
بسم الله الرحمن الرحیم
بهار

Tehran University
of Medical Sciences
School of Public Health
National Center of Excellence
for Public Health Education

دانشگاه علوم پزشکی و
خدمات بهداشتی درمانی تهران
دانشکده بهداشت
قطب علمی آموزش
علوم بهداشتی کشور

The Diagnostic Methods for Viral Vaccine-Preventable Diseases (Measles, Mumps and Rubella)

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Measles

Measles

- Acute, febrile rash illness
- Transmission: Direct contact with infectious droplets or airborne route
- Highly contagious (90% of susceptible household contacts)
- R0: 12–18 (in an unvaccinated population)

Measles – Clinical Case Definition

- **Fever (up to 40.5 °C)**
- **Rash**
- At least 1 of "The 3 C's"
  - Cough
  - Coryza (runny nose)
  - Conjunctivitis

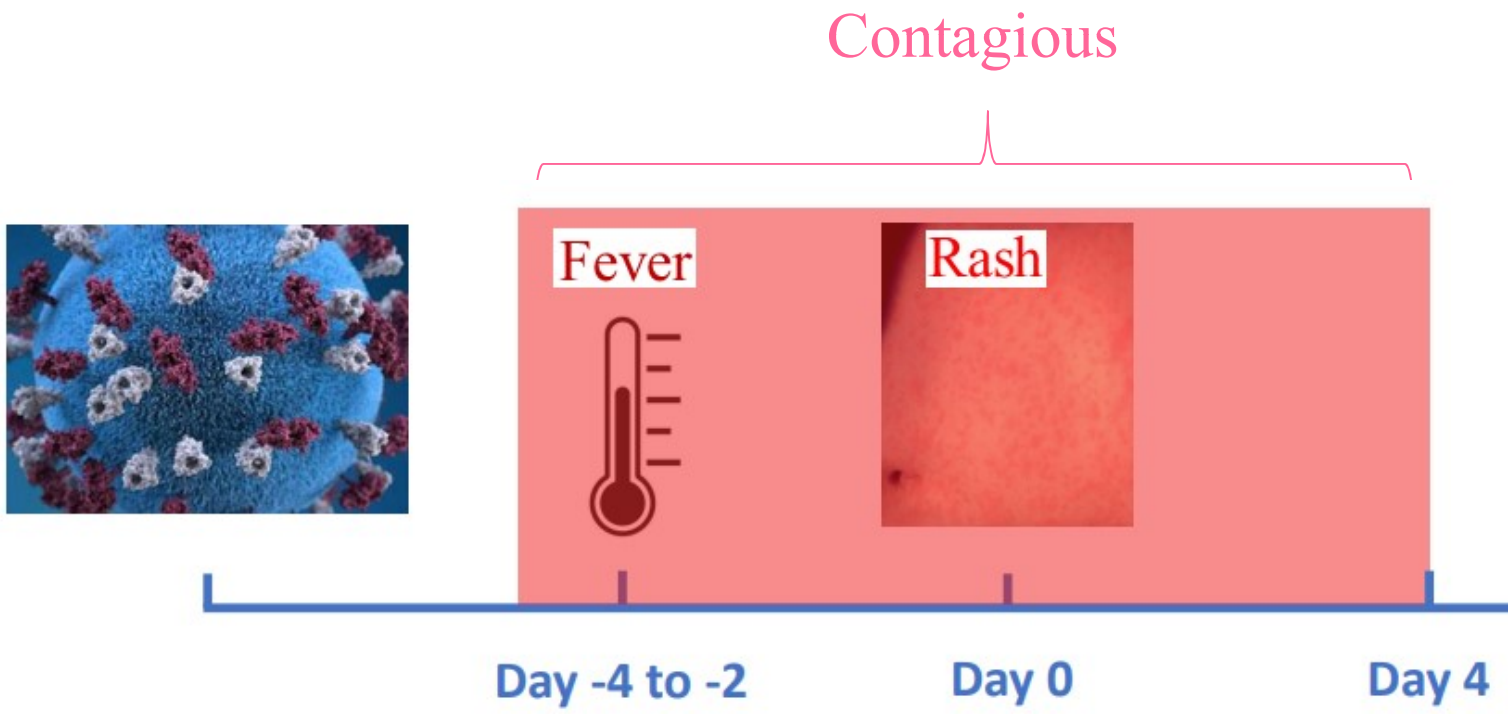
Measles Rash

- Maculopapular rash
- Starts on face, at hairline, or behind ears
- Spreads downwards to neck, trunk, extremities
- Koplik spots may be present on buccal mucosa

Koplik Spots

Measles – Typical Timeline

- The incubation period:
  - 14 days (range 7-21) from exposure to rash onset
- Contagious: 4 days prior to 4 days after rash onset

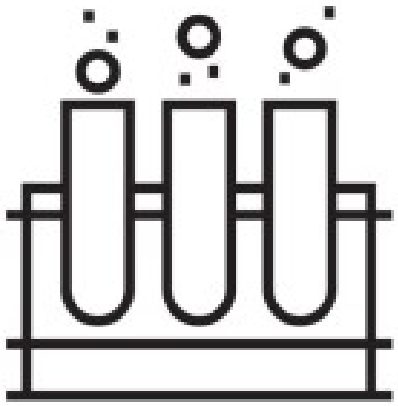


Measles Complications

Complications	Incidence rate
Diarrhea	8%
Otitis media	7-9%
Pneumonia	1-6%
Encephalitis	1 per 1000 cases
Subacute Sclerosing Panencephalitis (SSPE)	7-11 per 100,000 cases
Hospitalization	1 in 4 cases
Death	1-3 per 1000 cases

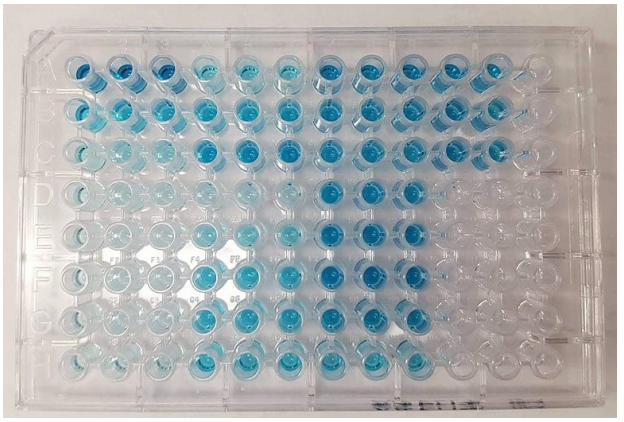
[1]Bellini WJ et al. J Infect Dis 2005:192:1686–93.

