Variola virus & smallpox: Past, present, or future tense?

- Orthopoxvirus Laboratory (DVRD/NCID/CDC)
- World Health Organization Reference Center for Orthopoxviruses

Inger Damon: Lab Director

Yu Li, Richard Kline

Russell Regnery

Poxviruses (I)

- Large complex “brick shaped” virions
- Double stranded DNA
- Cytoplasm of host cells is the ONLY environment permissive for growth

Poxviruses (II)

2 Subfamilies:

Chordopoxvirinae (vertebrate poxviruses)

- Orthopoxvirus (variola, cowpox, vaccinia, monkeypox, raccoonpox, camelpox, skunkpox, volepox, ectromelia, taterapox)
- Parapoxvirus (orf, pseudocowpox, …)
- Avipoxvirus (canarypox, fowlpox…)
- Capripoxvirus (goatpox, lumpy skin disease…)
- Leporipoxvirus (myxoma, fibroma…)
- Molluscipoxvirus (molluscum contagiosum)
- Yatapoxvirus (tanapox, Yaba monkey tumor)

– Entomopoxvirinae (insect poxviruses)

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Orthopoxviruses: Spectrum of human disease in normal host

- **Localized infections: vaccinia, cowpox**
- **Systemic illness: monkeypox, variola**

vaccinia vs. variola

- 96% nucleotide identity
- Essential virion proteins with >98% AA identity
- Envelope (glyco)proteins important in humoral recognition over 93% AA identity
- Proteins predicted to, or demonstrated to be involved in immune evasion or host range demonstrate a greater range of homology
  - Variola encodes 24 ORFS whose Vaccinia homologs are truncated or absent
  - Vaccinia encodes 7 ORFS whose Variola homologs are truncated

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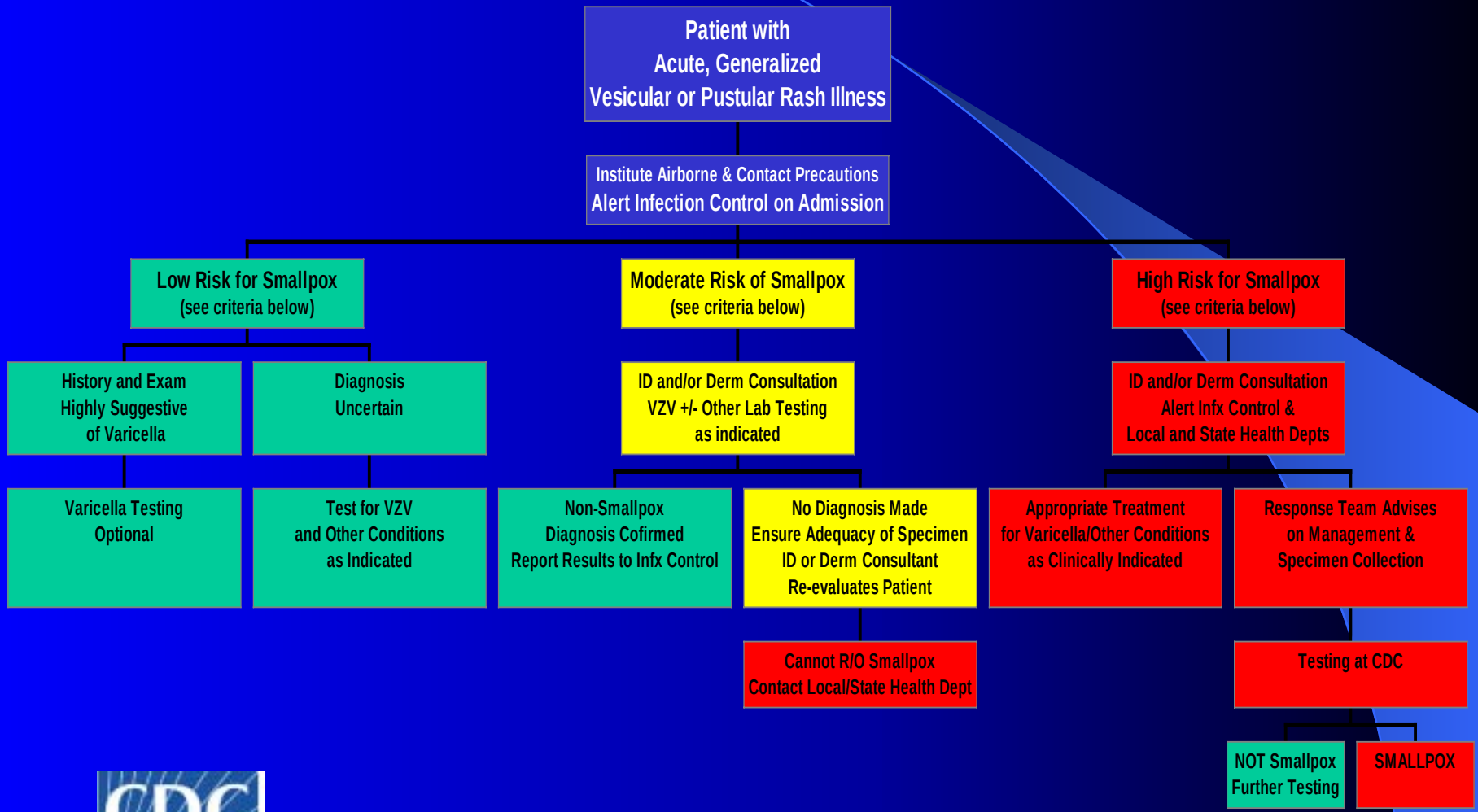
Febrile, vesicular rash illness algorithm



