Myxoviruses

Learning objectives

At the end of this class student should be able to know about

- Influenza and Parainfluenza viruses
- Mumps virus
- Measles virus
- Nipah virus and hendra virus
- Respiratory syncytial virus
- Metapneumovirus
- Rubella virus

Myxoviruses

- Group of viruses that bind to mucin receptors on the surface of RBCs (*myxo* in Greek meaning ‘mucin’).
- Mucin receptors - Clumping of RBCs together to cause hemagglutination.

CLASSIFICATION-

Properties	Orthomyxoviridae	Paramyxoviridae
Size	80-120nm	100-300 nm
Shape	Spherical; Rarelyfilamentous	Pleomorphic;
Nucleic acid	Negative sense ssRNA, Segmented; eight pieces	Negative sense ssRNA Un-segmented; single piece
Genetic recombination	Seen	Not seen
Antigenic variation	Seen	Not seen
Site for RNA Replication	Nucleus	Cytoplasm
Important human pathogens	Influenza virus	Parainfluenza virus Mumps virus Measles virus Respiratory syncytial virus Metapneumovirus

ORTHOMYXOVIRIDAE

- Influenza viruses are the members of Orthomyxoviridae family.
- Major causes of morbidity and mortality and have been responsible for several epidemics and pandemics of respiratory diseases in the last two centuries.

INFLUENZA VIRUSES – Morphology

- Influenza viruses consist of thee genera- Influenza A, B, and C.
- Spherical and 80–120 nm in size
- Helical nucleocapsid(9nm),surrounded by an envelope
- **Viral RNA** comprises of *multiple segments* of negative sense single stranded RNA. Each segment codes for a specific viral protein having a specific function.
  - Influenza A & B contain *eight segments* of RNA
  - Influenza C contains seven segments of RNA. The segment coding for neuraminidase is absent.

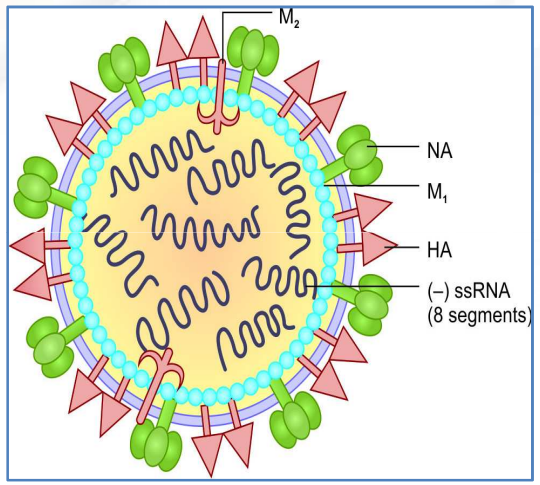
Morphology

- **Site of RNA replication**- nucleus
- **Viral proteins**

RNA segments	Coded Protein(s)	Function of proteins
Segment-1	PB2	RNA Transcription and replication
Segment-2	PB1	
Segment-3	PA	
Segment-4	HA (hemagglutinin)	Binds to receptors of RBC to cause hemagglutination.
Segment-5	NP (nucleoprotein)	Associates with RNA to form helical nucleocapsid
Segment-6	NA (neuraminidase)	Replaces HA from RBCs to cause elution (reversal of hemagglutination)
Segment-7	M1&M2	M1-forms a shell underneath the envelope M2-formsion channels
Segment-8	NS1&NS2	NS1-is interferon antagonist &inhibits pre-mRNA splicing NS2-is nuclear export factor

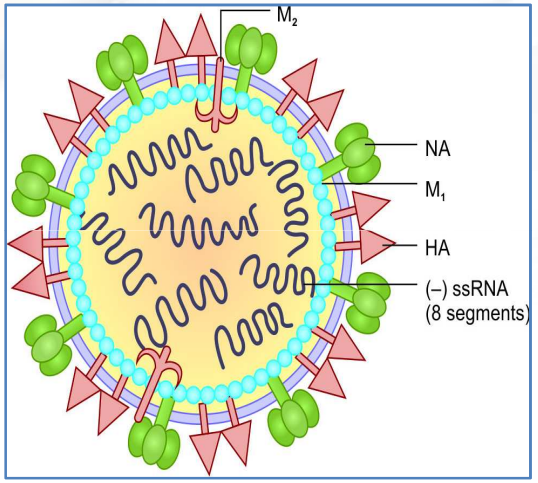
Morphology (cont..)

- Envelope is lipoprotein in nature.
  - Lipid part is derived from the host cell membrane.
  - Proteins or the peplomers arevirus coded,10nm long glycoproteins that are inserted into the lipid envelope.
  - Two peplomers are present-
    - ***Hemagglutinin (HA)***
    - ***Neuraminidase (NA)***



Morphology (cont..)

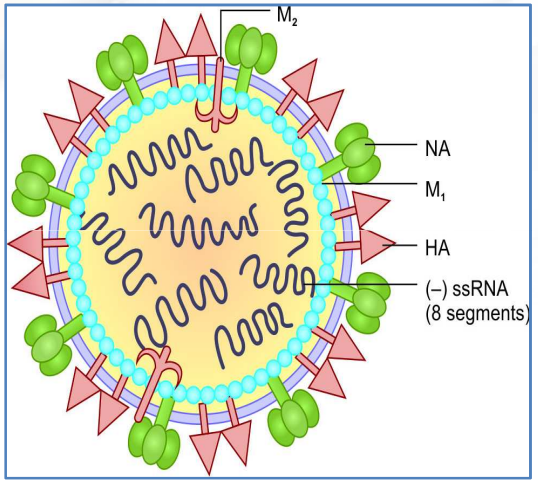
- ***Hemagglutinin (HA):***
  - Triangular shaped peplomer, binds to mucin or sialic acid receptors on RBCs, resulting in clumping of RBCs to cause **hemagglutination.**
  - Binds to the receptors on respiratory epithelial cells, thus facilitating viral entry.



Morphology (cont..)

Neuraminidase (NA):

- Mushroom-shaped peplomer, present in fewer number than HA.
- Sialidase enzyme that degrades the sialic acid receptors on RBCs; thus helps in-
  - Displaces HA from RBCs resulting in reversal of hemagglutination called **elution.**
  - Facilitates release of virus particles from infected cell surfaces during budding process by preventing self-aggregation of virions to the host cells.
  - NA helps the virus to pass through the mucin layer in the respiratory tract to reach the target epithelial cells.



