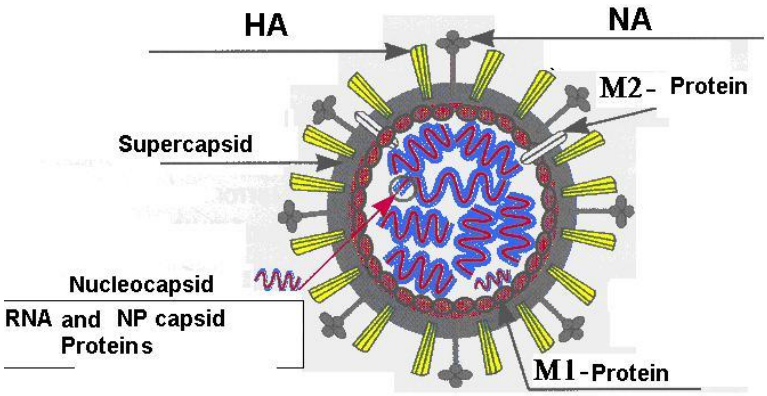
RNA ENVELOPED VIRUSES
(Orthomyxoviuses**)**

INFLUENZA VIRUS

Family Orthomyxoviridae (GK. myxa mucus): Three immunologic types are known, influenza A, B and C viruses. **Influenza A** virus causes influenza in human and influenza in animals (pigs, horse) and birds. **Influenza B** and **C viruses** are pathogenic only for human. The viral aetiology of type A influenza was ascertained in 1933 by W. Smith, C. Andrewes, and P. Laidlaw.

Influenza is an acute viral infection, for which intoxication and defeated respiratory tract are typical. This is pandemic infection.



Morphology: The influenza virus is spherical or oval (polymorphs: can be filamentous, is frequent in freshly isolated strains; rod shape) middle sized virus with the diameter of 80-120 nm. The nucleocapsid is formed of a RNA helix (connected with **endonuclease**) enclosed in an outer lipid-carbohydrate-protein membrane (enveloped virus), which is connected with core by **M matrix protein.** The virus is composed of single-stranded RNA of negative polarity. The negative sense single - stranded RNA is segmented and exists as eight pieces. Viral RNA-dependent RNA polymerase is present, which is essential for transcription of the viral RNA in infected host cell. The nucleocapsid is surrounded by an envelope, which has an inner membrane protein layer and an outer lipid layer (lipoprotein layer).The membrane protein is known as the matrix "M" protein. From the envelope two types spikes are projecting **Hemagglutinin (HA)** and **Neuraminidase (NA),** which are the important antigens that determine antigenic variation of influenza virus and host immunity.

Resistance: Influenza viruses are sensitive to high temperature. The virus loses infectivity more rapidly at 50°C. They are sensitive to ether, which destroys infectivity.

Antigenic structure: The influenza viruses have two antigens: **S (solutio)** internal soluble antigen, which is connected with the RNA. Because it is found free in the infected tissues and occurs in the supernatant when the virus containing fluid is centrifuged, it was also called soluble "S" antigen. By S antigen the influenza virus is subdivided into 3 serotypes: A, B and C. They possess no-cross reactivity among the three groups. Revealing of this antigen by CFR and immunoprecipitation tests. The next is **V (viral)** antigen, which is on the surface of spike like membrane, by chemical structure is glycoprotein HA and NA, they are used to subtype the viruses. 14 subtypes of HA (H1-H14) and nine subtypes of NA (N1-N9), in many different combinations, have been revealed from birds, animals, or humans. Three **HA (H1-H3)** and two **NA (N1, N2)** subtypes have been revealed covered from humans.

Hemagglutinin is complex glycoprotein. It is responsible for hemagglutination and hemadsorbtion. It binds viral particles to susceptible cells and is the major antigen against which neutralizing (protective) antibodies are directed. HA provided entering of viruses into the cell.

