possible
- Variety of assay formats
  - nested PCR and electrophoresis
  - real time SYBR green or probes
- Can multiplex with other viruses (HSV)
- Rapid: 6 hrs (nested) – 2.5 hrs (real time)
- Clinically sensitive and specific: CSF, ocular fluid, vesicle fluid, blood



# Nucleic Acid Testing for VZV

- Nested multiplex PCR for 4 herpes viruses: VZV, HSV-1, HSV-2, CMV



# Varicella Zoster: comparative sensitivity of diagnostic techniques from skin lesions

Sauerbrei et al (1999)

- 100 VZV infected patients
- 95 (95%) PCR
- 82 (82%) IFA
- 48 (48%) serology
- 20 (20%) culture

J Clin Virol (1999) 14: p31-36

Beards et al (1998)

- 132 vesicular lesions
- 53 VZV PCR pos, 64 HSV PCR pos
- Relative sensitivities:
  - 100% PCR
  - 60% EM
  - 47% culture

J Med Virol (1998) 54: p155-157

Schmutzhard et al (2004)

- 110 dermal samples
- 51 (46%) PCR (nested and real time)
- 15 (14%) culture

J Clin Virol (2004) 29: P120-126

Espy et al (2000)

- 253 dermal samples
- 44 (17%) PCR (real time, 2 primer sets)
- 23 (9%) shell vial MRC-5

J Clin Microbiol (2000) 38: 3167-3189

# NAT testing in assorted Varicella Zoster Complications

## CNS Infection

- VZV DNA detected in CSF of 10/12 VZV infected patients with CNS involvement
- 7/7 zoster patients with CNS symptoms PCR pos
- 3/5 post chicken pox cerebellitis patients PCR pos
- 2/5 PCR neg were high dose acyclovir treated

Puchhammer -Stockl et al (1991) J Clin Micro 29: 1513-1516

## Pneumonitis

- VZV DNA detection by PCR in BAL

Cowl C et al (2000) Am J Respir Crit Care Med 161: p753-754

## Post-herpetic neuralgia

- VZV DNA detected by PCR in PBMC 11/51 PHN patients
- VZV DNA detected by PCR in PBMC 019 zoster patients
- Role of VZV persistence?

Mahalingam R et al (1995) J Neurovirol 1: p130-3

## Congenital varicella syndrome

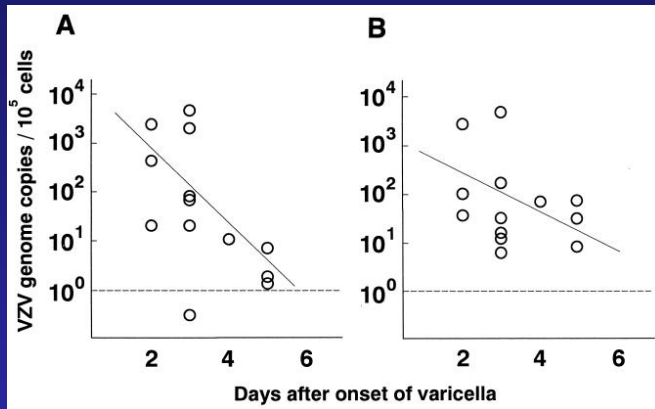
- 107 amniotic fluids from VZ infected pregnant women before 24/40
- 9/107 (8.4%) VZ PCR pos, 2/107 (1.8%) VZ culture pos
- 3/107 (2.8%) congenital varicella cases