Laboratory Diagnosis of Measles



Measles Serology…

- Increase the window of measles diagnosis (**encountering delays** in diagnostic or reporting)



- **IgM detection**: 1–3 days after rash up to 6–8 weeks
  - May disappear rapidly, be delayed, or not appear at all in vaccinated persons

- **Saliva sample** for IgM detection (during first 5 weeks) (when serum not available)

…Measles Serology

- Problematic IgM testing in settings with **low measles incidence**

  - **Due to vaccine …**

  - **Cross-reactivity** with other causes of febrile rash illness
    - Parvovirus B19, HHV6, Enteroviruses, Dengue viruses

  - **False positive results** when measles likelihood is low:
    - There isn't local active transmission and patients have not traveled
    - Patients have been fully vaccinated and have no known exposure

Measles real time RT-PCR

rRT-PCR

- **Proper specimen** collection (time, type, quality), storage, and processing

- Type: Nasopharyngeal/throat swabs and urine
- Time: within 3 days up to 10 days after rash onset (as soon as possible)
- Quality: Expert person, monitoring specimen integrity (reference gene: Human RNase P mRNA).
- A part of **MeV N gene** RNA.

Measles rRT-PCR vs. Serology

- Sensitivity and specificity much higher than serology
- False positivity much less common
- Time Limitation for sample collection

Measles Genotyping

- To perform **molecular surveillance**
- To establish and sustained **elimination**
- **Outbreak** detection and tracking

- In people recently exposed both to wild virus and vaccine strain:

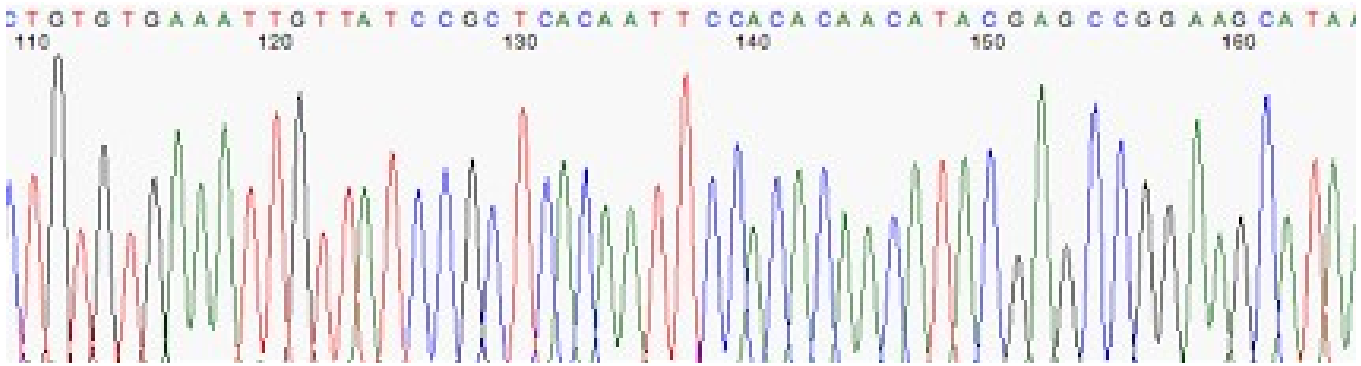
  Differentiation if measles virus is **vaccine-derived** or **from community transmission**

Measles Genotyping

Conventional RT-PCR & Sequencing

- Based on nucleotide sequencing of **H and N genes**
- **M-F noncoding region** nucleotide sequencing for **molecular surveillance**
- Eight clades (A – H), divided into 24 genotypes

* 1990: A*, B2, B3, C1, C2, D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, G2, G3, H1, H2
* Since 2018: **B3**, D4, D8, H1



Circulating Measles Genotypes in Iran…

- Molecular Epidemiology of Measles Virus before and after the 2003 Mass Vaccination Campaign for Measles/Rubella in Iran (Iran J Public Health. 2011; 40(1): 41–49.)

✓ Genotype **D4**

- Implementation of a National Measles Elimination Program in Iran: Phylogenetic Analysis of Measles Virus Strains Isolated during 2010–2012 Outbreaks (PLOS ONE April 15, 2014)

✓ Genotypes **B3, D8, D4 and H1**

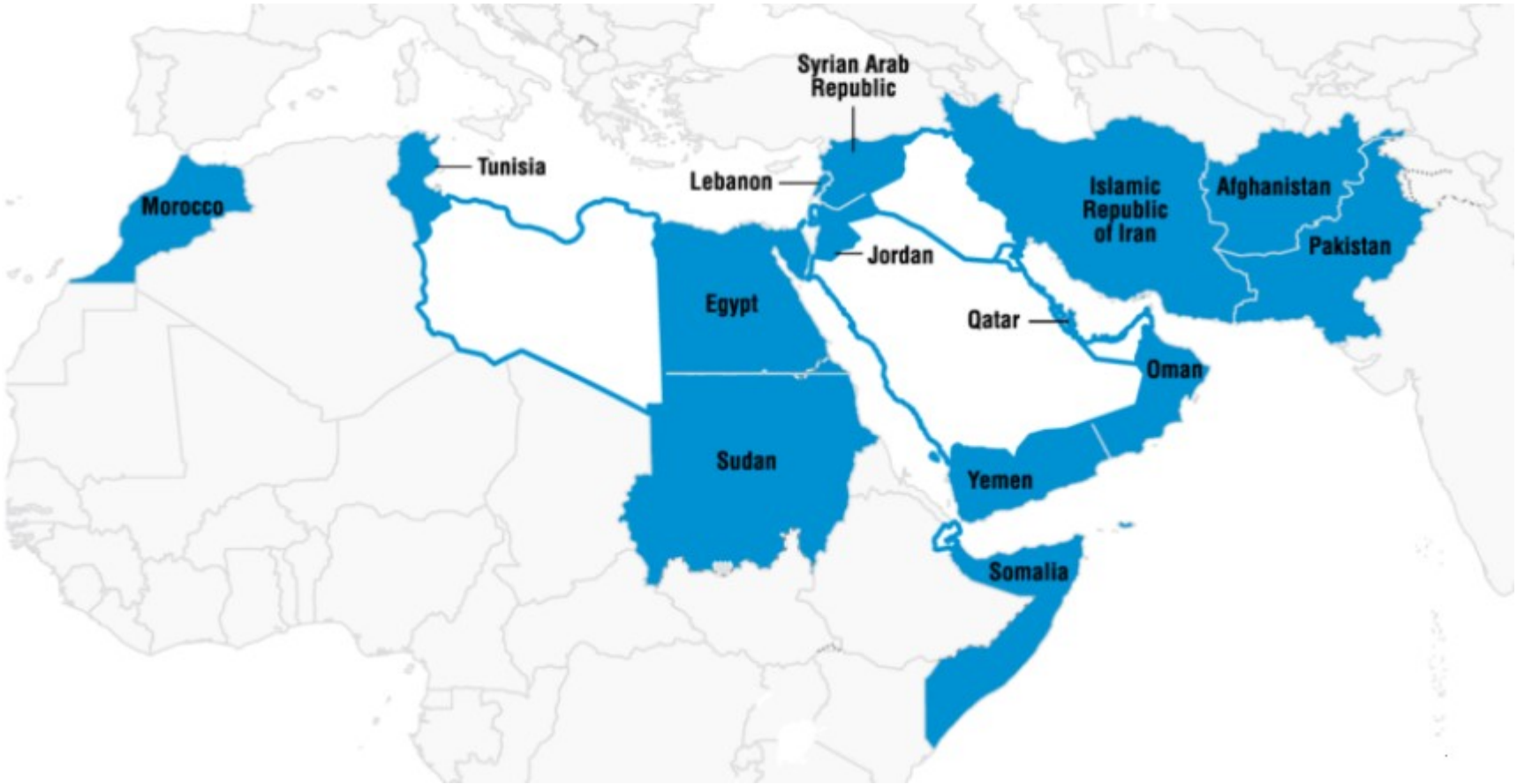
…Circulating Measles Genotypes in Iran

- Genetic Characterization of the H Gene of MeV Strains (H1, B3, and D4) Recently Circulated in Iran for Improving the Molecular Measles Surveillance in the National Measles Lab (Iran J Public Health. 2023 Aug; 52(8): 1730–1738)
  - ✓ Genotypes **H1, B3, D4**

- Recent data (unpublished)
  - ✓ Genotype **B3**, D8

Measles/Rubella Elimination in Iran

Iran received the certification for m**easles** and **rubella elimination** in May, 2019.