Functions of hemagglutinin are:

- Adhesion
- Antigenicity
- Penetration
- Hemagglutination
- Protective
- HA is a strain-specific antigen and capable of great variation. Neuraminidase:

The antigenicity of **Neuraminidase** is also important in determining the subtype of influenza virus isolates. The NA functions at the end of the viral life cycle. It is a sialidase enzyme. It facilitates release of viral particles from the infected cell surface during the budding process and helps prevent self-aggregation of virions by removing sialic acid residues from viral glycoproteins. It is not effective in protection as the antihemagglutinin antibody. It is a strain-specific antigen and exhibits variations.

Influenza viruses are remarkable because of the frequent antigenic changes that occur in HA and NA. Antigenic variants of influenza virus have a selective advantage over the parental virus in the presence of antibody directed against the original strains. This phenomenon is responsible for the unique epidemiologic features on influenza. Incidence of antigenic variation is highest in Influenza virus A, less in B and it has not been demonstrated in Influenza C. Depending on the degree of antigenic change of H and N, two distinct forms of antigenic variation are known:

Antigenic drift (minor antigenic changes) is due to the accumulation of point mutations in the gene, resulting in amino acid changes in the protein. Sequence changes can alter antigenic sites on the molecule such that a virion can escape recognition by the host's immune system. A variant must sustain two or more mutations before a new, epidemiologically significant strain emerges.

Antigenic shift (major antigenic changes in HA and NA) reflects drastic changes in the sequence of a viral surface protein, changes too extreme to be explained by mutation.

Influenza virus replication: The first stage is interaction of the virus with the host cell. The virus attaches to the glycoprotein receptors of the host cell. Viral particles are internalized within endosomes then. The next step involves fusion of the viral envelope and cell membrane. The next is uncoating by action of proteolytic enzymes.

Biosynthesis: Transcription and translation. The influenza virus has single-stranded RNA of negative polarity as genetic material. The influenza virus' RNA hasn't mRNA function. mRNA is synthesized by action of virus specific RNA-dependent RNA polymerase. After that translation takes place on host cell's ribosomes for virus-specific proteins synthesis (virus-specific RNA polymerase, HA, NA, the structural proteins). The next stage is maturation. The virus matures by budding from the apical surface of the cell. Individual viral components arrive at the budding site by different routes (the nucleocapsids are assembled in the nucleus and move out to the cell surface, the matrix protein synthesized in the cytoplasm). The viral multiplication cycle proceeds rapidly. New progeny viruses are produced within 8-10 hours.

Epidemiology: The three types on influenza vary markedly in their epidemiologic patterns. Influenza C is least significant. It causes mild, sporadic respiratory disease but not epidemic influenza. Influenza B sometimes causes epidemics, but for influenza type A virus is typical massive epidemics called pandemics. Every 10-40 years, when a new subtype of influenza A appears, a pandemic results (There are in 1918 (H1N1-Spanish); 1957 (H2N2); 1968 (H3N2-Hong-Kong); 1977 (H1N1 re-emerged). The reason why the virus is able to cause epidemics and pandemics is its ability to undergo antigenic variations.

The incidence of influenza peaks during the winter.

Pathogenesis: Influenza virus spreads from person to person by airborne droplets or by contact with contaminated hands or surface. The influenza virus penetrates the cells of the surface epithelial layer of the upper respiratory tract mucosa. The influenza virus is strictly pneumotropic and the first reproduction on this membrane occurs. After, the virus enters into the blood (viremia occurs). As the virus multiplies and the infection develops, the trachea, bronchi, bronchioli, and alveolal epithelial cells gradually become involved in the process. Influenza infections cause cellular destruction and desquamation of superficial mucosa of the respiratory tract. This is attributed to loss of ciliary clearance, dysfunction of phagocytic cells. They enter into the lymph nodes and infect the lymphocytes and acquired immunodeficiency occurs. It serves as a favourable medium for the development of secondary bacterial infections (bronchitis, pneumonia, encephalitis, influenzal meningitis).