Antigenic subtypes and Nomenclature

- **Three Genera-**Based on RNP and M proteins, influenza viruses are divided into four genera: A, B and C and D
- **Subtypes-** Based on HA and NA antigens,
- Influenza A has distinct 18 H subtypes (H1 to H18) and 11 N subtypes (N1-N11),
  - Most of the subtypes infect animals and birds, rarely human major epidemics and pandemics.
  - Example- Six HA (H1, H2, H3, H5, H7 & H9) and two NA (N1&N2) subtypes have been recovered from humans.
- Influenza B and C viruses though have subtypes; but are not designated.
- Influenza D virus primarily infects cattle and are not pathogenic to humans.

Antigenic subtypes and Nomenclature

Standard nomenclature system: Any influenza isolates should be designated based on the following information:

- Influenza Type/ host (indicated only for non-human origin)/ geographic origin/strain number/year of isolation/(HA NA subtype).
- Examples:
  - Human strain-
    - Influenza A/Hong Kong/03/1968(H3N2)
  - Nonhuman strain-
    - Influenza A/swine/Iowa/15/1930(H1N1)

Antigenic variation

- Antigenic variation is the unique property of Influenza viruses, which is due to the result of antigenic changes occurring in HA and NA peplomers.
  - ***Antigenic drift***
  - ***Antigenic shift***

Antigenic drift

- Minor change occurring due to ***point mutations*** in the HA/NA gene.
- Results in alteration of amino acid sequence of the antigenic sites on HA/NA, such that virus can escape recognition by the host's immune system.
- New variant must sustain two or more mutations to become epidemiologically significant.
- Seen in both Influenza virus type-A&B
- Results in outbreaks and minor periodic epidemics
- Antigenic drift occurs more frequently every 2-3 years.

Antigenic shift

- Abrupt, major drastic, discontinuous variation in the sequence of a viral surface protein (HA/NA)
- Occurs due to ***genetic re-assortment*** between genomes of two or more influenza viruses infecting the same host cells,resulting in a new virus strain, unrelated antigenically to the predecessor strains.
- Occurs only in Influenza A virus
- Results in pandemics and major epidemics- e.g. H1N1 pandemics of 2009.
- Antigenic shift occurs less frequently every 10-20 years.

Pathogenesis

Pathogenesis	Explanation
Transmission	• Via aerosols • Small-particle aerosols (<10μm) are more efficient in the transmission.
Target cell entry	• Viral HA attaches to specific sialic acid receptors on the host cell surface • Ciliated columnar epithelial cells are most commonly infected.
Multiply locally	• Virus replicates in the infected cells and infectious daughter virions spread to the adjacent cells.

Pathogenesis

Pathogenesis	Explanation
Spread	Very rarely, virus spreads to the lower respiratory tract or spills over blood stream to involve extra pulmonary sites.
Local damage	• Cellular destruction and desquamation of superficial mucosa of the respiratory tract • Edema and mononuclear cell infiltrations occur at local site leading to cytokine influx, which accounts for local symptoms. • Local damage predisposes to secondary bacterial invasion.

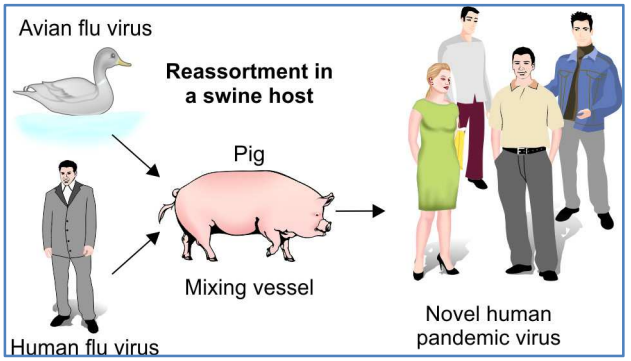
Sialic acid receptors

- Sialic acid receptors - found on the host cell surfaces are specific for HA antigens
- **α 2-6 sialic acid receptors:**
  - Specific for human influenza strains and are found abundantly on human upper respiratory tract epithelium but not on lower upper respiratory tract.
- **α 2-3 sialic acid receptors:**
  - Specific for avian influenza strains
  - Found abundantly on bird's intestinal epithelium.
  - In humans, they are present in very few numbers on lower upper respiratory tract.

Sialic acid receptors

- **Why pigs are the most common mixing vessels?**
  - Both α 2-3 &α 2-6 sialic acid receptors are found on the respiratory epithelium of pigs and swine flu strains have specificity for both the receptor types.
  - Hence pigs can be infected simultaneously by human, swine and avian strains, thus serving as a mixing vessel.

o Reassortment between the segments of various strains takes place inside the same swine cell.



Host immune response

- Humoral immunity - predominant
- Type and subtype-specific and long lasting.
- Antibodies against HA and NA – protective
- Antibodies to HA - prevent initiation of infection by inhibiting viral entry
- Antibodies to NA decrease the severity of the disease and prevent the transmission of virus to contacts
- Antibodies against other viral proteins are not protective.

Host immune response

- Antibodies against the ribonucleoprotein - useful in typing viral isolates as influenza A or B or C.
- All the three types of influenza viruses (i.e. A,B &C) are antigenically unrelated and there is no cross-protection.
- Immunity incomplete - reinfection with the same virus can occur.

Host immune response

- **Original antigenic sin:**
  - When a previously infected individual gets a repeated infection with a different antigenic variant of influenza virus, antibodies are produced against both the subtypes
  - But predominant response would be against the original strain, a phenomenon called "original antigenic sin."
- Components of both cell mediated immunity (e.g. cytotoxic T cells) and innate immunity (NK cells, interferons) are also important in providing immunity against influenza infections.

Clinical Manifestations

- ***Incubation period -*** 18-72 hours
- ***Uncomplicated Influenza (Flu syndrome*)**:
  - Asymptomatic or develop minor upper respiratory symptoms such chills, headache, and dry cough, followed by high grade fever, myalgia and anorexia.
  - Self-limiting condition.

Complications

Pneumonia:

- Secondary bacterial pneumonia - most common
- Common agents are staphylococci, pneumococci and *Haemophilus influenzae.*
- Primary Influenza pneumonia is rare but leads to more severe complication.
- Combined viral-bacterial pneumonia- It is three times more common than primary influenza pneumonia, most common co-infection being *S.aureus.*

Clinical Manifestations

- **Other** pulmonary complications:
  - Worsening of COPD
  - Exacerbation of chronic bronchitis and asthma.
- **Reye's syndrome**

Clinical Manifestations

- The following are at increased risk of complications:
  - Age- children <2yrs& old age (>65yrs)
  - Pregnancy
  - Underlying chronic lung, cardiac, renal, hepatic, and CNS conditions
  - Low immunity(HIV infected people)
  - Children have high risk of developing croup, sinusitis, otitis media, high-grade fever, and diarrhoea.