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Diagnostic aims and goals

- Mitigate generation of false-positive results
- Provide laboratory capacity to confirm causes of febrile, vesicular, rash illness, including smallpox
- What combination of diagnostic(s) confers adequate sensitivity and specificity?
- How best to determine what is appropriate at the various levels (under different scenarios)?
  - "pre-event", probability of smallpox is remote
  - "post-event", vaccination would be implemented

Overview: laboratory methods for confirmation of Orthopoxvirus dx

- Virus culture
- Immunohistochemistry
- Electron microscopy
- Various PCR
- Serology
  - Antigen detection (IFA, EIA ag capture)
  - IgM capture
  - Neutralization antibodies **
  - IgG ELISA **

What about the Past? and Future??

Virus Culture: The measure of infectious virus

- The gold standard to which all measures of sensitivity are measured.
- Important source of reference material for analysis (e.g., detailed DNA analysis)
- If it isn't infectious, it isn't infectious (hoax scenario implications)
- N.B. Issues associated with culture as a diagnostic (facilities and treaty).

PCR strategies

- Essential, conserved genes (E9L, A25R)
  - Difficult to discriminate amongst species of orthopoxviruses (i.e. vaccinia and variola): typically species generic
  - Unlikely to be manipulated

- Nonessential, variable genes (HA, ATI, crmB)
  - Able to discriminate amongst species of orthopoxviruses: species specific
  - Sources of potential manipulation

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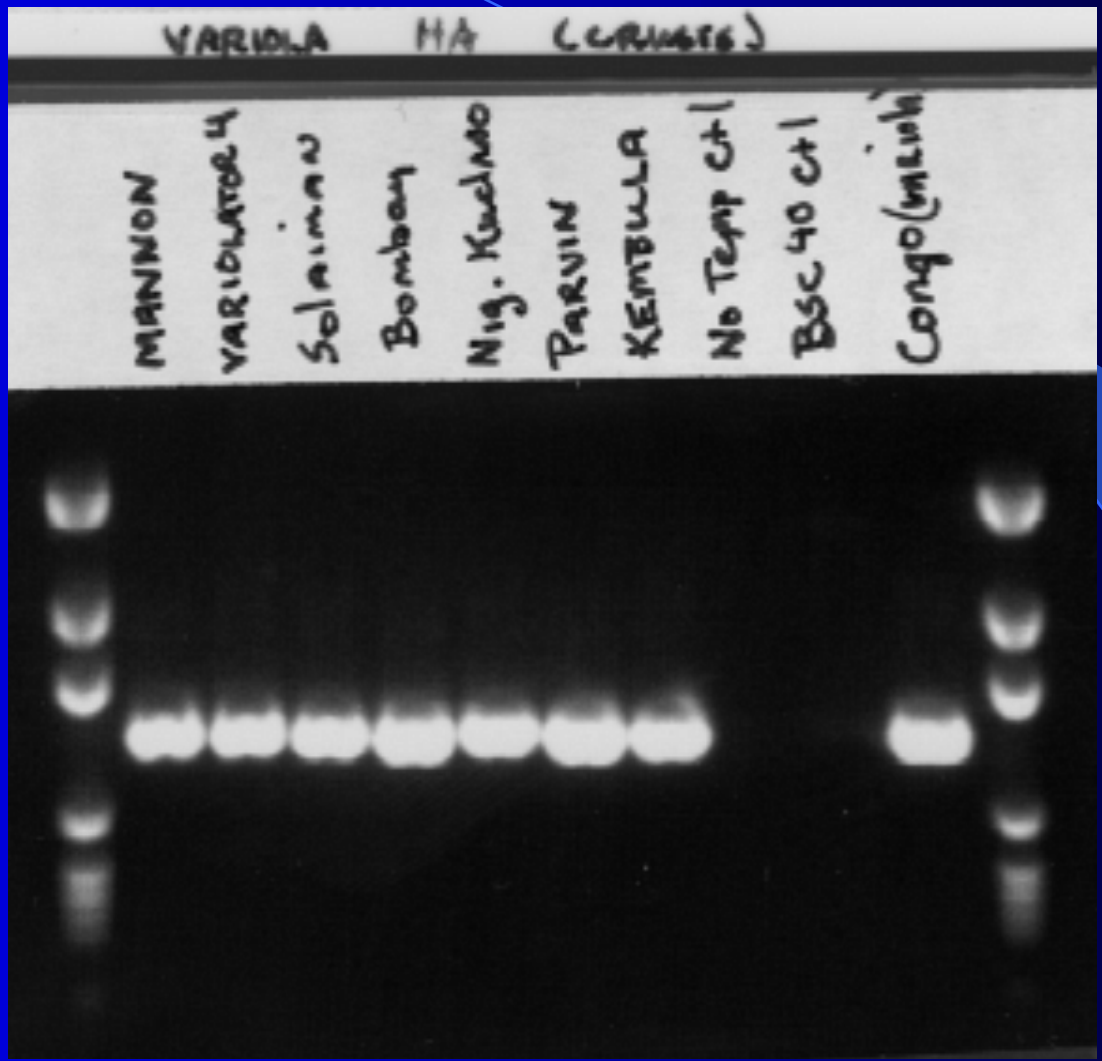
HA locus PCR: Variola scabs/crusts