Additional available Testing

Serology

- **Paired** (acute and convalescent) **IgG** Testing
  - When other tests (IgM detection and rRT-PCR) are inconclusive

- **Avidity Testing (IgG)**
  - Evaluation of vaccine efficacy in population with unknown immune status for measles
  - Evaluation of measles breakthrough among previously vaccinated people

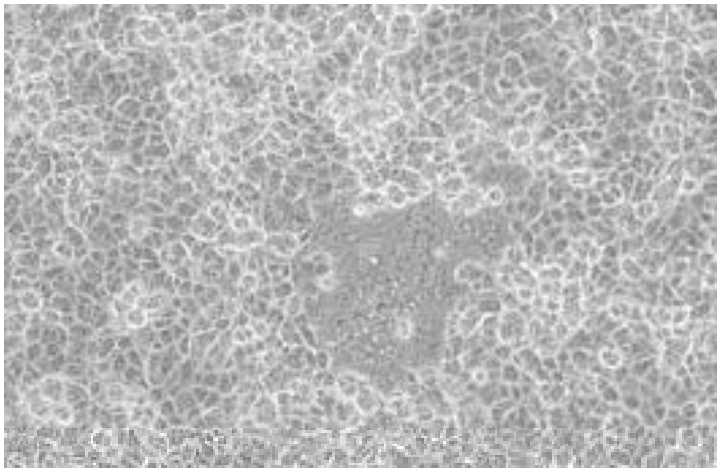
Laboratory Diagnostic Concerns

- Measles serology: (IgM detection) useful but buggy
  - **Cross-reactivity** with other causes of febrile rash
  - High **dependence on disease prevalence**

- Measles diagnosis should include:
  - **Both** molecular testing (rRT-PCR) and serology
  - Consideration of the **clinical** and **epidemiologic context** (seasonality, age, Endemicity/ geographic location, outbreaks)

Measles cell culture

- Research purpose not diagnosis
- Vero cell line, Vero/SLAM cell line
- Proper sample:
  - Urine sediment
  - Throat swab



- Cytopathic effect (**CPE**), produced by **measles** virus replication in **cell culture** is easily observed

Rubella

Rubella

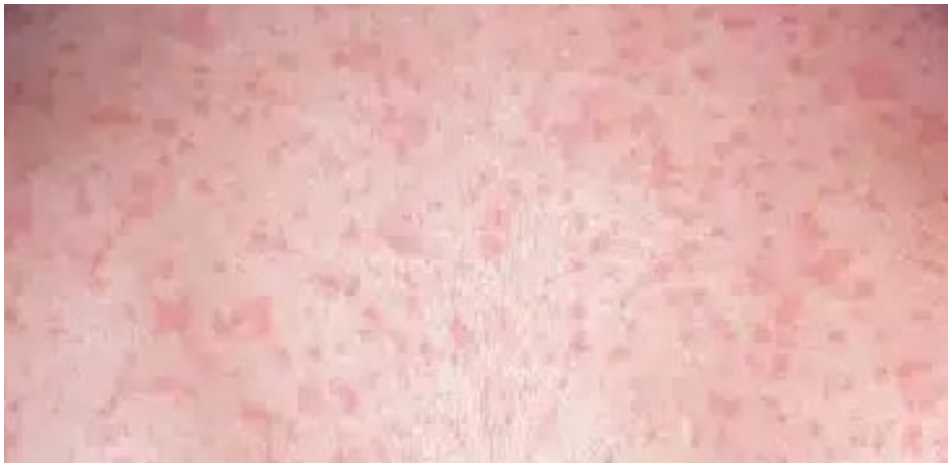
- Mild febrile rash illness
- Transmission: Direct contact with infectious droplets
- Much less contagious than measles
- R0: generally low (<5)

Rubella – Clinical Case Definition

- **Mild fever (37.2°–37.8°C)**
- **Rash**
- Swollen, tender lymph nodes (usually in the back of the neck or behind the ears)
- Mild polyarthritis in adult

