Polioviruses.
Enteroviruses.
Rotaviruses.

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Taxonomy of Polioviruses

Group: Group IV ((+)ssRNA)
Order: Picornavirales
Family: Picornaviridae
Genus: Enterovirus
Species: Enterovirus C (three serotypes of polioviruses)

It is possible to suffer 2-3 times from poliomyelitis (even from paralytic form), because three different serotypes of the virus are known.
Classification of Viruses

**Naked viruses**
- dsDNA
  - Adenoviridae
  - Papillomaviridae
  - Polyomaviridae
- ssDNA
  - Parvoviridae
  - Circoviridae
- dsRNA
  - Reoviridae
- ssRNA
  - Picornaviridae
  - Caliciviridae
  - Astroviridae
  - Out of family

**Enveloped viruses**
- dsDNA
  - Poxviridae
  - Herpesviridae
  - Hepadnaviridae
- ssRNA
  - Orthomyxoviridae
  - Paramyxoviridae
  - Coronaviridae
  - Retroviridae
- ssRNA
  - Filoviridae
  - Rhabdoviridae
  - Bunyaviridae
  - Arenaviridae
  - Togaviridae
  - Flaviviridae
Families of + RNA viruses
Electron Microscopy of Polioviruses
Morphology and Structure

Polioviruses are small spherical naked viruses with icosahedral capsid, enclosing a linear single-stranded positive RNA genome. Virion is approximately 30 nm in diameter. The naked enterovirus genome is infectious and resembles messenger RNA (mRNA). The icosahedral capsid has 60 capsomers, grouped in 12 pentameric vertices, each of which is composed of five protometric units of proteins. The protomers are made of four virion polypeptides (VP1, VP2, VP3, and VP4).
Structure of Polioviruses: Naked viruses with Icosahedral Capsid
Antigens of Polioviruses

The main enterovirus antigens are capsid proteins (e.g., VP1). Viruses have group specific complement-binding antigen, identified in complement fixation reaction, and type specific antigens for each serotype, defined in neutralization tests in mice or cell cultures. Human polioviruses have 3 serotypes.
Replication of Polioviruses
(Initial Stages of Poliovirus Penetration)

Picornaviruses (e.g., enteroviruses) bind the specific receptors on the susceptible cells. Then the capsid releases VP4, the capsid is weakened and VP1 forms a channel directly across the membrane of the cell, by which the viral genome (“+”ssRNA) enters the cytoplasm, and binds to the ribosomes.
Scheme of the capsid of poliovirus.
The icosahedral capsid of the virus is represented with the three external proteins VP1, VP-2 and VP-3 constituting a protomer. The 5-fold, 3-fold and 2-fold axis of symmetry are indicated. The canyon recognized by the cellular viral receptor CD155 is shown.
The canyon recognized by the cellular viral receptor CD155 is shown.
First Stages of Interaction of Poliovirus with the Susceptible Cell
Replication of Polioviruses

(continuation)

“+”ssRNA acts as mRNA. A single large polyprotein, containing all viral protein sequences, is translated within 10-15 minutes of viral penetration. The polypeptide is cleaved into different proteins (RNA-dependent RNA polymerase, protease, and new capsid proteins). RNA polymerase synthesize “-“strand RNA from genomic “+”strand RNA. The“-“strand RNAs serve as templates for new mRNAs and genomes.

The translated polyprotein is cleaved initially in VP0, VP1, and VP3 by viral-encoded protease. The proteins assemble into subunits, five subunits form pentamers, 12 pentamers are packaged into the procapsid, and after assembly around the genome, VP0 is cleaved into VP2 and VP4 to finish the maturation of the virion.