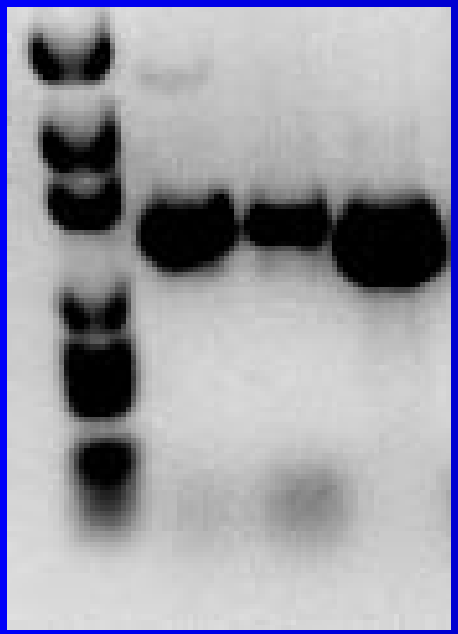
Sensitivity: 8/8

Correlates with > day 10 rash

PCR: Species generic

HA locus (5-6 hrs)

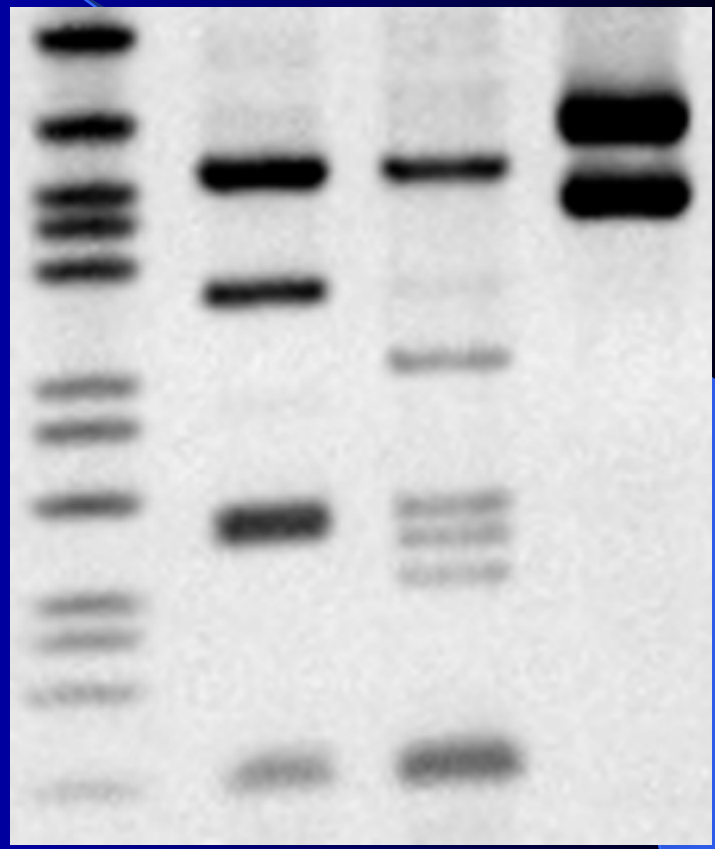
VAC MPX VAR



+ enzyme: *Taq*I

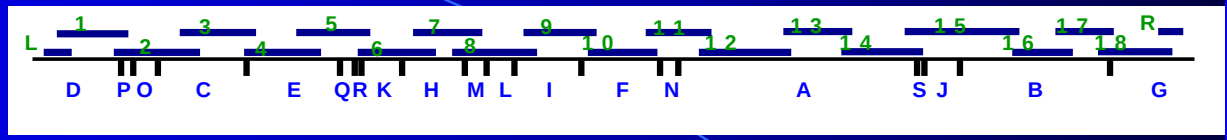
7-8 hours total

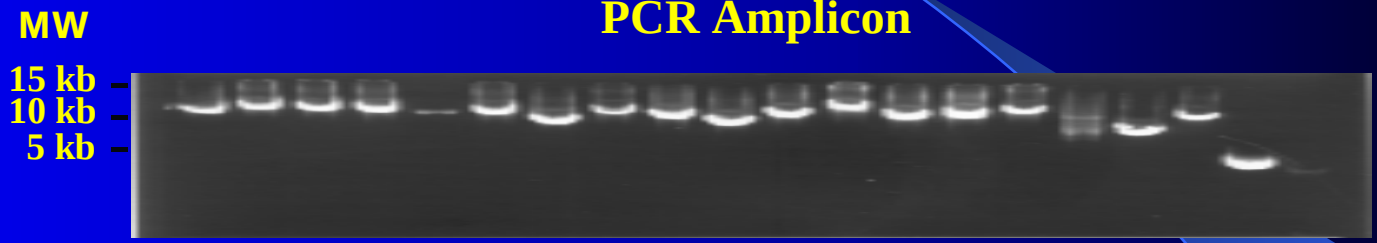
VAC MPX VAR

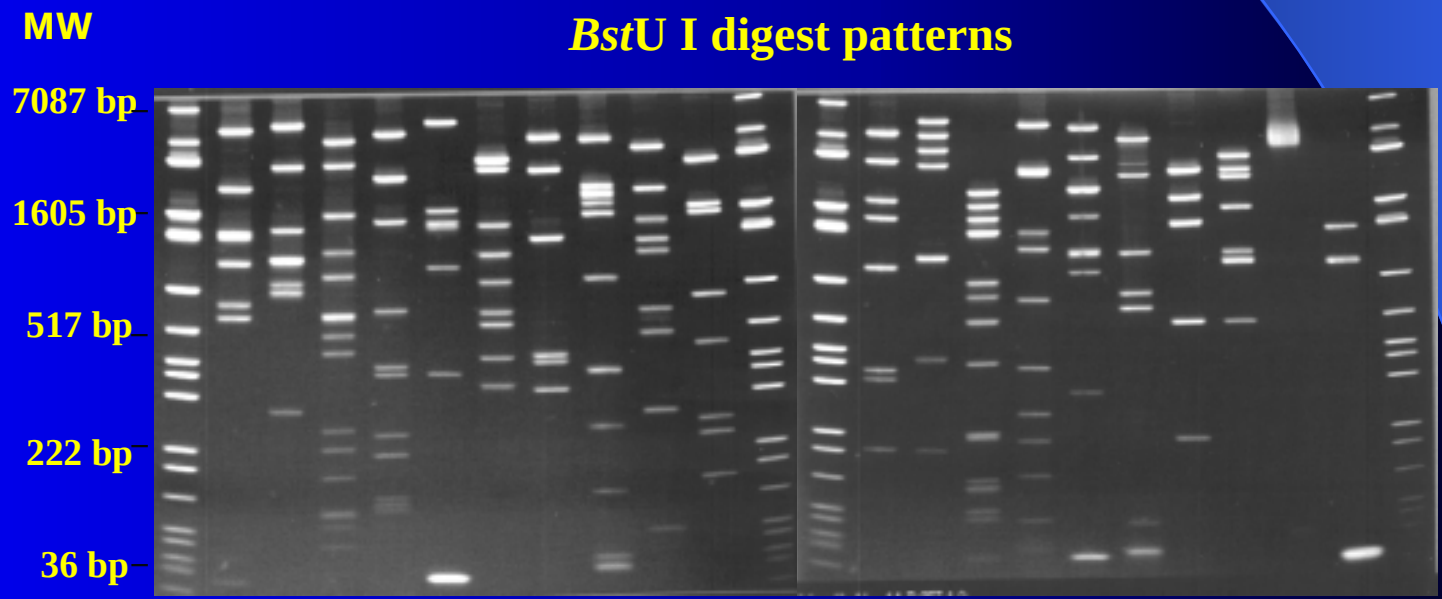


RFLP: Species specific

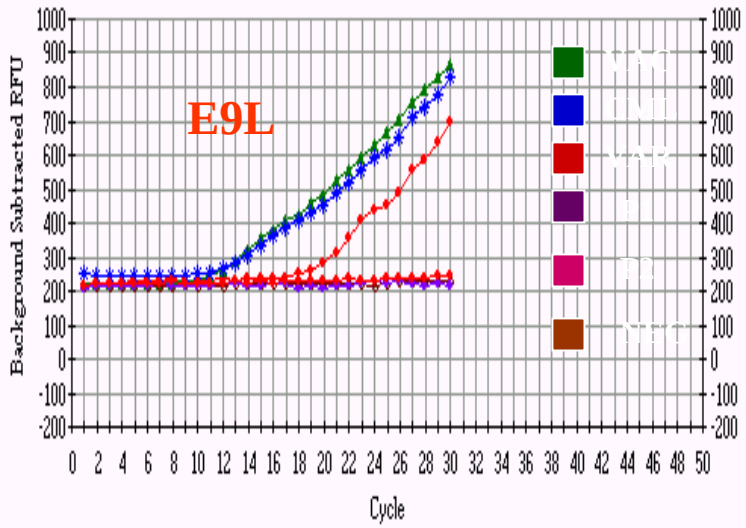
Extend-PCR/RFLP variola Bangladesh 1975

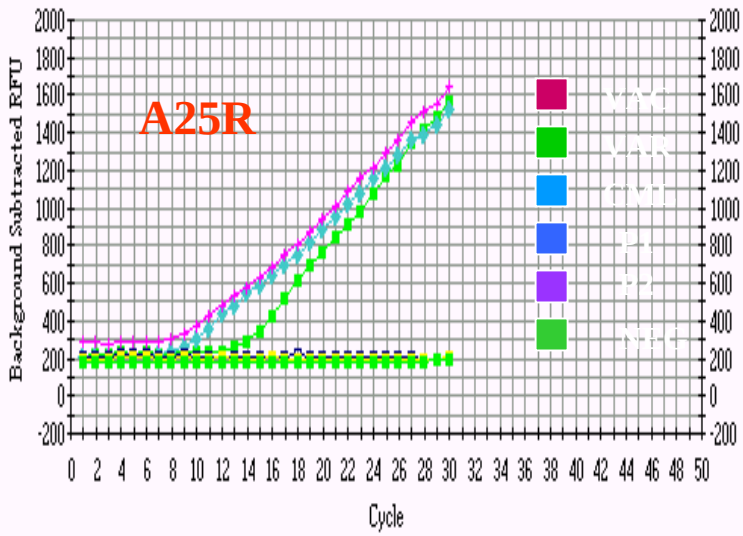