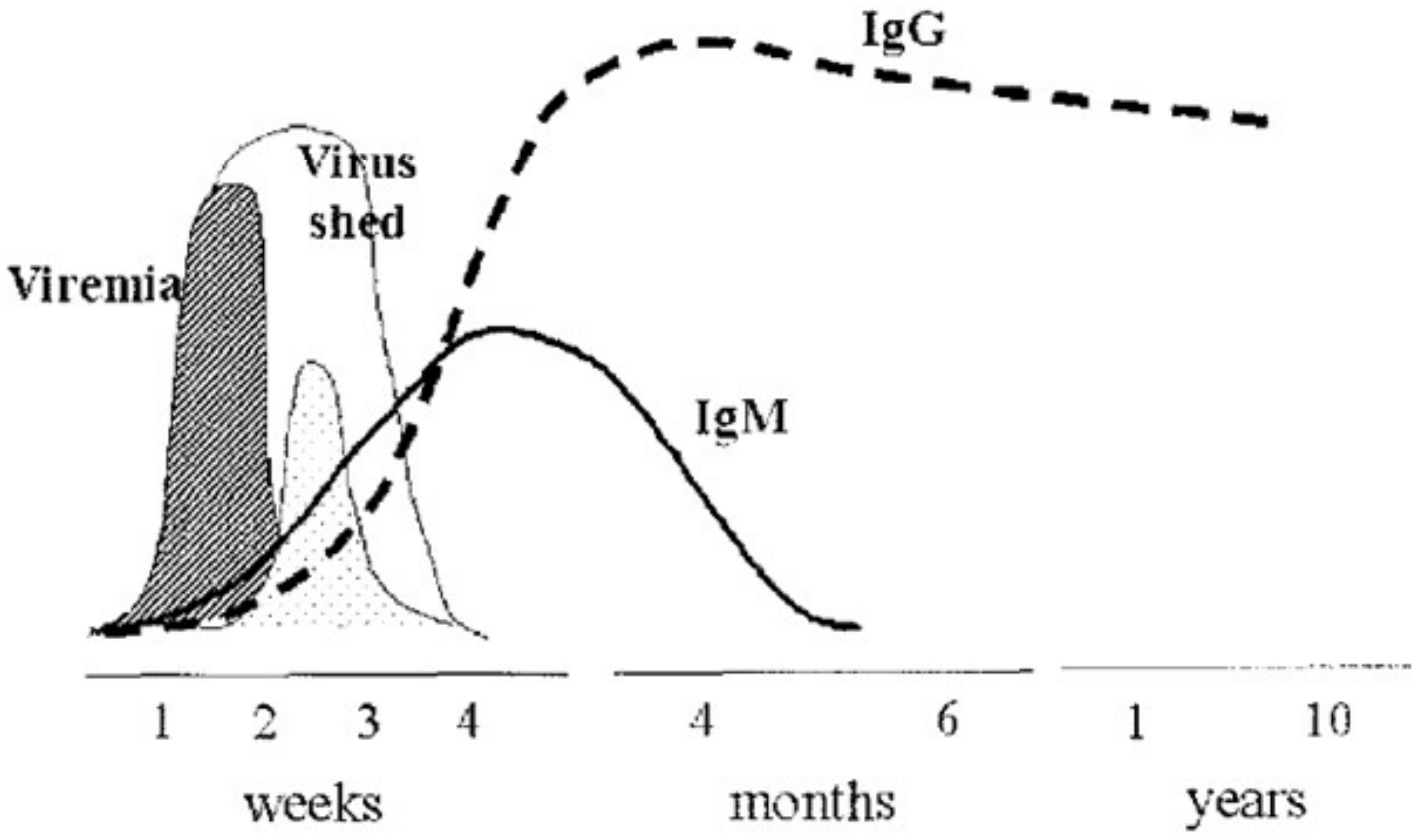
Rubella Rash

- In 1/2 of all cases
- Fine, pink macules
- Starts on the face and spreads downward
- Fades within 48 hours



Rubella – Typical Timeline

- ✓ Incubation period: 2-3 weeks (18 days)
- ✓ The infectious period: from 7 days before to 5–7 days after rash onset



Rubella Complication

- ✓ In the absence of pregnancy, it is usually clinically manifested as a mild self-limited infection.

- ✓ During pregnancy, the virus can have potentially devastating effects on the developing fetus.

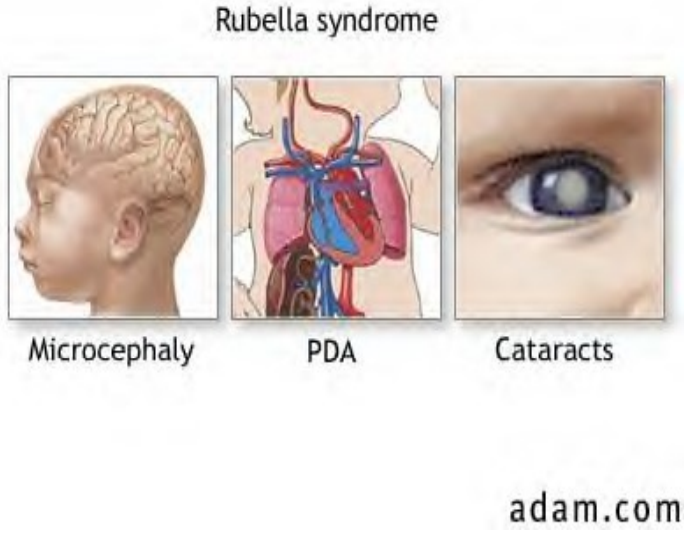
Congenital Rubella Syndrome (CRS)

- ➢ **Primary rubella infection** during the first trimester (and the forth month) of pregnancy

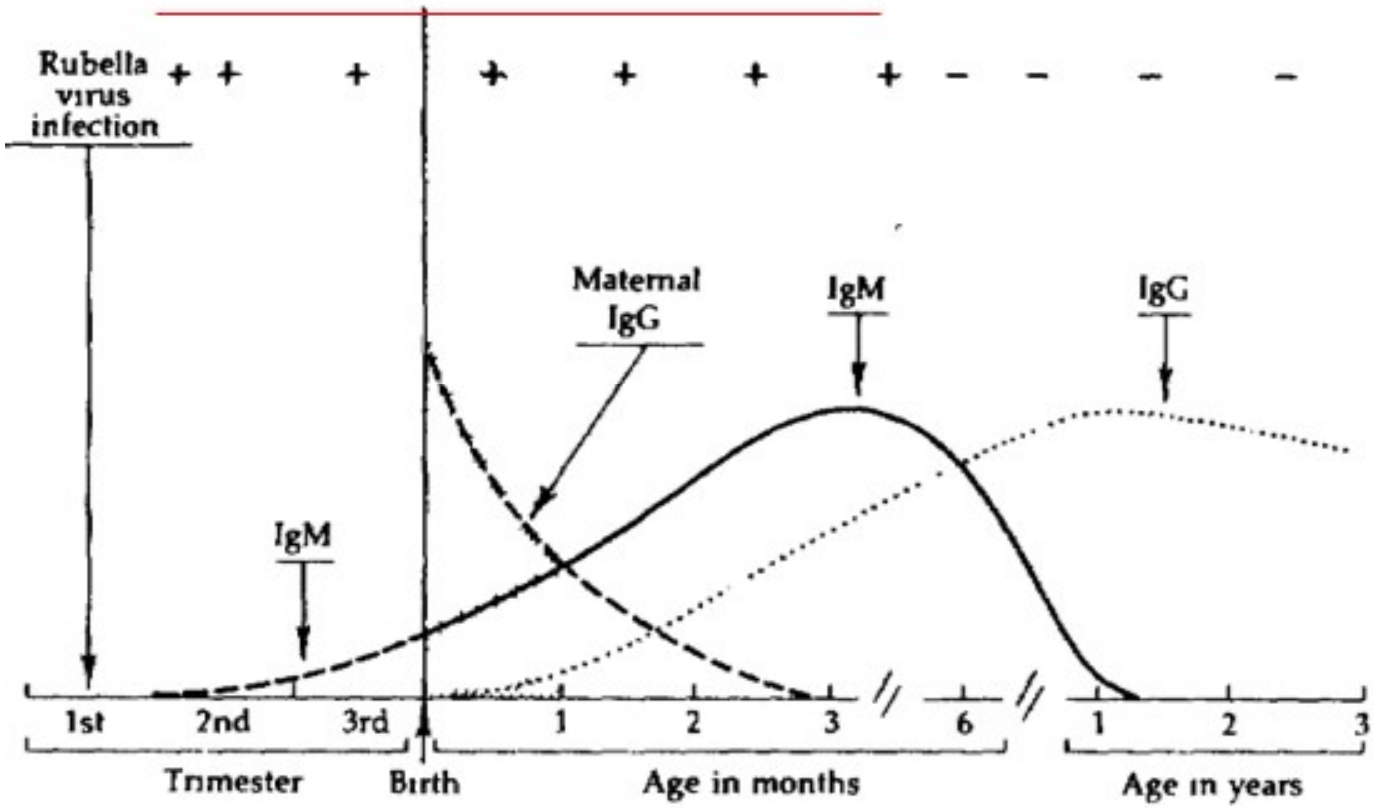
Congenital Rubella Syndrome (CRS)