Epidemiology

- **Incidence:** 3–5 million cases of severe illness and 2.5–5 lakhs of deaths occur worldwide.
- **Seasonality:** Common during winters. The most common seasonal flu strain varies from season to season and from place to place (e.g. H3N2 in Pondicherry in 2018)
- **Epidemiological pattern:** Depends upon the nature of antigenic variation that occurs in the influenza types.

Epidemiology

- Major influenza outbreaks:

Years	Subtype	Extent of Outbreak
1889–1890	H2N8	Severe pandemic
1900–1903	H3N8	?Moderate epidemic
1918–1919	H1N1[a] (HswN1) (Spanish flu)	Severe pandemic
1933–1935	H1N1[a] (H0N1)	Mild epidemic
1946–1947	H1N1	Mild epidemic
1957–1958	H2N2 (Asian flu)	Severe pandemic
1968–1969	H3N2 (Hong Kong flu)	Moderate pandemic
1977–1978[b]	H1N1 (Russian flu)	Mild pandemic
2009–2010	H1N1	Pandemic

Avian Flu

- Birds - primary reservoir
- All influenza subtypes (16H types and 9N types) are found in birds and some of the subtypes can be transmitted to mammals (e.g.; H1, H2, H3, and H5 to humans; H1 and H3 to swine; and H3 and H7 to horses).
- Avian flu strains- highly virulent as they possess ***PB1F2*** protein, which targets host mitochondria and induces apoptosis.

Avian flu infection in birds

- Bird flu strains - highly lethal to chickens and turkeys (but avirulent to ducks) and are the major cause of economic loss in poultry causing severe mortality in chickens.
- Avian flu multiplies in intestinal tracts of birds and shed through feces into water.
- Influenza viruses do not undergo antigenic variation in birds, because of the short life span of birds.

Avian flu infection in humans

- Till date, all human pandemic strains have originated by reassortment between avian and human influenza viruses and the mixing has occurred in pigs.
- ***A/H5N1*** is the most common avian flu strain that has been endemic in the world for the past 15 years.
- *Origin*-It was first reported from Hong Kongin 1997and has spread to various countries including India within few years.

Avian flu infection in humans

- Less morbidity and more mortality
- *Clinical feature*- H5N1 avian flu strains are associated with higher rates of pneumonia and extra-pulmonary manifestations such as diarrhoea and CNS involvement.
- ***Other avian flu strains*** that can cause human infections are-
  - A/H7N7(Netherlands)
  - A/H9N2 (Hong Kong)
  - A/H7N9 (caused an outbreak in China, 2013)

Laboratory Diagnosis

- Avian flu strains can be identified by real time reverse transcriptase PCR detecting specific HA and NA genes.

H1N1 in India

Seasonal flu:

- After the pandemic in 2009, A/H1N1 has become a seasonal influenza strain circulating in India along with the other two seasonal strains (A/H3N2 and type B).
- Most cases in a year occur during the winter season (Dec-Jan).

H1N1 in India (cont..)

Epidemiological Surveillance for Influenza

- Integrated Disease Surveillance Program (IDSP) under NCDC, Government of India has established a network (of 12 regional centers) for epidemiological surveillance for H1N1 and other influenza like illness (ILI).
- Also monitors the changes in the circulating influenza strain (if any).

H1N1 in India (cont..)

- **Government of India (NCDC) report for H1N1, 2018:**
- Between 2010- 2017, about 1,15,630 cases and 8,681 deaths due to H1N1 were reported from India.
- **Varied with geographical regions:** Maharashtra was the worst-hit state with 23,958 cases and 2,710 deaths, followed by Gujarat and Rajasthan. Sikkim and Lakshadweep are the only two states/Union territories with no cases over the past seven years.
- **Varied with year: Year 2015 (maximum), 2017 and 2010** recorded highest number of cases

Clinical Features

- Uncomplicated influenza: Mild upper respiratory tract illness and diarrhea
- Complicated/severe influenza - high-risk groups - secondary bacterial pneumonia, dehydration, CNS involvement, and multi-organ failure.

Categorization of Seasonal influenza A/H1N1

- Guideline on categorization of Seasonal Influenza A/H1N1 cases during screening for home isolation, testing, treatment and hospitalization (issued by Ministry of Health & Family Welfare, Govt. of India)

Guideline on categorization

Category	Definition	Guideline for laboratory testing for H1N1*, treatment** and isolation
Category A	Mild fever plus cough / sore throat with or without bodyache, headache, diarrhoea and vomiting	Laboratory testing for H1N1- not required Treatment- only symptomatic, antiviral drugs not required. Isolation- Confine patients at home, avoid contact with public and high risk members in the family.
Category B	Category A plus any one: (i) High grade fever and severe sore throat or (ii) Presence of risk factors: Children, age >65 years, pregnant women, patients with lung/ heart/liver/kidney/ neurological disorders, diabetes, cancer or HIV; on long term steroid therapy.	Laboratory testing for H1N1- not required Treatment- Symptomatic treatment required. Antiviral drug (oseltamivir) may be required. Isolation- Confine patients at home, avoid contact with public and high risk members in the family.

Guideline on categorization

Category	Definition	Guideline for laboratory testing for H1N1*, treatment** and isolation
Category C	Category B plus any one: (i)Breathlessness, chest pain, fall in blood pressure, sputum mixed with blood, bluish discolouration of nails; (ii)Children with influenza like illness who had a severe disease as manifested by the red flag signs (inability to feed well, convulsions, difficulty in breathing, etc). (iii)Worsening of underlying chronic conditions.	Laboratory testing for H1N1- required . Immediate hospitalization required. Treatment- start antiviral drug (oseltamivir) immediately without waiting for lab result. Isolation- all components droplet precaution to be followed. (refer prevention of influenza section)

* Real time reverse transcriptase PCR can detect and quantify the specific HA and NA genes of H1N1.

** Oseltamivir (Tamiflu) tablet or Zanamivir (inhalational form)

GISRS

- Influenza surveillance has been conducted globally through WHO's Global Influenza Surveillance and Response System (GISRS).
- Monitors the evolution of influenza viruses and provides recommendations in areas including laboratory diagnostics, vaccines and treatment.
- Serves as a global alert mechanism for the emergence of influenza viruses with pandemic potential.