The replication cycle is completed in 3-4 hours. The mechanism of viral release is still unclear, but each degrading cell release up to 10,000 viable polio viruses.
Genome organization of polioviruses, translation and processing

A Viral (+) strand genome

Translation/processing
Transmission of Poliomyelitis

Polioviruses are transmitted by the fecal-oral route (intestinal source). Polioviruses can be spread also by oral-oral route (oropharyngeal source).
The upper respiratory tract, the oropharynx, and the intestinal tract are the portals of entry for enteroviruses. Viral replication is initiated in the mucosa and lymphoid tissue of the tonsils and pharynx, and the virus later infects lymphoid cells of Peyer’s patches underlying the intestinal mucosa. Primary viremia spreads the virus to receptor-bearing target tissues (reticuloendothelial cells of the lymph nodes, spleen, and liver), where a second phase of viral replication may occur, resulting in symptoms and a secondary viremia. In the case of poliovirus, the virus must cross the blood-brain barrier or may gain access to the brain by infecting skeletal muscle and travelling up the innervating nerves to the brain, somewhat like rabies does. Viral shedding from the oropharynx can be detected for a short time before symptoms begin and can occur up to a month.
Pathogenesis and Clinical Manifestations

Poliovirus has one of the narrowest tissue tropism, recognizing a receptor expressed on anterior horn cells of the spinal cord, dorsal root ganglia, motor neurons, skeletal muscle cells, lymphoid cells, and few other cells. Coxsackieviruses and echoviruses recognize receptors expressed on more cell types and tissues and cause more repertoires of diseases. Receptors for these enteroviruses are present on cells of the central nervous system, heart, lung, pancreas, mucosa, and other tissues. Most enteroviruses are cytolytic, replicating rapidly and causing direct damage to the target cell.
Scheme of Interaction between Poliovirus and Neuron
Types of Poliovirus infections

Poliovirus infections are

1) usually asymptomatic illness (>90%);

2) minor illness, or abortive poliomyelitis (≈5-7%): pharyngitis or gastroenteritis;

3) aseptic meningitis, or nonparalytic poliomyelitis ((≈1-3%): the prodromal symptoms include generalized, nonthrobbling headache; fever of 38-40 °C; sore throat; anorexia; nausea; vomiting; and muscle aches. These symptoms may or may not subside in 1-2 weeks, then meningitis can follow;

4) major illness, or paralytic polio (≈0.1-1%); in which spinal cord motor neurons are killed, and flaccid paralysis results, and, in severe cases, motor cortex of the brain is disrupted (bulbar poliomyelitis).
Pathogenesis of Poliomyelitis

Day 0:
- Small intestine: Primary infection, replication

Day 1:
- Mesenteric lymph nodes: Replication

Day 2:
- Bloodstream: Primary viraemia

Day 3:
- Control focus of replication

Day 4:
- Initial appearance of antibodies

Day 5:
- CNS: Infection, replication, intraneural spread

Day 6:
- High level of antibody in serum

Day 7:
- Paralysis

Day 8:
- Excretion of virus in faeces
Polio Spread

Wild Polio Virus—1988–2005

- 1988: 350,000 estimated paralytic polio cases
- 2005: 2033 cases (1979 wild type)

99% decline in cases

Countries with confirmed wild polio virus cases

Source: WHO: Summary, Global Immunization Coverage, 2005
Egyptian stele of polio victim (see right leg) – (1403–1365 BC).
Paralytic Poliomyelitis
Paralytic Poliomyelitis
Immunity

Antibody is the major protective immune response to the polioviruses. Secretory IgA can prevent the initial establishment of infection in the oropharynx and gastrointestinal tract, and serum antibody prevents viremic spread to the target tissue and therefore disease. Cell-mediated immunity is not usually involved in protection but may play a role in pathogenesis.
Laboratory Diagnosis of Poliomyelitis

Clinical specimen: nose and throat swabs, stool, cerebrospinal fluid; serum, etc.

Methods of diagnosis:
1. Virological method (polioviruses grow in monkey tissue culture, coxsackieviruses and echoviruses grow in primary monkey or human embryo kidney cells with identification in neutralization tests with type specific serums).
2. Serological method (detection of antigens in samples and fourfold increase in paired serums between the time of illness and the period of convalescence by CFT, ELISA, RIA, neutralization tests).
3. Immunofluorescent method.
Specific Prophylaxis of Poliomyelitis

Active immunization is used. Two types of poliovirus vaccine exist: the inactivated polio vaccine (IPV), developed by Jonas Salk, and a live attenuated oral polio vaccine (OPV), developed by Albert Sabin. OPV is trivalent and contains polioviruses type 1, 2, and 3. Three doses of the vaccine (OPV or IPV) at the intervals of 6 weeks are to be given before the age of six months. The live vaccine elicits lifelong immunity and local immunity after three doses of vaccine.