The incubation period is 12-48 hours. Symptoms of influenza usually appear abruptly and include chills, headache, and dry cough, followed closely by high fever, generalized muscular aches, malaise, and anorexia.

Clinical symptoms of influenza in children are similar to those in adults, although children may have higher fever and a higher incidence of gastrointestinal manifestations. Influenza A viruses are important cause of croup in children under 1 year of age.

Immunity: Antibodies against HA and NA are important in immunity to influenza. They have virus neutralization property. They form stable immunity. The three types of influenza viruses are antigenically unrelated and therefore induce no cross-protection. Cytotoxic T cells and macrophages take place in immune response too, but the role of cell-mediated immune responses in influenza is unclear. Protection correlates with secretory IgA antibodies too.

Treatment: Amantadine, rimantadine, antiviral immunoglobulin, Interferon are used. Antibiotics are used for prevention of secondary infections.

Prevention: Influenza spread by the air-droplet route. The source of infection is a patient who may infect healthy people when sneezing, coughing, and talking. Influenza is highly contagious. Spread of

infection is prevented by isolation of patients, regularly ventilating the rooms and cleaning them a damp cloth (moistened in chloramine solution).

Viral vaccines are the primary means of prevention of influenza. Inactivated, live vaccines are used. Now purified HA and NA is used as a vaccine which is few reactogene and toxic.

Treatment: Amantadine, Rimantadine, antiviral immunoglobulin, Interferon are used. Antibiotics are used for prevention of secondary infections.

Laboratory diagnosis (picture1-2): Clinical characteristics of viral respiratory infections can be produced by many different viruses. Consequently, diagnosis of influenza relies on isolation of the virus, identification of viral antigens in the patient's cells, or determination of a specific immunologic response by the patient.

Virological method: Isolation and identification of virus. Classically, embryonated eggs and primary monkey kidney cells have been the isolation methods of choice for influenza viruses.

Rinocytoscopic method.

Immunofluorescence.

Serological: This based on hemagglutination inhibition and complement fixation reactions.

MEASLES

Measles is acute viral infection. Measles is anthroponose infection with severe intoxication, catarrhal syndrome, rhinitis, and laryngitis and skin rash.

Measles virus is in the family Paramyxoviridae, genus Morbilivirus. The virus has the general morphology of Paramyxoviruses. It is a spherical, 150-500 nm in diameter. It has spiral nucleocapsid which is surrounded by the lipoprotein envelope carrying on its surface hemagglutinin(H) spikes. The measles virus does not possess neuraminidase(N) activity. The envelope also has the F protein which mediates cell fusion and haemolytic activities. The genome consists of single-stranded RNA, negative polarity. It contains internal S antigen and surface V antigen and has single serotype (antigenically is uniform).

Resistance: The causative agent is very susceptible to high temperatures and is destroyed quickly at 58° C. Outside the body it survives for not longer than 30minutes. It is sensitive to exposure to sunlight.

Cultivation: The virus cultivated on various tissues and on monkey kidney cells or on human amnion cells. Measles virus grows slow and typical cytopathic effects are develops (syncytium formation, multinucleate giant cells are also formed in lymphoid tissues of patients).

Pathogenesis: Human is the only source of infection. Infection prevails in children. Patients become infective from the first day of the prodromal period and remain so until the fourth and fifth day after the appearance of eruption. Measles is spread by the air-droplet route. The disease prevails in winter. The route of entry is the mucous membrane of upper respiratory tract. After entering the upper respiratory tract the virus replicates (primary reproduction) and invades the blood and viremia occurs. Virus affects the capillary endothelium and maculopapular rash appears. Except it, the virus suppresses the activity of T-lymphocytes and secondary immunodeficiency develops. The virus can enter CNS and cause encephalomyelitis.