Classic triad :

- ✓ Sensorineural deafness (58% of patients)
- ✓ Eye abnormalities (43% of patients)
  (retinopathy,cataract & microphthalmia)
- ✓ Cogenital Heart disease (50% of patients)
  (patient duct arteriosus)

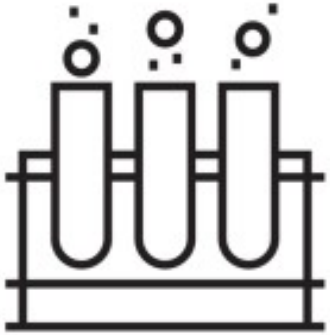


Viral shedding / Ab response in congenital rubella



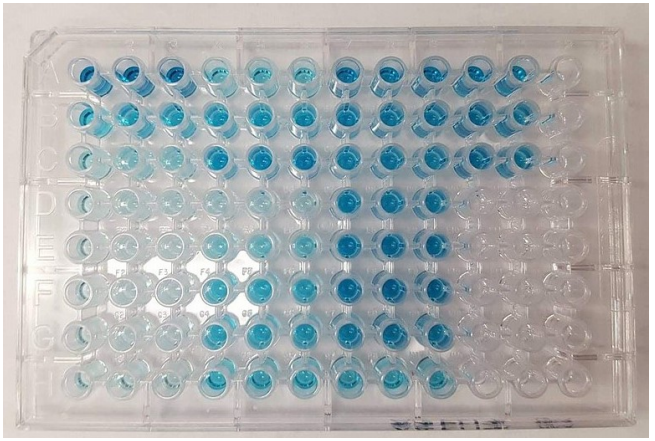
Pattern of viral excretion (+) and the infant's antibody response in congenital rubella (From S. Krugman and R. Ward, "Infectious Diseases of Children and Adults," 5th Ed. Mosby, St Louis, 1973)

Laboratory Diagnosis of Rubella



Rubella Serology

- Increase the window of diagnosis



- IgM detection: 1–3 days after rash up to 6–8 weeks
  - May disappear rapidly, be delayed, or not appear

- **IgG Avidity Assay**

Problematic Rubella IgM testing

In low rubella incidence era

- **Cross-reactivity** with other causes of febrile rash illness:
  - Measles, B19, CMV, EBV

- **False positive results** when the likelihood of rubella is low:
  - There isn't local active transmission and patients have not traveled
  - Patients have been fully vaccinated and have no known exposure

- **IgM positivity** for a **long time** or in rubella **reinfection**

IgG Avidity

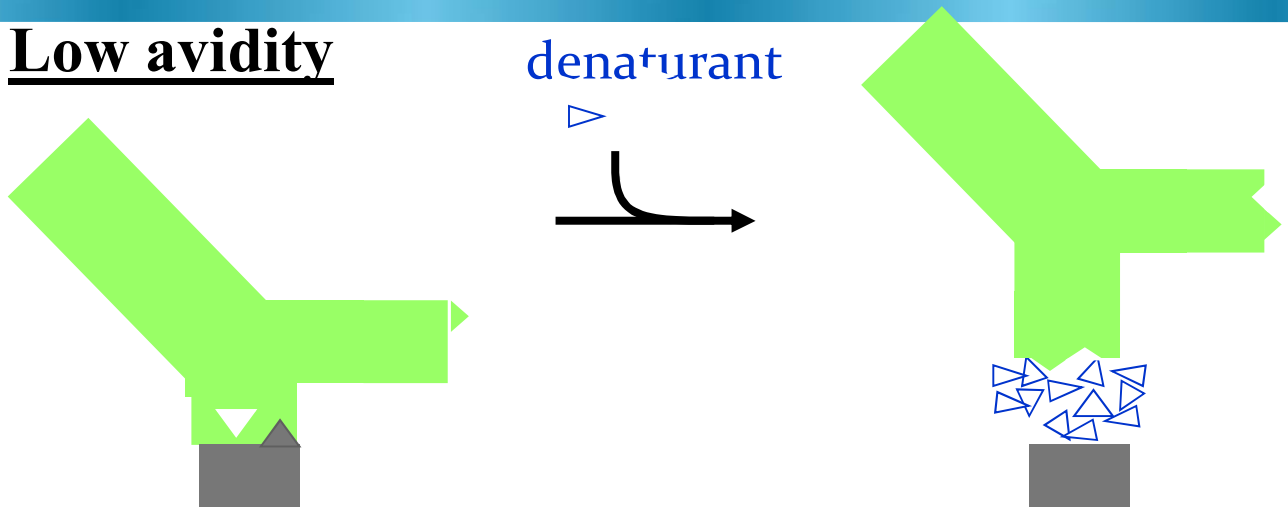
Avidity is the binding strength of IgG to antigen

- ✓ **Low avidity** IgG indicates **primary infection**
- ✓ High avidity IgG indicates non primary/past infection

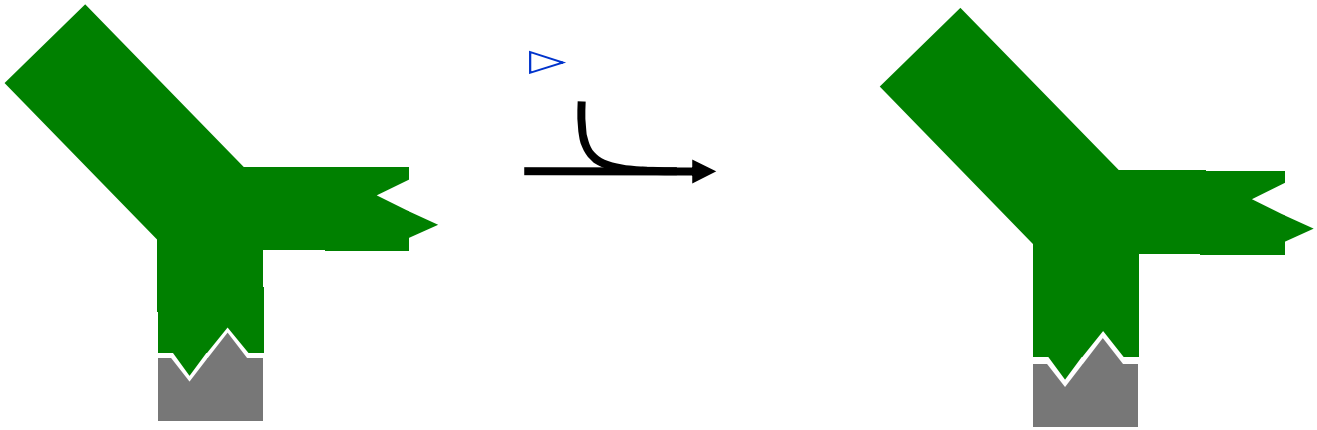
- ➢ **The most important application**: Diagnosis of **primary rubella** infection during the **first four months of pregnancy**