Russian National Immunization Programme includes 2 doses of inactivated polio vaccine (IPV) initially, and then the live vaccine is administered in the third dose (OPV) in Russian Federation.
Advantages of Live Polio Vaccine

The attenuated vaccine (OPV) have several important advantages over inactivated vaccine:
lower cost,
ease of administration,
spread to contacts resulting in a herd effect,
mucosal as well as humoral immunity.
Side effects, which can be caused by Specific Prophylaxis of Poliomyelitis

The major drawback of the live vaccine is that there is a remote potential for the virus to revert to its virulent form and cause paralytic disease. The incidence of this is estimated to be 1 per 4 million doses administered (versus 1 in 100 people infected with the wild-type poliovirus). The risk of vaccine associated paralytic poliomyelitis is increased in immunocompromised people, but the disease is more likely to occur on susceptible adults than in susceptible children. Such vaccine-associated poliomyelitis can occur in the contacts as well as the recipients of the vaccine.
Polio Vaccine

Inactivated (J. Salk)

Live OPV (A. Sabin)
Treatment of Poliomyelitis

Pleconaril can be used for therapy of poliomyelitis.

Pleconaril prevents the poliovirus from attachment to the host cell, because pleconaril binds to a hydrophobic pocket in the VP1 protein.
Enterovirus Infections

Enterovirus infections is a large group of infectious diseases, caused by many serotypes of enteroviruses, which are characterized by different clinical manifestations of target tissues and organs (rash, mucous lesions (e.g., herpangina, conjunctivitis), common cold-like symptoms, myocarditis, pericarditis, pleurodynia, meningitis, encephalitis, paralysis, pancreatitis, hepatitis, etc.).
Enteroviruses

**Family:** Picornaviridae  
**Genus:** Enterovirus  
**Species:** Currently twelve Enterovirus species are classified (Human enteroviruses are species A-D), there are the following main groups within the species by traditional historical classification:  
- **Coxsackievirus A** (types 1-22, 24)  
- **Coxsackievirus B** (types 1-6)  
- **Echovirus** (types 1-9, 11-27, 29-33)  
- **Enterovirus** (types 68-71 types).
Antigens of Enteroviruses

The main enterovirus antigens are capsid proteins (e.g., VP1). Viruses has group specific complement-binding antigen, identified in complement fixation reaction, and type specific antigens for each serotype, defined in neutralization tests in mice or cell cultures. Human pathogenic enteroviruses are polioviruses (3 serotypes), coxsackieviruses A (23 serotypes), coxsackieviruses B (6 serotypes), echoviruses (31 serotypes), enteroviruses (4 serotypes).
The virus family he discovered was eventually given the name Coxsackie, for the town of Coxsackie, New York, a small town on the Hudson River where Dalldorf had obtained the first fecal specimens.
Groups of *Coxsackieviruses*

Coxsackieviruses are divided into group A and group B viruses based on early observations of their pathogenicity in mice. Group A Coxsackievirus were noted to cause a flaccid paralysis, which was caused by generalized myositis, while group B Coxsackievirus were noted to cause a spastic paralysis due to focal muscle injury and degeneration of neuronal tissue. At least 23 serotypes (1-22, 24) of group A and 6 serotypes (1-6) of group B are recognized.
Coxsackieviruses Infects Suckling Mice but not Adult Mice
Pathogenesis of Coxsackievirus Infection

Coxsackieviruses are transmitted primarily via the fecal-oral route and respiratory aerosols, although transmission via fomites is possible. The viruses initially replicate in the upper respiratory tract and the distal small bowel. They have been found in the respiratory tract up to 3 weeks after initial infection and in feces up to 8 weeks after initial infection. The viruses have been found to replicate in the sub mucosal lymph tissue and disseminate to the reticuloendothelial system. Further dissemination to target organs occurs following a secondary viremia.
Enterovirus Pathogenesis