Mouly F et al (1997) Am J Obstet Gynecol 177: p894-8

# PCR for VZV DNA in Human Vitreous

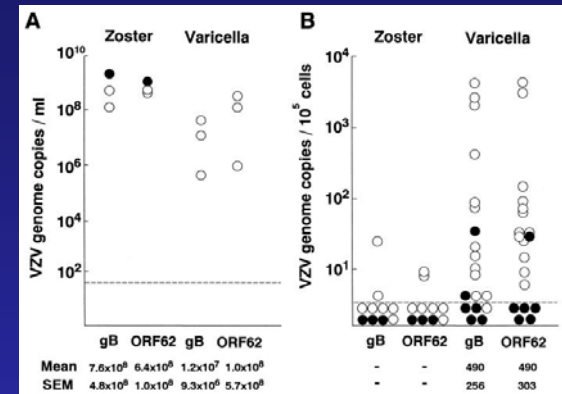
Clinical Diagnosis	No. of cases	No. Positive for VZV DNA
HIV positive		
VZV retinitis (PORN)	14	11
CMV retinitis	72	1
Other retinitis (CD4 count, [is less than] 1 00/[mm.sup.3])		
Presumed HIV negative		
Vitreoretinal inflammation	23	0
Vitreous hemorrhage	14	0
Macular hole/epiretinal membrane/retinal detachment	38	2

# Varicella Zoster: Virus Load in the Blood



Viral loads and days after onset in patients with varicella.

J Clin Microbiol. 2000 June; 38(6): 2447–2449.



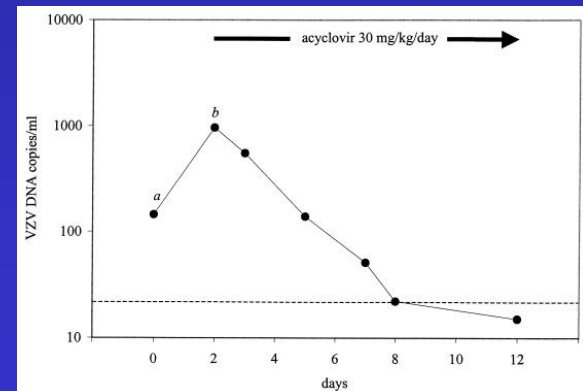
Quantitation of VZV DNA in vesicles and PBMC. (A) Vesicle fluid obtained from three patients with zoster or varicella. (B) PBMC obtained from 10 patients with zoster and 19 patients with varicella.

J Clin Microbiol. 2000 June; 38(6): 2447–2449.

Comparison of viral loads in PBMC between acyclovir-treated and untreated groups

Treatment	No. of patients <sup>a</sup>	Days after onset (mean ± SEM)	No. of VZV genomes (mean ± SEM/ $10^5$ cells) determined with:	
			gB gene	ORF62
Acyclovir	5	4.0 ± 0.3	7 ± 7 <sup>b</sup>	5 ± 5 <sup>c</sup>
None (untreated)	10	3.7 ± 0.3	510 ± 354 <sup>b</sup>	382 ± 339 <sup>c</sup>

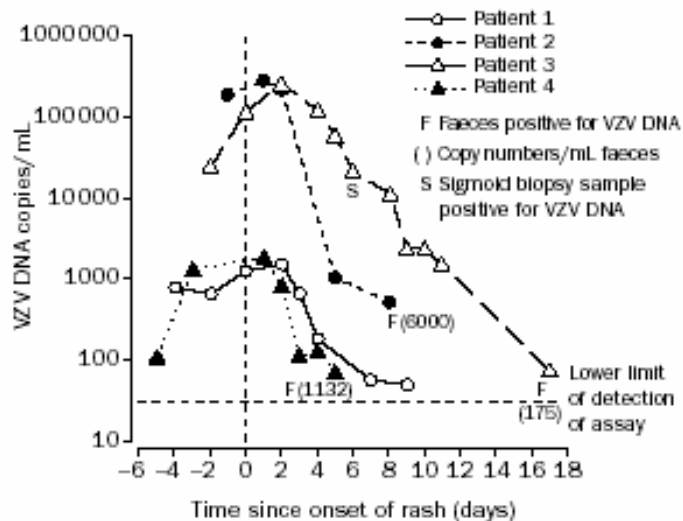
J Clin Microbiol. 2000 June; 38(6):2447-2449



Q-PCR of seven consecutive plasma specimens from a patient who progressed from unidermatomal herpes zoster.

J Clin Microbiol. 2000 July; 38(7): 2568–2573.