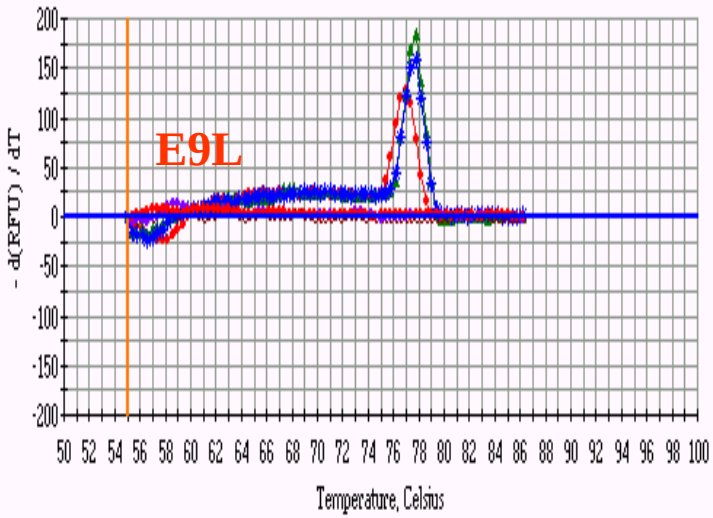


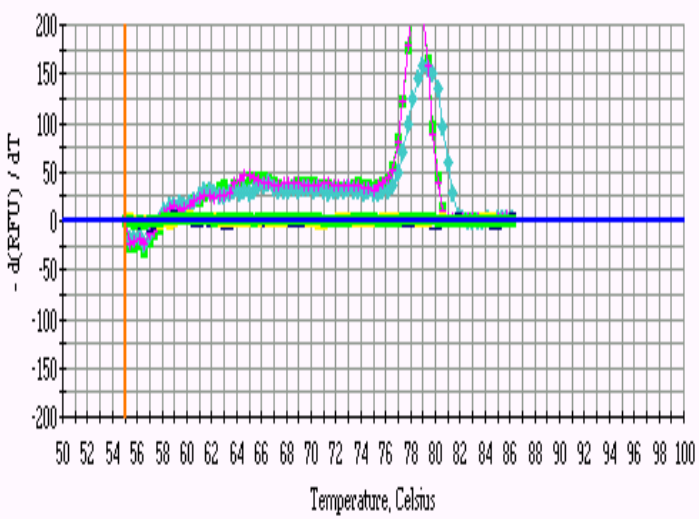
Real time PCR of essential orthopoxvirus genetic locii: Rapid, high throughput screening test: NOT SPECIES SPECIFIC





Melt Curve analysis of PCR products





Real time PCR "TaqMAN" assay development
Essential gene target: E9L (DNA polymerase)
Variola SPECIFIC
monkey blood samples

Monkey#	d8 std (HA)	d8real time	d11std (HA)	d11real time	d14real tim
C083	Positive	Positive	Negative	Positive	Positive
C003	Positive	Positive			
57-394	Positive	Positive	Negative	Positive	Negative
C271	Positive	Positive (3039)	Negative	Negative	Negative
C282	Positive	Positive (2059)	Negative	Positive (290)	Negative
C835	Indeterm	Positive (647)	Negative	Negative	Negative
57-245	Negative	Positive	Negative	Negative	Negative
C677	Negative	Positive	Negative	Negative	Negative
C382	Indeterm	Positive	Indeterm	Positive	Negative
C409	Negative	Positive	Negative	Negative	Negative
48-48	Negative	Positive	Negative	Negative	Negative

Controls: monkeypox (10 to$10x10^6$ copies negative)
varicella (negative)