The principals of ELISA IgG Avidity Assay

Low avidity



High avidity



Rubella real time RT-PCR…

rRT-PCR

- **Proper specimen** collection, storage, and processing
- Type: **Nasopharyngeal/throat swabs** and urine
- Time: one week before to one/two weeks after the onset of the rash (as soon as possible)
- Quality: Expert person, monitoring specimen integrity (reference gene).
- **GC rich genome**, problem in amplification
- A part of rubella **p150 gene** (154 nt region)

Rubella rRT-PCR vs. Serology

- Higher sensitivity and specificity
- False positivity much less common
- Time limitation for sample collection

Rubella Genotyping

Conventional RT-PCR & Sequencing

- Important to establish and sustained **elimination**

- **Outbreak** detection and tracking (all rRT-PCR positive specimens)

- In people recently exposed both to wild virus and vaccine strain:

Differentiation if rubella virus is vaccine-derived or from community transmission

- Nucleotides sequencing of E1 protein coding region

Rubella genotypes

13 genotypes based on 8-10% nucleotide differences in E1(739 nt)

- ✓ Rubella sub group 1: 1a,1B,1C,1D,1E,1F,1G,1H,1I,1J
  Europe, Japan, America, western hemisphere

- ✓ Rubella sub group 2: 2A, **2B**, 2C
  Asia (china, India)

Iran: 2B

Additional available Testing

Serology

- Paired (acute and convalescent) IgG Testing
  - When other tests (IgM detection and rRT-PCR) are inconclusive
  - Not able to distinguish primary vs. non primary rubella infection

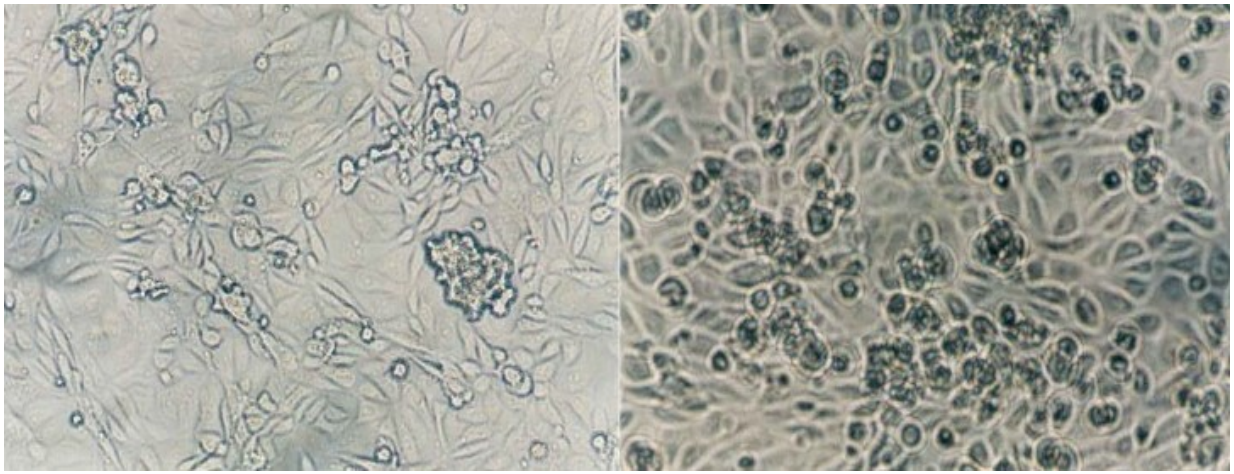
Laboratory Diagnostic Concerns

- Rubella serology: useful but buggy
  - Cross-reactivity with other causes of febrile rash
  - High dependence on disease prevalence
  - IgM positivity for a long time (even 5 years)
  - Rubella reinfection

- Rubella diagnosis should include:
  - Both molecular testing (rRT-PCR) and serology
  - Consideration of the clinical and epidemiologic context

Rubella cell culture

- Research purpose not diagnosis
- Vero cell line, RK13 cell line
- Proper sample:
  - **Throat swab**
  - Urine sediment
- No obvious cytopathic effect (**CPE**) …



Rubella vaccine & Pregnancy

* Potential risk to the developing fetus

(1.3% in 1-2 weeks before to 4-6 weeks after conception)

* The isolation rate of virus from fetus : 3%

* No CRS has been reported

(515+116 healthy infants after vaccination in pregnancy)

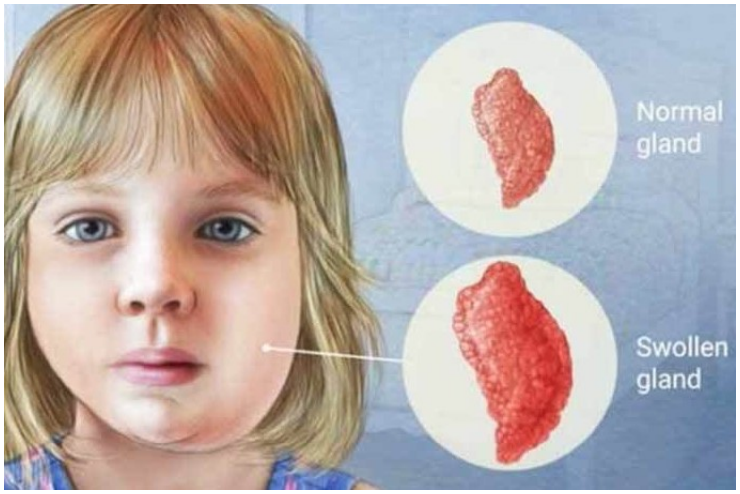
Mumps

Mumps

- Acute, febrile illness
- Transmission: Direct contact with infectious droplets or airborne route
- Highly contagious
- R0: 10-12 (in an unvaccinated population)