**ENTEROVIRUS PATHOGENESIS**

Entry via aerosol or ingestion

- Replication Oro-pharynx tonsils
- Replication Peyer’s patches

- Virus in feces

**Secondary viremia**
- Target tissue
  - Polio Cox
  - Echo, Polio Cox
  - Hep A
  - Echo Cox A
  - Echo Cox A B

**Primary viremia circulation**
- Brain
- Meninges
- Liver
- Skin
- Muscle
- Hand foot mouth disease
  - Rash Herpangina

**Encephalitis Paralysis**
- Hepatitis A
- Myocarditis Pericarditis Pleurodynia
Cytopathic Effect (CPE) of Enteroviruses

Area of red callout box has been enlarged via Photoshop to better view CPE. Arrows point to cells showing pyknosis (shrunken, dense, poorly shaped cells).
Coxsackievirus Infection

- Both group A and group B Coxsackievirus can cause nonspecific febrile illnesses, rashes, upper respiratory tract disease, and aseptic meningitis.
Coxsackievirus Infection

- In general, group A coxsackieviruses tend to infect the skin and mucous membranes, causing herpangina, acute hemorrhagic conjunctivitis (AHC), and hand-foot-and-mouth (HFM) disease.
Acute Hemorrhagic Conjunctivitis

- Infection that affects the whites of eyes
- Starts as eye pain
- Followed by red, watery eyes
- Causes eye swelling and light sensitivity
- Blurry vision may occur
Coxsackie virus syndrome, a type of Coxsackie virus syndrome, causes painful red blisters in the throat and on the tongue, gums, hard palate, inside of the cheeks, and the palms of hands and soles of the feet.
Hand, Foot, and Mouth Disease

Hand, foot and mouth disease usually affects infants and children, and is quite common. It is highly contagious and is spread through direct contact with the mucus or faeces of an infected person. It typically occurs in small epidemics in nursery schools or kindergartens, usually during the summer and autumn months.
Hand, Foot, and Mouth Disease

• Type of Coxsackie Virus syndrome
• Causes painful red blisters on:
  – Throat
  – Tongue
  – Gums
  – Cheeks
  – Palms of hands
  – Soles of Feet
Hand, Foot, and Mouth Disease
Hand, Foot, and Mouth Disease
Herpangina

Herpangina, an infection of the throat which causes red-ringed blisters and ulcers on the tonsils and soft palate, the fleshy back portion of the roof of the mouth.
Myocarditis can be a serious disease

- Group B Coxsackievirus tend to infect the heart, pleura, pancreas, and liver, causing pleurodynia, myocarditis, pericarditis, and hepatitis
Coxsackie B3 Causes Myocarditis

Coxsackie B3 has been found to be one of the main causes of certain debilitating or life-threatening diseases, such as viral myocarditis.

In about 20% of the cases, there can be progressive disease or recurrence of symptoms; the heart damage can be extensive, causing arrhythmias, weakened left ventricular functions.
Pleurodynia

Also called Bornholm disease (Born Holm word is a place where the disease is identified)

Causes painful spasms in the muscles of the chest and upper abdomen

Males: may have pain in the testicles
Echoviruses

The first echoviruses were accidentally discovered in human faeces, unassociated with human disease during epidemiological studies of polioviruses. The viruses were named echoviruses (enteric, cytopathic, human, orphan viruses).

These viruses were produced CPE in cell cultures, but did not induce detectable pathological lesions in suckling mice.