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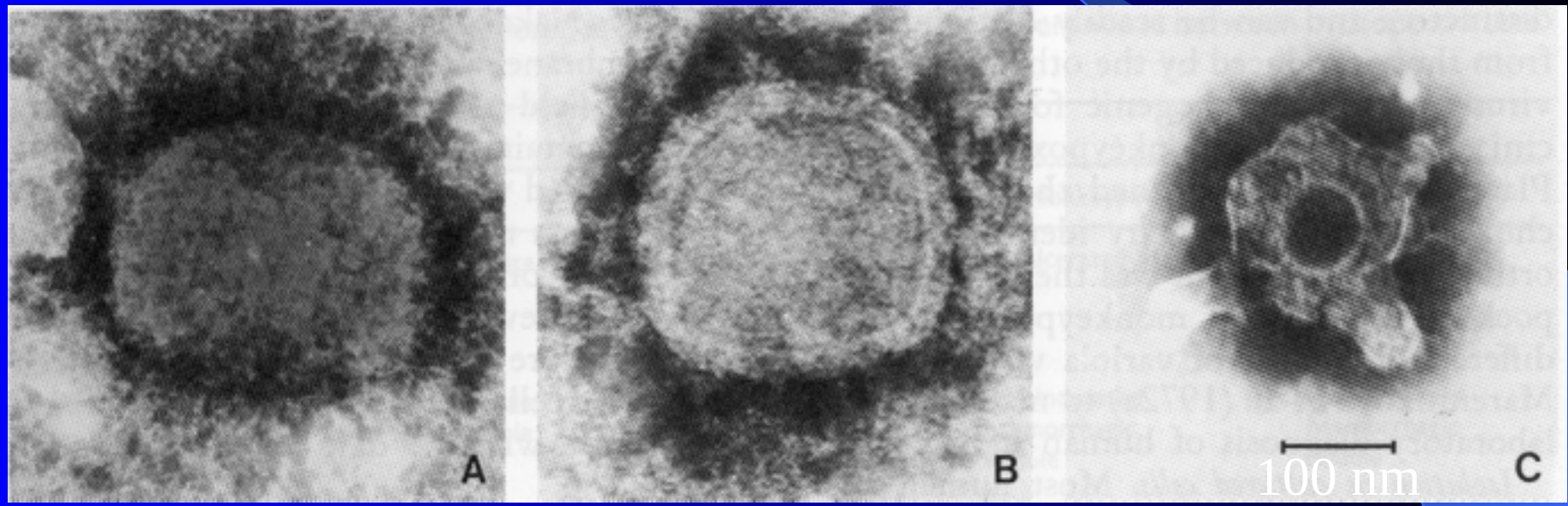
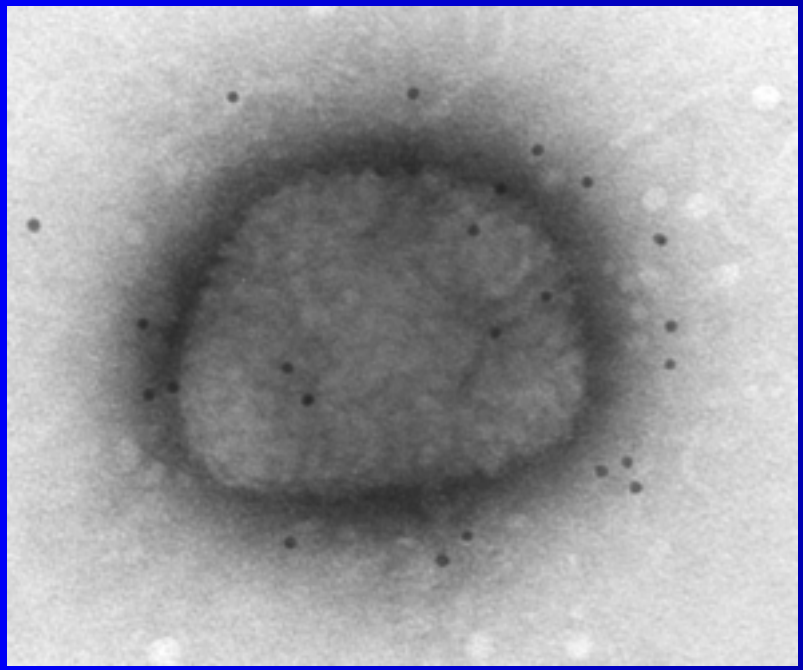
Negative stain electron microscopy

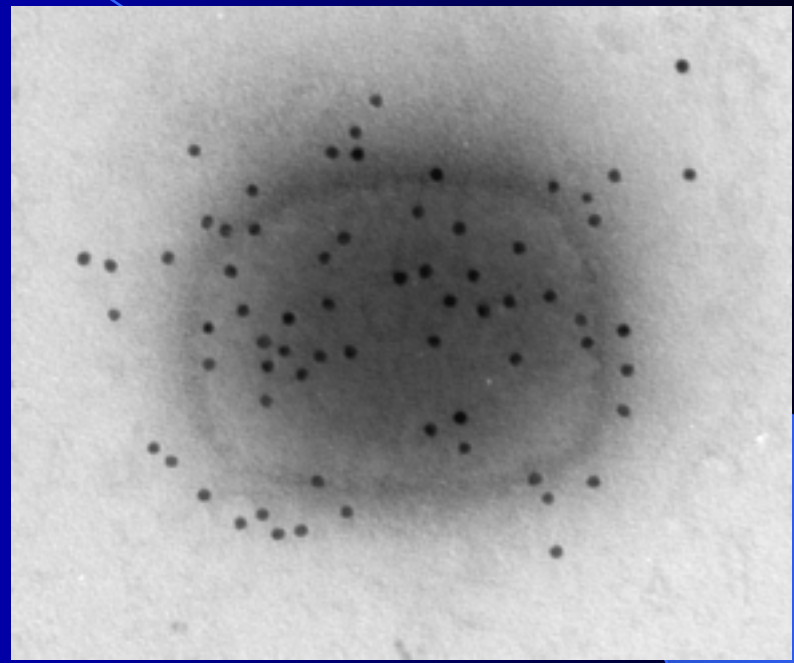
variola vs. varicella



variola variola varicella

Immuno electron microscopy: orthopoxvirus (not species-specific)





Virus:	**vaccinia**	**monkeypox**
Primary:	**1:5000 rabbit anti-variola**	**1:2000 mouse anti variola HMAF**
Secondary:	**12 nm colloidal gold conjugated , species specific**	


1. Center MediRule over area to be meas
2. Each circle is numbered in centimeters
affected area.
3. The cross hairs are ruled in millimete
Use Only Once!
Dispose At Bedside!

Day 4 rash

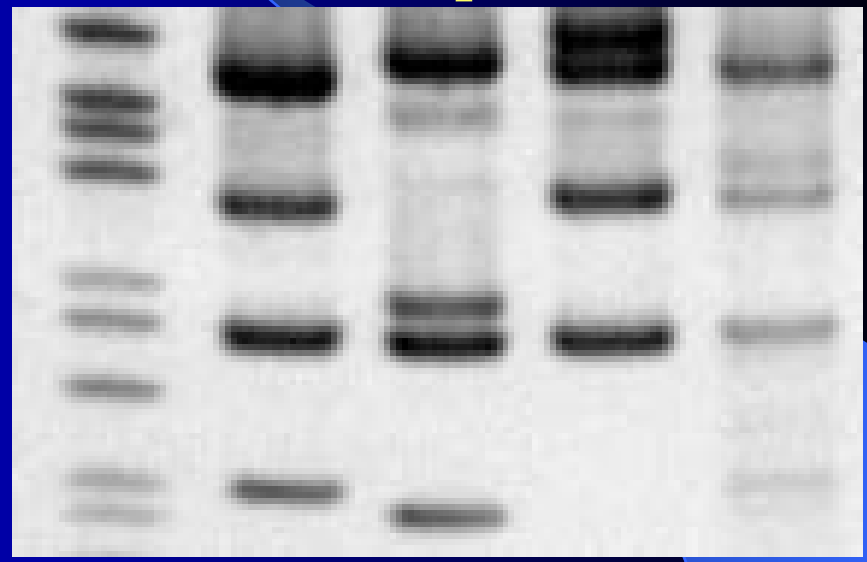
Speciation of clinical sample by single gene PCR-RFLP analysis: ATI gene