Mumps Clinical Features

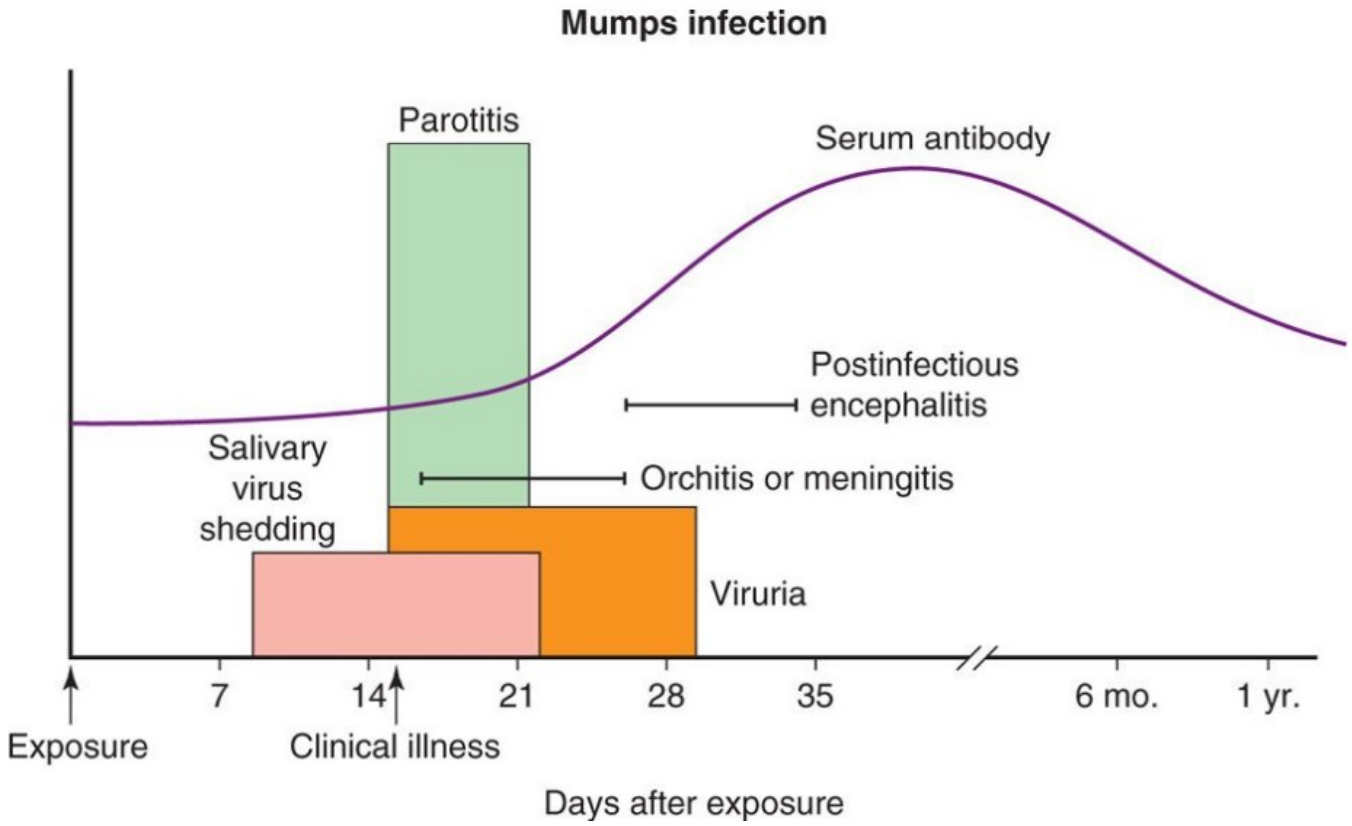
- **Low grade Fever**



- Nonspecific prodrome:
  - Myalgia, malaise, headache
- Unilateral or bilateral tender, swelling of the parotid or other salivary glands
- Painful testicular swelling (orchitis)
- Rash may occur

Mumps – Typical Timeline

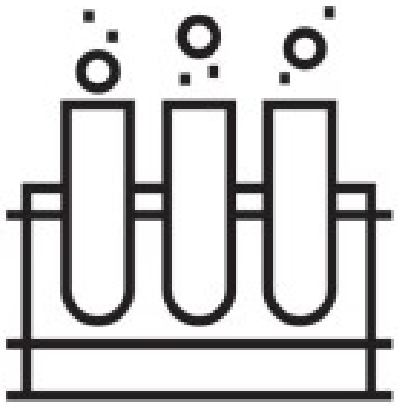
- Incubation period: 14-21 days
- Contagious: 6 days before to 9 days after facial swelling



Mumps Complications

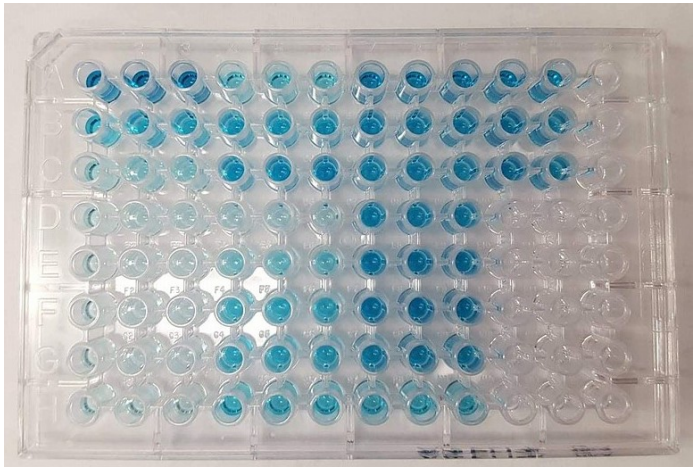
- Aseptic meningitis
- Encephalitis
- Hearing loss
- Sterility
- Arthritis
- Oopharitis
- Nephritis
- Myocarditis
- Miscarriages

Laboratory Diagnosis of Mumps



Mumps Serology

- Increase the window of diagnosis
- IgM detection: 3–7 days after parotid swelling
  - May disappear rapidly, be delayed, or not appear at all in vaccinated persons
- Problems in IgM testing
  - **Cross-reactivity**
  - **False positive results**



Mumps real time RT-PCR

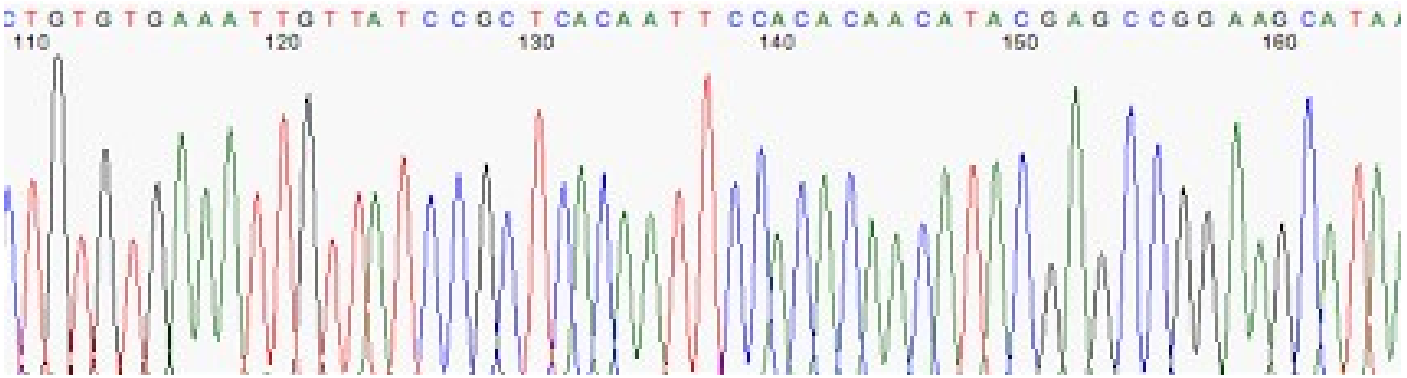
- **Proper specimen** collection (time, type, quality), storage, and processing
- Type: Buccal/Oral swabs, urine, and CSF (in specific situations)
- Time:
  - 0-5 days after swelling: buccal swab
  - 6-9 days after swelling: buccal swab and urine
- Quality: Expert person, monitoring specimen integrity (reference gene).

- A part of SH gene is more sensitive
- F gene can be used with less sensitivity.