Laboratory Diagnosis

- **Specimen Collection:**
  - Ideal specimens - nasopharyngeal swab or lavage fluid, nasal aspirate or to less extent throat swab
  - Swabs *with a synthetic tip (e.g. polyester or Dacron* swabs) are best for specimen collection
  - Cotton or alginate swabs are unsatisfactory
- Transport: Swabs are immediately put inside the viral transport media, kept at 4°C during transport up to 4days, thereafter at –70°C.

Isolation of virus

- Embryonated eggs and primary monkey kidney cell lines have been the methods of choice for the isolation of influenza viruses in the past.
- The viral growth in cell line was detected by hemadsorption or hemagglutination test.
- Because of technical difficulty, isolation is not routinely followed for diagnostics.

Direct immunofluorescence test

- Viral antigens coated onto epithelial cells can be directly detected in nasal aspirates by using fluorescent antibodies.
- Rapid but less sensitive than isolation.

RT-PCR (reverse transcriptase PCR)

- Highly sensitive, specific and rapid (turnaround time of <1 day).
- Also detect the specific type and subtype of influenza virus

Real-time RT-PCR

- **Currently the gold standard** (recommended by Govt of India).
- Quantitative, higher sensitivity and specificity than RT-PCR with turnaround time of 2–3 hours.
- Simultaneously detects the three common seasonal flu strains (A/H1N1, A/H3N2 and type B).

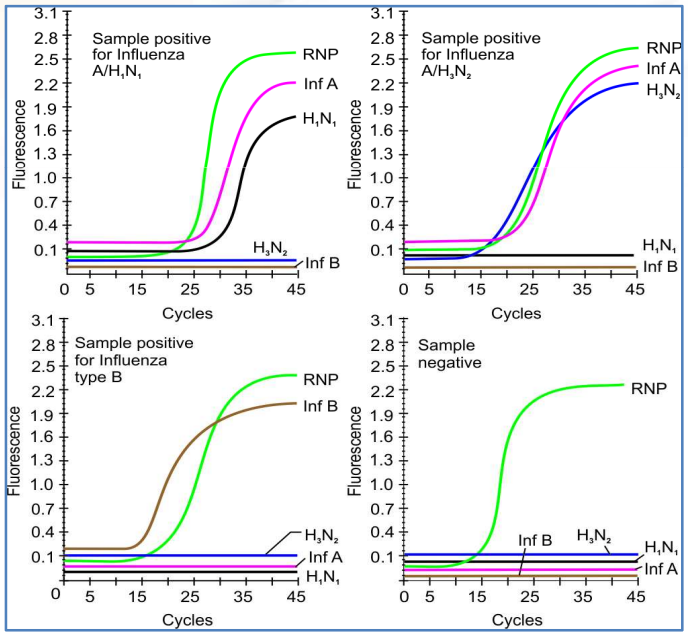
Real-time RT-PCR

- Result is expressed as the emission of fluorescence during the cycles

Table 44.5: Real time RT-PCR for seasonal Influenza types

Simultaneously detects five genes	Specimens positive with influenza type			Influenza negative
	A/H1N1	A/H3N2	Type B	
Influenza A (matrix gene)	+	+	–	–
H1N1 (HA gene)	+	–	–	–
H3N2 (HA gene)	–	+	–	–
Influenza B (HA gene)	–	–	+	–
RNP (ribonucleoprotein)*	+	+	+	+

*RNP (ribonucleoprotein) is an internal control; indicates presence of human DNA in specimen. If it is not detected, then the test is considered invalid.



Antibody detection

- Fourfold rise in the antibody titer between acute and convalescent sera is more significant than a single high titer.
- **HAI test** (Hemagglutination inhibition): Obsolete
- **Neutralization test**- Though it is the most specific & the best predictor of susceptibility to infection, is time-consuming and difficult to perform.
- **ELISA -** More sensitive than other assays; detects antibodies against HA and/or NA antigens.

Treatment

- **Neuraminidase inhibitors** (such as zanamivir, oseltamivir, and peramivir) can be administeredfor influenza A and influenza B infections.
  - Drug of choice for A/H1N1 2009 flu, A/H5N1avian fluand influenza-B.
  - Dosage-
    - Oseltamivir (Tamiflu 75mg tablets)
    - Zanamivir (10mg, inhalational form)
  - Schedule-
    - For treatment- given twice a day for 5 days
    - For chemoprophylaxis- given once daily. Duration depends on the clinical setting.

Treatment

- **Matrix protein M2 inhibitor:**
  - Amantadine and rimantadine can be given for some strains of influenza A infection.
  - Strains of A/H1N1 2009 flu and A/H5N1 avian flu and influenza B have developed resistance.

General preventive measures

- Measures of droplet precaution:
  - **Strict hand hygiene** to be followed.
  - **Isolation room:** Patients should be kept at isolation room or cohorting to be followed.
  - **Containment of coughs and sneezes**
    - Respiratory hygiene and cough etiquette
    - Use of gloves and mask for staff and patient
  - **Work restriction:** CDC recommends that people with influenza-like illness remain at home until at least 24 hours after they are free of fever (100° F) without the use of fever-reducing medications.

Vaccination Prophylaxis

- **Vaccine strains:**
- Based on WHO recommendations, influenza vaccines are prepared every year.
- **Strains to be included** in the vaccine depend upon the strains isolated in the previous influenza seasons and strains that are anticipated to circulate in the upcoming season.
- **Formulations**: Most of the influenza vaccines are cocktails containing two type A and one or two type B influenza strains.
  - *Trivalent form:* This is the most common form available; comprises of three strains: A/H1N1, A/H3N2 and influenza B strain.
  - *Quadrivalent form:* In addition to trivalent form, this contains another type B strain. (Yamagata lineage, 2013).
- **GISRN**: WHO's Global Influenza Surveillance and Response System (GISRS) reviews the vaccine composition on an annually for both northern and southern hemisphere.

Vaccination Prophylaxis

- **For 2018-19 season:** The WHO recommended vaccine:
  - For northern hemisphere is a *Quadrivalent form* vaccine :
  - Type A/H1N1(Michigan lineage, 2015)
  - Type A/H3N2 (Singapore lineage, 2016)
  - Type B (Yamagata lineage, 2013)
  - Type B (Victoria lineage, 2017)
  - For southern hemisphere is a trivalent vaccine: same as that for northern hemisphere except the Type B (Victoria lineage, 2017).
- **Types:** Both injectable (inactivated) and nasal spray (live attenuated) vaccines are available.