Case: v01-I-02

Specimens: vesicular fluid (v), skin roof from vesicle(sk)

RFLP (BglII)

Vac Mpx Var Sk1

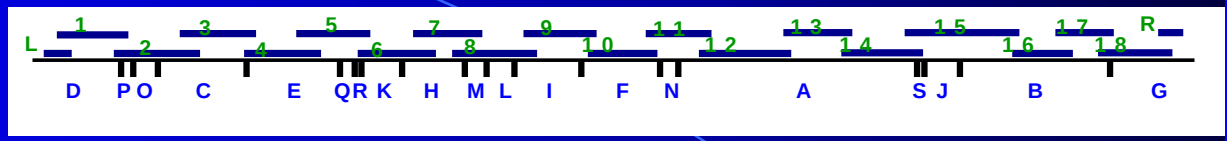


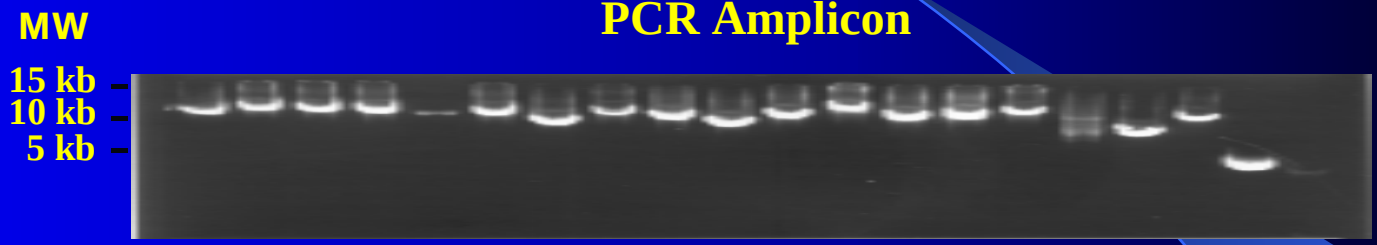
V MVSk v sk v v sk v sk v

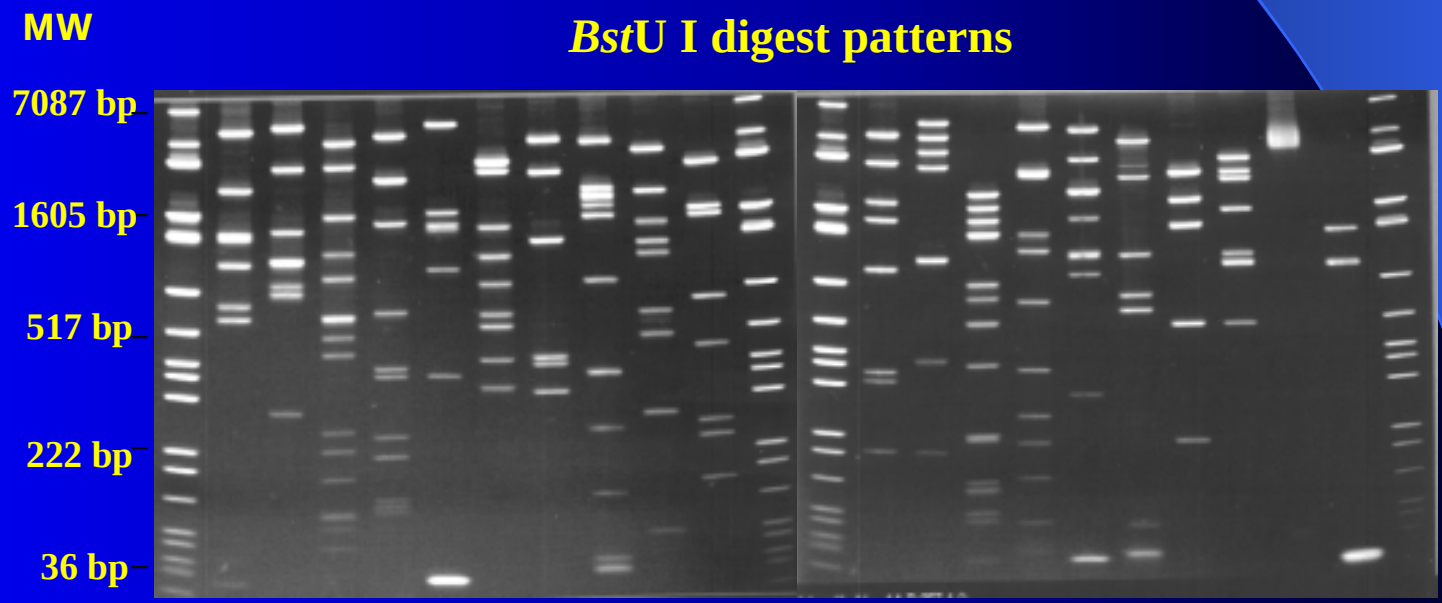
PCR amplified product yield: skin>vesicular fluid samples