# Varicella Zoster Virus: NAT in visceral zoster



- Disseminated VZV may present with severe abdominal pain before/without rash
- Especially in bone marrow transplant
- Grave outcome if diagnosed and treated as GVHD
- VZV retrospectively shown present in blood prior to rash in 4 subjects
- VZV present in blood/faeces after rash

de Jong et al (2001) Lancet 357 p2101-2102

# Varicella Zoster: Misdiagnosis as Herpes Simplex

Zoster: face and leg

- 110 patients with vesicular eruptions
  - Clinical diagnosis HSV (45) and zoster (65)
  - 65/65 clinical zoster diagnoses confirmed by VZ PCR
  - 36/45 clinical HSV diagnoses confirmed by HSV PCR
  - 9/45 (12%) clinical HSV diagnoses VZ PCR pos
  - Small eruptions periocular/nasolabial groove/thigh

Rubben et al (1997) Br J Dermatol 137: p259-261

Zoster: genital region

- 6210 patients with genital lesions; 2225 PCR pos
- 2185 (97%) HSV, 65 (2.9%) VZV
- 40/65 female, variety genital sites both sexes
- 5 clinically diagnosed VZV, 10 uncertain HSV/VZV
- VZV clinical diagnosis more common in children/elderly

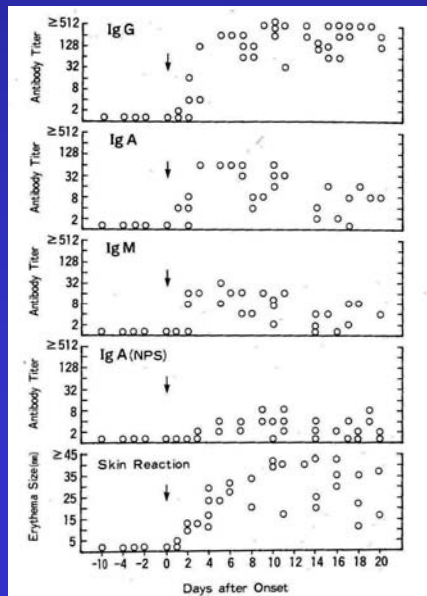
Birch et al (2003) Sex Transm Inf 79: p298-300



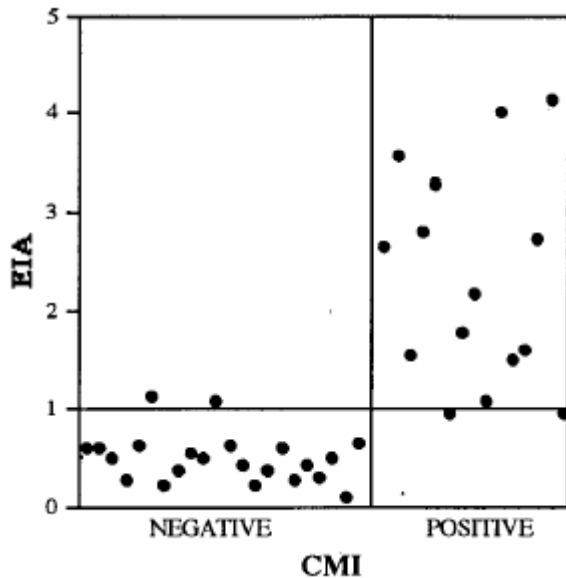
# Varicella Zoster: Serology



- IgM and IgG responses measurable within days of rash
- Many assay formats: IFA, EIA etc
- Adjunct to virus detection only
- Immunity of interest in at-risk exposures
  - Pregnant women
  - Nosocomial exposure
- Rapid formats often used eg latex agglutination



# Varicella Zoster: Determination of Immunity



- Fluorescent anti-membrane antigen (FAMA) is serological gold standard
- EIA and LA correlate well and widely available
- EIA and LA validated against VZV CMI
- EIA (BioWhittaker)
  - 87% sensitive
  - 91% specific
  - 87% PPV
  - 91% NPV
- Performance improved using "grey zone" of VI 0.9-1.2
- LA (Becton Dickinson)
  - 96% sensitive
  - 91% specific
  - 97% PPV
  - 90% NPV
- Performance improved using 1:8 dilution rather than 1:2

Weinberg A et al (1996) J Clin Microbiol 34: p445-6.

Weinberg A et al (1996) J Clin Microbiol 34: p445-6.