Injectable Vaccines

- Most widely used vaccines in immunization programmes.
- **Types:** There are three types of injectable vaccines.
  - *Inactivated Influenza Vaccine (IIV)*
  - *Cell Culture-based Inactivated Influenza Vaccine (ccIIV3);*
  - *Recombinant Influenza Vaccine (RIV)*

Injectable Vaccines (cont..)

- **Schedule:** Single dose administered by intramuscular (IM) route; except for 6 month-8 year of age (2 doses are required ≥ 4 weeks apart).
- **Timing of vaccination:** Optimally before onset of influenza season, i.e. by end of October.
- **Efficacy:** 25–67 % (25% for H3N2, 42% against type B and 67% against 42%). The efficacy is lower if vaccine virus doesn't match to currently circulating viruses. Immunity lasts for 6-12 months.
- **Side effects:** Mild reactions can occur in 5% of cases such as redness at injection site, fever and aches. Serious side effects - rare.

Injectable Vaccines (cont..)

- **Indication:** Routine annual influenza vaccination is recommended for all persons aged ≥6 months who do not have contraindications. If facility are not feasible, then high-risk groups should be given first priority for vaccination.
  - Age: ≥ 6 month to <5 years and ≥50 years
  - Persons with chronic pulmonary, cardiovascular, renal, hepatic, neurologic, hematologic, or metabolic disorders.
  - Low immunity (e.g. HIV infection)
  - Pregnant women
  - Those receiving aspirin
  - Extremely obese (body mass index ≥40)
  - Caregivers and contacts of those at risk: e.g. health care workers and household contacts

Injectable Vaccines (cont..)

- **Contraindication**: IV should not be administered to people who have allergy to eggs or have history of hypersensitivity to previous dose of vaccine.
- **Travelers:** If travelling to an area of increased influenza activity; can consider vaccination, preferably ≥2 weeks before departure.

Live Attenuated Influenza Vaccine (LAIV)

- Generated by reassortment between currently circulating strains of influenza A and B virus with a cold-adapted attenuated master strain which can grow at 33°C (upper respiratory tract) but not at 37°C (lower respiratory tract).
- May cause mild flu like symptoms but never infect lower respiratory tract, hence never cause serious adverse effects.
- Trivalent vaccine
- Administered by intranasal spray.

Live Attenuated Influenza Vaccine (LAIV)

- Indication: It can be given to all healthy persons of 2–49 years age (except in pregnancy), but is not given to high risk groups.
- However due to efficacy issues, LAIV is not recommended for use in any population for 2017-18.

Chemoprophylaxis

- Recommended only for post exposure and during outbreak situations in hospitals.
- **Indication:** Following exposure to an influenza case, it is recommended to the following groups: (i) if not vaccinated or vaccinated recently (<2weeks), (ii) HIV infected people.
- **Duration:**
  - *Non-outbreak exposure* (e.g. in community): It should be started as soon as possible following exposure (within 48 hours) and continued for 7 days.
  - *During outbreaks in hospitals* (for elderly persons & children and health care workers)- duration for a minimum of 2 weeks, and continuing up to 1 week after the last known case was identified.

Chemoprophylaxis

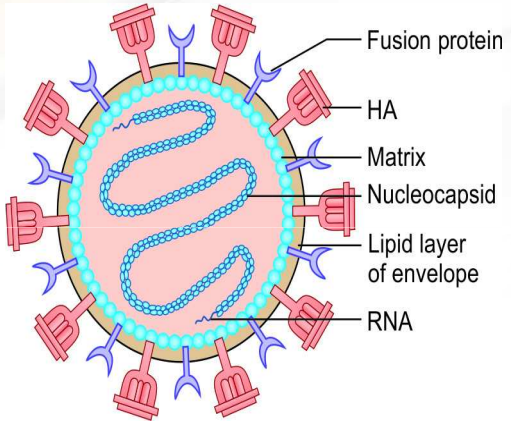
- **Antiviral drugs** recommend are:
  - Oseltamivir is the drug of choice. It is given as 75mg per oral once a day for 7 days.
  - Zanamivir : 10 mg (two 5-mg inhalations) once daily for 7 days.
- **Efficacy:** The efficacy of chemoprophylaxis is about 70% to 90% in preventing influenza.

PARAMYXOVIRIDAE

- Group of viruses, which are transmitted via the respiratory tract following which-
  - Cause localized respiratory infection in children
  - Disseminate
- Rubella virus is though not a paramyxovirus, because of its resemblance clinically and epidemiologically to measles virus; it has been discussed in this topic.

MORPHOLOGY

- **Size**- Vary from 100-300 nm
- Possess a **helical nucleocapsid** of 18 nm size
- **RNA-**Contains single-stranded RNA which is linear, non-segmented and negative-sense.
- **Contains six structural proteins**
- **Envelope**- The nucleocapsid is surrounded by a host derived lipid envelope in which the following virus-coded peplomers (glycoproteins) are inserted.



Glycoproteins of paramyxoviruses

- *F-glycoproteins*
- *Larger glycoproteins*

Characteristics of Family Paramyxoviridae

Subfamilies	**Paramyxovirinae**				**Pneumovirinae**	
Genera	Respirovirus	Rubulavirus	Morbillivirus	Henipavirus	Pneumovirus	Metapneumovirus
Human viruses	Parainfluenza 1, 3	Mumps, Parainfluenza 2, 4a, 4b	Measles	Hendra, Nipah (Zoonotic)	Respiratory syncytial virus	Human metapneumovirus
Nucleocapsid	18 nm	18 nm	18 nm	?	13 nm	13 nm
Large Glycoproteins	HN type	HN type	H type	G type	G type	G type
Hemagglutinin	+	+	+	-	-	-
Neuraminidase	+	+	-	-	-	-
Fusion protein	+	+	+	+	+	+
Hemolysin	+	+	+	?	-	-
Inclusions	Cytoplasm	Cytoplasm	Cytoplasm& Nucleus	?	Cytoplasm	?

PARAINFLUENZA VIRUSES

- Major causes of LRTI in young children.
- It has five serotypes (1-4)-
  - Types 1&3 belong to the genus *Respirovirus*
  - Types 2,4a&4b belong to the genus *Rubulavirus*
- **Pathogenesis and clinical manifestations**
  - Transmission is by respiratory route (by direct salivary contact or by large-droplet aerosols)
  - The incubation period appears to be 5–6 days.