Mumps Genotyping

Conventional RT-PCR & Sequencing

- Molecular epidemiology
- Based on nucleotide sequencing of SH+NCRs
- 12 genotypes: (A to N), recent years only (C, D, F, G, H and K)
- Genotype H was isolated from a patient in Iran (1986)

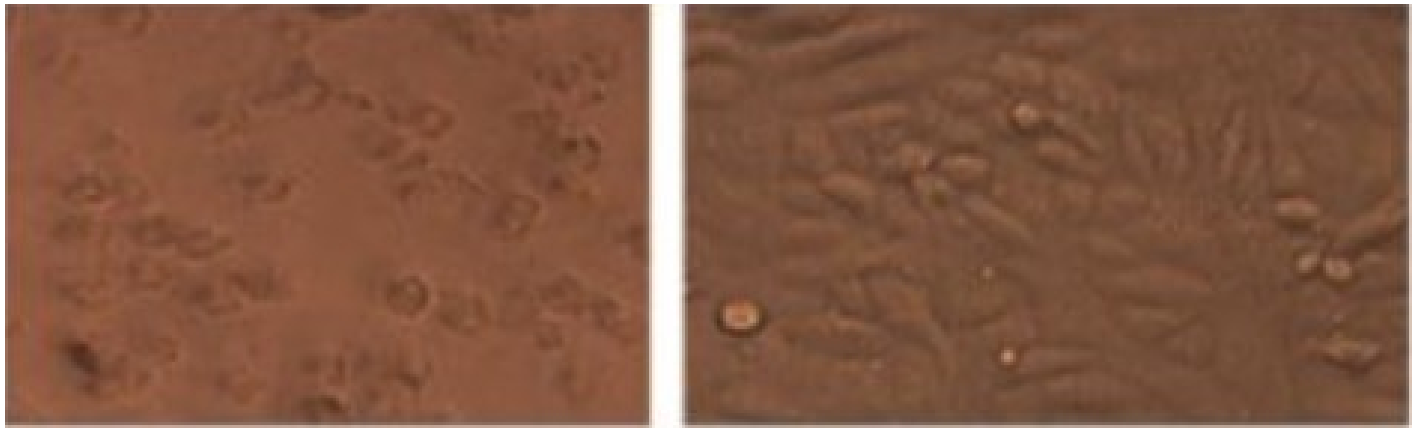


Laboratory Diagnostic Concerns

- Mumps serology:
  - False positivity
  - Cross reactions
- Mumps diagnosis should include:
  - Both molecular testing (rRT-PCR) and serology
  - Consideration of the clinical and epidemiologic context

Mumps cell culture

* Proper sample: Saliva, Urine, CSF
* Culturing in Chick Embryos, Primary Monkey kidney, green monkey kidney cells, Vero cells, HeLa cells
* Typical Paramyxoviruses cytopathic effects



Anti-mumps virus activity by extracts of Mimosa pudica, a unique Indian medicinal plant

Take Home Message

MMR diagnostic methods

- Serology: IgM detection (screening of acute infection)
- Complementary serology test: IgG avidity assay (specially to detect rubella primary infection)

- Molecular test:
  - Real time RT-PCR for detection
  - Conventional RT-PCR for genotyping

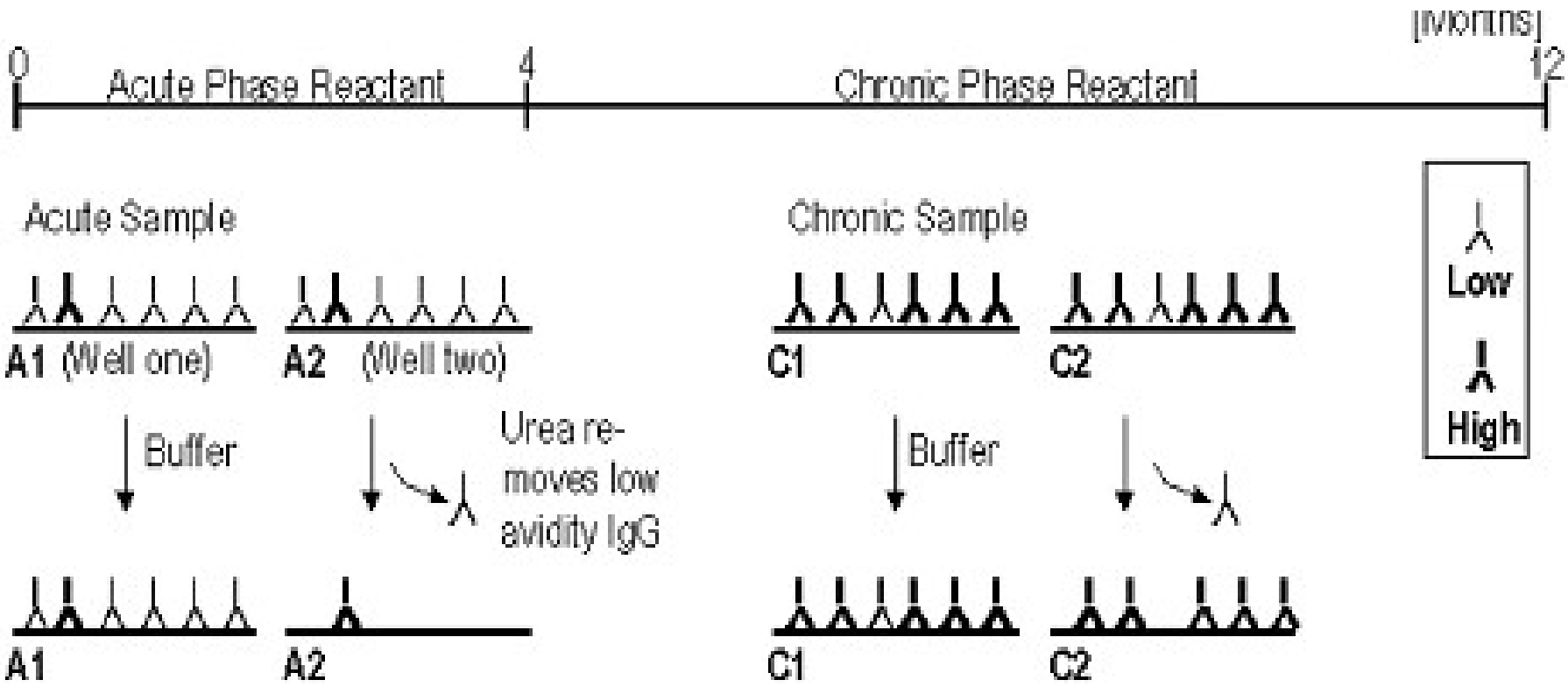
- Cell culture for research


Thanks for your attention

Estimated Herd Immunity thresholds for vaccine preventable diseases

Disease	Herd Immunity
Diphtheria	85%
Measles	83-94%
Mumps	75-86%
Pertussis	92-94%
Polio	80-86%
Rubella	83-85%
Small Pox	80-85%

The principals of ELISA IgG Avidity Assay



Curdt I et al. J. Clin. Microbiol. 2009;47:603-613

Primary rubella infection in pregnancy

The chance of fetus Infection

✓ 0–28 days before conception	------ ?
✓ 0–12 weeks after conception	90%
✓ After 20 weeks	rarely
✓ Third trimester	again rise