1/8 amplicons sufficient yield for speciation by RFLP: Vaccinia

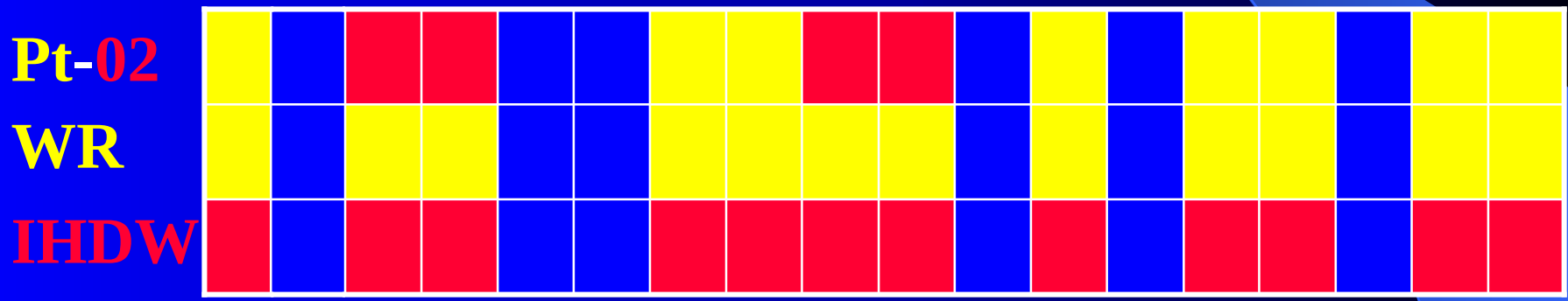
Extend-PCR RFLP variola Bangladesh 1975







E-PCR RFLP evidence for multiple crossover events to produce Patient-02 isolate from two common lab vaccinia strains



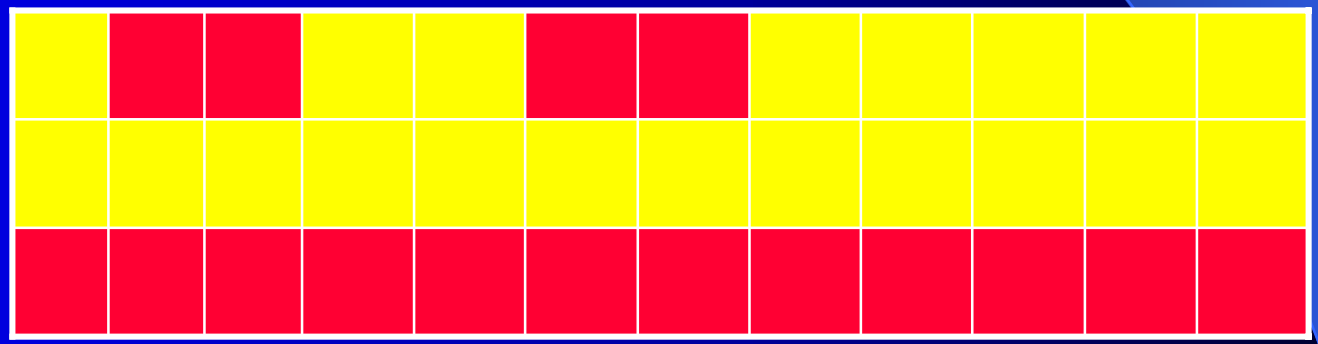
***Bst*UI digestion of amplicons 1-18**

E-PCR RFLP evidence for multiple crossover events to produce Patient-02 isolate from two common lab vaccinia strains

Pt-02

WR

IHDW



***Bst*UI digestion of amplicons 1-18,**

6 common band patterns not presented

Febrile vesicular rash illness example: disseminated vaccinia lessons summarized

- DFA-VZV negative
- EM – no viral particles seen:
  - Optimize specimen collection: utilize grid to lesion method
- TaqMAN: correct answers in our lab
  - Need to standardize species specific assays, better characterize their sensitivity and specificity
  - Potential utility to screen for orthopoxvirus
- Orthopoxvirus IgM + at day 4 rash (first specimen)
- Culture positive <24 hours
- Interesting, intelligent answers possible

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Opportunities for pessimism

- "Dark optimism":
  - Smallpox was once an EID event (probably zoonotic) that subsequently benefited from thousands of years to evolve very clever mechanisms to optimize transmission and survival in a limited, host-specific, human context (prevaccination).
  - Reminder of the need to be responsive to future possible EID poxvirus events (perhaps not completely unlike possible BT events).

- **Sources for possible optimism:**
  - **Febrile Vesicular Rash Algorithm is a creative way to enhance good medicine by better identifying smallpox look-alike diseases, and focus reference diagnostic resources on finite suspect smallpox cases.**
  - **A proven strategy for smallpox control exists (just in case).**
  - **Considerable immune cross-reactivity (the basis of a proven vaccine). Implications beyond vaccines.**
  - **Strategies for vaccines with less adverse rx's over the horizon.**
  - **Increasingly "intelligent" analytic tests.**
  - **Increasingly sophisticated understanding of basic orthopoxvirus virology leads to potential vulnerabilities.**