Clinical manifestations

- Mild common cold syndrome such as rhinitis and pharyngitis is the most common presentation, seen with all serotypes
- Croup (laryngotracheobronchitis)
- Pneumonia or bronchiolitis
- Otitis media - most common complication
- Reinfections are common, but less severe.
- There is no cross protection between the serotypes.
- Immunocompromised people are susceptible to severe infections.

Epidemiology

- Worldwide in distribution.
- Type 3 - most prevalent serotype and exists as endemic throughout the year with annual epidemics occur during spring.
- Types 1 and 2 - less common and seasonal
- Type 4a&4b - milder illness
- Important cause of outbreaks in paediatric wards, day care centres and in schools.

Laboratory Diagnosis

- **Antigen detection -** Viral antigens in the infected exfoliated epithelial cells of the nasopharynx can be directly detected by immunofluorescence test by using specific monoclonal antibodies. It is rapid but less sensitive than viral isolation.
- **Viral isolation:**
  - *Specimens* such as nasal washes, bronchoalveolar lavage fluid and lung tissue can be used. Specimen should be inoculated as early as possible to obtain best results.
  - *Primary monkey kidney cells* are most sensitive, alternative is a continuous monkey kidney cell line- LLC-MK2.
  - They produce little or no cytopathic effect.

Laboratory Diagnosis

- **Serum antibodies** can be measured by neutralization test, hemagglutination inhibition test or ELISA. Presence of IgM or fourfold rise of IgG titer is indicative of active infection.
- **Reverse transcriptase PCR** assays are highly specific and sensitive but available only in limited settings.

MUMPS VIRUS

- Most common cause of parotid gland enlargement in children. In severe cases, it can also cause orchitis and aseptic meningitis.
- **Pathogenesis:**
  - *Transmission* - respiratory route via droplets, saliva, and fomites.
  - *Primary replication* occurs in the nasal mucosa or upper respiratory mucosa →infects mononuclear cells and regional lymph nodes → Spill over to blood stream resulting in viremia→ dissemination.
  - *Target sites*– Mumps virus has a special affinity for glandular epithelium. The classic sites include the salivary glands, testes, pancreas, ovaries, mammary glands, and CNS.

Clinical manifestation

- **Incubation period** is about 19 days (range, 7–23 days).
- **Inapparent infection**- Up to half of the infected people are either asymptomatic or present with non-specific symptoms such as fever, myalgia and anorexia. This is more common in adults than in children.
- Bilateral parotitis
- Epididymo-orchitis
- Aseptic meningitis
- Oophoritis
- Pancreatitis
- Atypical mumps

Epidemiology

- Endemic worldwide
- Sporadic cases occurring throughout the year, with a peak in cases typically in winter and spring
- Epidemics occur every 3-5 years; typically associated with unvaccinated people living in overcrowded areas
- **Period of communicability**- Patients are infectious from 1 week before to 1 week after the onset of symptoms
- Most contagious period - within 1–2 days before the onset of symptoms.
- Infective material- Mumps virus is shed in saliva, respiratory droplets, and urine.

Epidemiology

- **Source**- Cases (both clinical and subclinical cases) are the source of infection.
  - There is no carrier state
  - Subclinical cases (30-40% of all cases)
- **Reservoir**- Humans
- **Incidence-** Annual incidence of mumps is about 100-1,000 cases per 10,000 populations (worldwide) .
- **Age**- *5-9 years* age are most commonly affected; however, no age is exempt if there is no previous immunity. Disease tends to be more severe in adults.
- **Immunity**- One attack (either by vaccine or infection) gives lifelong immunity.
- Secondary attack rate is high (86%)

Laboratory Diagnosis

- **Specimens-** Buccal or oral swab.Can also be detected in saliva, CSF, urine(shed up to two weeks), seminal fluid and rarely blood. Massaging the parotid gland area for 30 seconds prior to swabbing is recommended.
- **Direct viral antigen detection** - specific immunofluorescent staining of clinical specimens
- **Virus isolation:**
  - Monkey kidney cells are the preferred cell lines. Specimens should be inoculated immediately.
  - Viral growth after 1-2weekscan be detected by demonstration of cytopathic effect (cell rounding and giant cell formation) or hemadsorption.
  - Shell vial technique is followed for rapid detection in 1-2 days.

Laboratory Diagnosis

Serum antibodies detection:

- ELISA - most widely used assay.
- Separate ELISA formats are available for detecting mumps specific IgM and IgG separately.
- Mumps ELISA is highly specific, does not cross-react with parainfluenza antibodies.
- Detection of IgM antibodies (present up to 60 days of infection), or a rise in IgG titer indicates active infection.
- The traditional tests such as neutralization test, hemagglutination inhibition test and complement fixation tests are seldom used now.

Laboratory Diagnosis

- **Reverse-transcription PCR is available to detect mumps** specific RNA such as N gene(nucleoprotein) in clinical specimen.
- Highly sensitive and specific.
- **Genotyping is done targeting small hydrophobic (SH)** gene; the most variable region of mumps genome.

Treatment

- No specific antiviral drug available.
- Treatment is mostly symptomatic.
- Mumps immunoglobulin is available but not effective, hence not recommended for treatment or post exposure prophylaxis.

Prevention (Live attenuated vaccine)

- **Live attenuated Vaccine Strain:**
- Prepared in chick embryo cell line.
- **Mumps vaccine is available as:**
  - Trivalent MMR vaccine (live attenuated measles-mumps-rubella vaccine) or
  - QuadrivalentMMR-V vaccine (contains additional live attenuated varicella vaccine
  - Monovalent mumps vaccine (not commonly used)
- **Schedule**- Two doses of MMR is given by IM route at 1 year and 4-6 year (before starting of school)

MEASLES (RUBEOLA) VIRUS

- Measles is an acute, highly contagious childhood disease characterized by fever & respiratory symptoms, followed by typical maculopapular rash.

Pathogenesis

- **Transmission**
  - Droplets inhalation over short distances and, less commonly,
  - Small-particle aerosols that remain suspended especially in schools, hospitals, and enclosed public places in the air for longer period.
- **Spread-**The virus multiplies locally in the respiratory tract; then spreads to the regional lymph nodes → enter into the bloodstream in infected monocytes (primary viremia)→further multiply in reticuloendothelial system → spills over into blood (secondary viremia)→disseminateto various sites.