Altogether, There are 32 echoviruses (types 1-34; echovirus 10 and 28 were found to be other viruses and thus the numbers are unused)

There is no group echovirus Ag but heterotypic cross-reactions occur between a few pairs.
## Diseases associated with Enteroviruses

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Polio</th>
<th>Cox A</th>
<th>Cox B</th>
<th>Echo</th>
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<tr>
<td>Paralytic disease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Meningitis-encephalitis</td>
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<td>Carditis</td>
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<td>+</td>
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<tr>
<td>Neonatal disease</td>
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<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Pleurodynia</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>Herpangina</td>
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<td>+</td>
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<tr>
<td>Rash disease</td>
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<td>+</td>
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<tr>
<td>Haemorr. conjunctivitis</td>
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<tr>
<td>Respiratory infections</td>
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<tr>
<td>Undifferentiated fever</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diabetes/pancreatitis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Laboratory Diagnosis of Enterovirus Infection

**Clinical specimen:** nose and throat swabs, stool, cerebrospinal fluid; serum, etc.

**Methods of diagnosis:**

1. **Virological method** (polioviruses grow in monkey tissue culture, coxsackieviruses and echoviruses grow in primary monkey or human embryo kidney cells with identification in neutralization tests with type specific serums).

2. **Serological method** (detection of antigens in samples and fourfold increase in paired serums between the time of illness and the period of convalescence by CFT, ELISA, RIA, neutralization tests).

3. **Immunofluorescent method.**

4. **Molecular-genetic method** (RT-PCR).
Treatment and Prevention

There is no specific antiviral therapy available against enteroviruses other than polio.

Some authorities use human normal immune globulin in the treatment of neonatal infections or severe infections in immunocompromised individuals. HNIG have been to prevent outbreaks of neonatal infection with good results.

There is no vaccine available mainly because of the multiplicity of serotypes.
Rotaviruses are the main agents of the human infantile viral diarrhea.

Taxonomy:
Family: *Reoviridae*
Genus: *Rotavirus*
Species:  
A, B, C, D, E, F, G, H (human are mainly infected by species A (90% cases), also B and C species are infectious for humans).
Classification of Viruses

Naked viruses
- dsDNA
  - Adenoviridae
  - papilloma-viridae
  - Polyoma-viridae
- ssDNA
  - Paroviridae
  - Circoviridae
- dsRNA
  - Reoviridae
- ssRNA
  - Picornaviridae
  - Caliciviridae
  - Astroviridae
  - Out of family
  - Deltavirus

Enveloped viruses
- dsDNA
  - Poxviridae
  - Herpesviridae
  - Hepadnaviridae
- ssRNA
  - Orthomyxoviridae
  - Paramyxoviridae
  - Coronaviridae
  - Retroviridae
  - Filoviridae
  - Arenaviridae
  - Togaviridae
  - Flaviviridae
### Families of +RNA Viruses

<table>
<thead>
<tr>
<th>Reo</th>
<th>Birna</th>
<th>Calici</th>
<th>Picorna</th>
<th>Flavi</th>
<th>Toga</th>
<th>Retro</th>
<th>Corona</th>
<th>Filo</th>
<th>Rhabdo</th>
<th>Bunya</th>
<th>Orthomyxo</th>
<th>Paramyxvo</th>
<th>Arena</th>
</tr>
</thead>
</table>

The diagram illustrates various families of +RNA viruses, each represented by a unique visual symbol.
Morphology and Structure of Rotaviruses

Rotaviruses are spherical naked viruses of icosahedral morphology with a double-layered capsid (outer capsid, containing outer layer and intermediate layer, and inner capsid) and double-stranded segmented RNA genome, consisting 11 separate segments. Virion is 60-80 nm in diameter. It resembles a wheel in electron micrographs. The genome is generally monocistronic. The genomic segments encode 6 structural (VP) and 6 nonstructural (NSP) proteins. The outer capsid is composed of structural proteins that surround a nucleocapsid core that includes enzymes for RNA synthesis. Outer capsid contains glycoprotein VP7 and viral attachment protein VP4, forming spikes. The intermediate layer consists of major protein VP6. The nucleocapsid (inner capsid, covering double-stranded RNA) has 3 structural proteins (VP1, VP2, and VP3). VP1 has enzymatic activity of RNA-dependent RNA polymerase (transcriptase); VP3 has enzymatic activity, which acts as the mRNA capping enzyme.
Structure of Rotaviruses
Structure of Rotaviruses
Electron microscopy of rotaviruses.
They resemble a wheels in electron micrographs.
Antigens of Rotaviruses