Target sites

- The virus is predominantly seeded in the epithelial surfaces of the body, including the skin, respiratory tract, and conjunctiva.

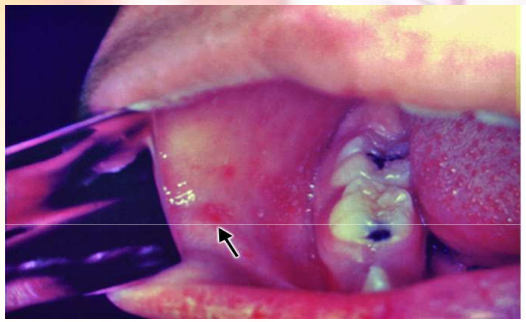
Clinical Manifestations

- *Incubation period* is about 10days which may be shorter in infants and longer (up to 3 weeks) in adults
- Disease can be divided into three stages.
- ***Prodromal stage:*** Lasts for four days (i.e. from 10th to 14th day of infection)
  - *Fever*
  - *Koplik's spots*
  - *Non-specific symptoms*

Clinical Manifestations

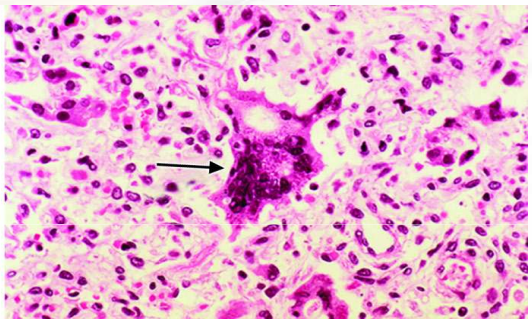
- ***Eruptive stage*:**
  - Maculopapular dusky red rashes
  - Rashes typically appear first ***behind the ears***→then spread to face, arm, trunk and legs→then fade in the same order afterfour days of onset
  - Fever (10^{th} day)→ Koplik's spot (12^{th} day) → rash(14^{th} day)
- ***Post measles stage***- It is characterized by weight loss and weakness. There may be failure to recover and gradual deterioration into chronic illness.

Measles



Koplik spot in buccal mucosa (measles)

Measles rashes (on face)



Multinucleated giant cell of measles infected cell lines

Complications

- ***Complications result from secondary bacterial infections:***
  - Otitis media and bronchopneumonia are most common
  - Recurrence of fever or failure of fever to subside with the rash
  - Worsening of underlying tuberculosis with a false positive Mantoux test
- ***Complications due to measles virus itself***
  - Giant-cell pneumonitis in immunocompromised children, and HIV infected people
  - Acute laryngotracheobronchitis (croup)
  - Diarrhoea, leads to malnutrition including vitamin A deficiency

Complications

- ***CNS complications***
  - *Post-measles encephalomyelitis*
  - *Measles inclusion body encephalitis*
  - *Subacute sclerosing panencephalitis (SSPE)*

Laboratory diagnosis

- **Specimens-**Nasopharyngeal swab, conjunctival swab, blood, respiratory secretions, and urine are the ideal specimens.Synthetic swabs are recommended.
- **Antigen detection-**Measles antigens in the infected cells can be detected directly by using anti-nucleoprotein antibodies.

Laboratory diagnosis

- **Virus isolation:**
  - Cell lines- Monkey or human kidney cells or a lymphoblastoid cell line (B95-a) are optimal cell lines used for isolation of measles. Vero/hSLAM cell line is the CDC recommended cell line.
  - Cytopathic effect - ***Warthin- Finkeldey cells***

Antibody detection

- Detection of measles-specific IgM antibody in serum or oral fluid or four fold rise of IgGantibody titer between acute & convalescent-phase sera is taken as significant.
- Demonstration of high titre measles antibody in the CSF is diagnostic of SSPE.
- IgM are detected by capture ELISA whereas IgG are detected by indirect ELISA.

Reverse-transcription PCR

- Extremely sensitive and specific,
- Also permit characterization of measles virus genotypes for molecular epidemiologic studies
- It can distinguish wild-type from vaccine virus strains
- RNA can be detected in specimens up to 10-14 days post rashes, in contrast to virus isolation, which often becomes negative after 3 days of rash.

Treatment-

- There is no specific antiviral therapy available for measles. Treatment is symptomatic and consists of general supportive measures.
- Vitamin A has been effective in reducing the morbidity and mortality due to measles.

General Preventive Measures

- Airborne precaution such as isolation in negative pressure room, use of PPEs such as N95 mask, etc. must be followed while handling measles cases

Live attenuated Measles vaccine

- Schwartz strain (currently serves as the standard in much of the world)
- Edmonston-Zagrebstrain
- Moraten strain
- Vaccine is prepared in chick embryo cell line
- *Reconstitution*- Vaccine is available in lyophilized form and it has to be reconstituted with distilled water and then should be used within 4 hours.
- Vaccine is thermolabile, hence it must be stored at -20^0C.
- One dose (0.5ml) containing >1000 infective viral units is administered subcutaneously.

Live attenuated Measles vaccine

- **Side effects include-** Mild measles like illness, toxic shock syndrome
- **Contacts-** Susceptible contacts over 9-12months may be protected against measles if the measles vaccine is given within 3 days of exposure.

Measures following contacts

- Measles immunoglobulin can also be given within 3 days, at a WHO recommended dose of 0.25mg/kg of body weight.
- However, both should not be given together. At least 8-12 weeks of gap must be maintained.

Epidemiology-

- **Source-** Cases are the only source of infection. Carriers are not known to occur.In-apparentor sub-clinical infections are rare.
- **Reservoir-** Humans are the only reservoir of infection. There is no animal reservoir.
- **Infective material-** Virus is shed in the secretions of nose, throat and respiratory tract of cases of measles, especially during the prodromal stage and early stage of rash.
- **Period of communicability-** Patients are infectious from four days before to four days after the onset of rash. Patients are highly contagious, isolation is recommended from the onset of prodromal stage until third day of rash.

Epidemiology-

- **Secondary attack rate** is very high (>90%)
- **Age-** Measles is a childhood disease
- **Immunity-** No age is immune if there is no previous immunity.
  - There is single serotype hence one attack (vaccine or infection) gives lifelong immunity.
  - Infants are protected up to 6 months due to pre-existing maternal antibodies.

Measles Elimination

- The target timeline for South East Asia Region (including India) is set by 2020. The following objectives are set to achieve this target:
- (1) ≥ 95% coverage with two doses against measles and rubella
- (2) Develop and sustain a case-based surveillance system
- (3) Develop and maintain an accredited measles and rubella laboratory network
  (4) Strengthen support and linkages to achieve the above three strategic objectives.

NIPAH VIRUS AND HENDRA VIRUS

- Zoonotic paramyxoviruses.
- Hendra virus was first isolated in 1994 in Hendra (Australia) and Nipah virus was discovered in 1999 in Malaysia.
- **Reservoir: Fruit bats (flying foxes) are the natural host** for both Nipah and Hendra viruses
- **Geographical distribution:** Hendra virus infections are confined to horses in Australia, whereas Nipah viruses cause infection of pigs in Malaysia

Transmission

- Hendra virus is transmitted by exposure to infected body fluids and excretions of horses
- Transmission of Nipah virus to humans may occur after direct contact with infected bats, pigs, or persons.
- Consumption of infected raw date palm sap is thought to be another mode of transmission.

Clinical manifestations

- **Both the viruses can produce** of encephalitis in humans

Laboratory diagnosis

- Real-time PCR from throat and nasal swabs, CSF, urine, and blood should be performed in the early stages of disease.
- Antibody detection by ELISA (IgG and IgM) can be used later stage.
- Immunohistochemistry is performed on tissues collected during autopsy, which confirm the postmortem diagnosis.
- Prone to cause laboratory acquired infections and are classified as biosafety level 4 pathogens.

- **Treatment: No antiviral drug is available.**
- **Vaccine: A subunit vaccine, using the Hendra G protein,** produces cross-protective antibodies against Hendra and Nipah viruses. It has been recently used in Australia to protect horses against Hendra virus. It can be used in humans as well.

RESPIRATORY SYNCYTIAL VIRUS

- **Transmission-**RSV is spread by i)direct contact(contaminated fingers or fomites and by self-inoculation of the conjunctiva or anterior nares) or ii) by large droplets inhalation
- **Spread**- RSV replicates locally in the epithelial cells of the nasopharynx and may spread to the lower respiratory tract to cause bronchiolitis and pneumonia.
- **Pathology**- Lymphocytes in large numbers migrate to the site and secrete several cytokines which cause the following changes-
  - Further peribronchiolar infiltration of inflammatory cells
  - Submucosaledema
  - Necrosis of the bronchiolar epithelium and
  - Formation of plugs consisting of mucus, cellular debris, and fibrin which occlude the smaller bronchioles

Clinical Manifestations

- **Incubation period** is about 3–5 days.
- **Infants-** RSV is the most common cause of lower respiratory tract infection below 1 year of age
- Chest X ray shows peri-bronchial thickening, diffuseinterstitial infiltration and occasionally lobar consolidation
- Severe in premature infants and underlying congenital cardiac disease, bronchopulmonarydysplasia, nephrotic syndrome, or immunosuppression
- **Adults-** RSV produces influenza-like upper respiratory symptoms such as common cold, running nose, sore throat, and cough

Laboratory Diagnosis

1. Antigen Detection

- Direct immunofluorescence test detecting antigens on exfoliated cells or
- ELISA detecting antigens in nasopharyngeal secretions.

2. Virus isolation –CPE - **syncytium formation**

3. Reverse Transcriptase-PCR -- RT-PCR amplifying viral RNA (such as nucleoprotein N gene)

Laboratory Diagnosis (cont..)

- **Antibody Detection-** Serum antibodies are of less diagnostic importance; rather they are the markers of prevalence of infection

Treatment

- Ribavirin is the drug of choice
- Supportive care

Rubella

- **MORPHOLOGY:**
- Togaviridae *family, and is the only* member under genus *Rubivirus.*
- Enveloped, single-stranded RNA virus measuring 50–70 nm in size; surrounded by capsid (C) protein
- Envelope contains a lipid layer from which two types of spike-like glycoproteins (E1 and E2) are projected

TYPES OF RUBELLA INFECTIONS

- **Postnatal Rubella - N**eonatal age, childhood, and adult life.
- **Transmission-** respiratory droplets via upper respiratory mucosa.
- **Spread -** Rubella virus replicates locally in the nasopharynx, and then spreads to the lymph nodes. Subsequently, viremia develops after 7–9 days, and lasts until 14th day by which time both antibody and rashes appear almost simultaneously suggesting an immunologic basis for the appearance of rash.

- **Clinical Manifestations**
- Incubation period is about 14 days (range, 12–23 days)
- Rashes are often the first manifestations in children, but in older children and adults, 1 to 5-day prodrome often precedes the rash, which includes low-grade fever, malaise, and upper respiratory symptoms
- Rashes are generalized and maculopapular in nature
- Lymphadenopathy (occipital and postauricular)
- Forchheimer spots
- Complications - Arthralgia and arthritis are common in adults,

LABORATORY DIAGNOSIS

- Specimen: Nasopharyngeal or throat swabs
- Virus Isolation: In monkey or rabbit origin cell lines and then growth is detected by viral interference
- Antibody detection: By HAI or ELISA.
- RT-PCR is available detecting rubella specific RNA (nucleoprotein N gene) in clinical specimens.

Congenital Rubella Syndrome

- **Transmission -** Both the risk of transmission to fetus and severity of congenital infection are maximum if the mother acquires the infection during first trimester of pregnancy.
- Risk after 5th month of pregnancy is almost negligible (90% risk at 11 weeks vs 20% risk at 20 weeks of gestation).

Clinical Manifestations

Classical triad consists of:

- Ear defect: *Sensory neural deafness (most common* defect of congenital rubella syndrome)
- Ocular defects: Salt-and-pepper retinopathy is the most common ocular defect followed by cataract
- Cardiac defect: Patent ductus arteriosus (PDA)
- CNS defects *such as—microcephaly, mental retardation,* motor delay and autism.

Laboratory Diagnosis

- IgM antibodies do not cross placenta; their presence in a neonate is diagnostic of congenital rubella infection
- Reverse transcriptase PCR to detect viral RNA.

PREVENTION

- **General Preventive Measures -** Airborne precaution
- **Rubella Vaccine - RA 27/3 is a live attenuated vaccine for rubella, prepared** from human diploid fibroblast cell line.
- **Schedule: Single dose (0.5 mL) of vaccine is administered** subcutaneously
- **Indication: In India, rubella vaccine is indicated in** all women of reproductive age (first priority group) followed by all children (1–14 years).
- **Precautions:** Vaccine is contraindicated in pregnancy;Infants below 1 year should not be vaccinated due to possible interference from persisting maternal antibody.