Group specific antigen is protein VP6 (serogroups A, B, C, D, E, F, G, H are equivalent of species). Group A has 4 serotypes (type specific antigens are VP7 and VP4).
Antigens of Rotaviruses
Antigens of Rotaviruses
Replication of Rotaviruses (Reoviridae)
Replication of Rotaviruses

The rotavirus replication starts in the gastrointestinal tract from protease cleavage of external capsid proteins, forming intermediate/infectious subviral particles (ISVP). They attach via viral protein VP4 to a cellular receptor sialoglycoproteins and integrins on epithelial and other cells. Rotaviruses replicate inside cytoplasm of host cell. ISVP are partially uncoated in the endolysosomes by removal of the outer capsid (VP4 and VP7), and then penetrate through endosomal membrane in the cytoplasm.
Replication of Rotaviruses
(continuation)

Early transcription of the dsRNA occurs inside these double-layered particles by viral RNA-dependent RNA polymerase for each genomic segment. The “+”RNA (mRNA) are extruded in the cytoplasm and translated to generate different viral proteins. They also serve as a template for “-” RNA in the new cores, replicating the double-stranded genomes, which are packed later within new nucleocapsid core structures. Assembly occurs inside the cytoplasm. Mature virions with outer capsid are formed on the outside the endoplasmic reticulum and released after cell lysis.
Replication of Rotaviruses
(Reoviridae)
Replication of Rotaviruses

1. VP4 spikes attach to lining and outer shell is shed
2. Subparticle enters cytoplasm
3. Virus multiplies and produces toxin
4. New virus leaves infected cells to invade healthy ones
5. Epithelial cells die and fluids exit the body
Electron micrograph of a rotavirus-infected enterocyte (above) compared to an uninfected cell (bottom).
Pathogenesis and Clinical Manifestations of Rotaviral Infection

Incubation period is 1-2 days. Rotaviruses disrupt differentiated columnar epithelial cells at the tips of the small intestine villi, shortening them. Stool contains 10^10 viral particles per gram. Mononuclear cells infiltrate the lamina propria, leading to malabsorption of nutrients, electrolytes, and water, resulting in fever, vomiting, diarrhea with dehydration. The NSP4 protein acts in a toxin-like manner to promote calcium ion influx into enterocytes which disrupts the cytoskeleton and tight junction of the host cells. The NSP4 protein induces also release of cytokines and neuronal activators which alter water absorption. Mucosal humoral (IgA) and cell-mediated protective immunity is formed within 1 week, leading to recovery and persistently protect from reinfection.
Pathogenesis of Rotaviral Infection.
Clinical Manifestations of Rotaviral Infection, caused by Dehydration.
Mortality of Rotaviral Infection.

Under-5 mortality rate due to rotavirus disease per 100,000 population (<5 years of age)
Laboratory Diagnosis of Rotavirus Infection

Clinical specimen: feces; serum, etc.

Methods of diagnosis:
1. **Microscopical method** (electron microscopy and immune electron microscopy).
2. **Serological method** (detection of antigens in stool or antibodies in serum by ELISA, RIA, passive agglutination test, neutralization tests).
3. **Immunofluorescent method**.
4. **Molecular-genetic method** (RT-PCR for detection of genotype of rotavirus).
Laboratory Diagnosis of Rotavirus Infection

1. Electron microscopy

2. Serological method (ELISA for detection of rotaviruses)
Laboratory Diagnosis of Rotavirus Infection

3. Molecular-genetic method (genotyping)
Specific Prophylaxis of Rotavirus infection

Specific prophylaxis is based on administration of live attenuated rotavirus vaccine (ROTARIX) that contains 89-12 human strain from serogroup A and is 90% effective in protection against severe rotavirus infection.

ROTARIX is a vaccine indicated for the prevention of rotavirus gastroenteritis caused by G1 and non-G1 types (G3, G4, and G9) when administered as a 2-dose series. ROTARIX is approved for use in infants 6 weeks to 24 weeks of age.