The incubation period is 9-11 days. Clinically there are 3 periods: prodromal, catarrhal, convalescence. Infection starts as severe with intoxication, fever 39-40^{o} C, sneezing, coughing, running nose, redness of eyes, anorexia. This is the prodromal period which lasts 3-4 days. The main symptom in this period is appearance of Koplik's spots, which are small tiny red patches with central white specks on the oral mucosa opposite the molars. Later, the illness becomes weak, but on the 5th day the symptoms of prodromal period recover and the rash appears (Koplik's spots disappear). The red maculopapules rash of measles typically appears on the forehead first and spreads downwards progressively to the chest, the trunk, and the limbs, to disappear in the same sequence 3-6 days later leaving behind a brownish discolouration and finely granular desquamation. Symptoms are most marked when the rash is at its peak but subside rapidly thereafter. Most patients recover uneventfully but quite a few develop complications which may be due to the virus (croup, bronchitis) or to the secondary bacterial infections(pneumonia, otitis media). The most serious complication is involving the central nervous system. Acute encephalitis, meningoencephalitis occurs; late complication is subacute sclerosing panencephalitis (SSPE). The mortality rate in encephalitis associated with measles is about 15 per cent.

Immunity: Infection confers lifelong immunity. The presence of humoral antibodies indicates immunity. However, cellular immunity must also be relevant to protection.

Prevention and control: In specific prevention, attenuated live measles virus vaccine is used. Each child should receive two doses of measles vaccine, the first at 15 months of age and second just before entering school. The vaccine is given either by itself, or in combination, as the MMR vaccine. Prevention

includes isolation of patients. Sick children are normally not sent to the hospital but are isolated at home. Rooms which have been occupied by measles patient must be ventilated and be looked after in adequately hygienic conditions and immunisation of all individuals who have been in contact with patients should be done with anti-measles immunoglobulins.

Treatment: there are no available antiviral drugs effective against measles or its complications. Bacterial superinfections should be treated with antibiotics. Passive immunization indicates neonates, susceptible pregnant woman, and immunosuppressed patients. Passive immunity persists for 30 days. If a child was exposed to contact with measles patient for the second time, the gamma-globulin injection is repeated. Usually gamma-globulin does not completely provide protection from the disease, but delays its onset, significantly lessens its severity, and prevents fatal cases.

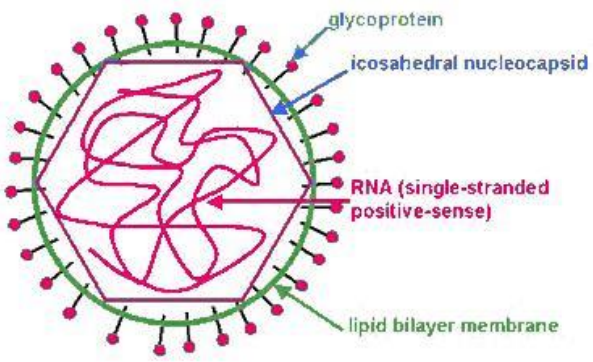
Laboratory diagnosis (picture1-2):

1.Serological-hemagglutination inhibition, complement fixation tests.

2.Virological.

Togaviridae family

Togaviridae family (toga-meaning the Roman mantle or cloak, and refers to the viral envelope) has two genuses: Alpha virus and Rubivirus. Rubella virus is in Rubivirus genus.



RUBELLA (German measles)

Virion is spherical, 50- 70nm in diameter. The genome is composed of single-stranded RNA, an icos ahedral nucleocapsid and a lipoprotein envelope. On the surface of virion there are **E1** and **E2** glycoprotein spoke-like projections. These surface spikes contain hemagglutinin. Rubella virus possesses hemolytic and neuraminidase activities. However, unlike the Paramyxoviruses, such as measles and mumps viruses, it has a positive strand RNA and therefore has no virion polymerase. The virus has a single antigenic type. Antibodies, against hemagglutinin, neutralizes infectivity. Humans are the natural hosts.

Antigenic structure: Rubella virus possesses nucleoprotein (nucleocapsid), protein **"C"** antigen, which is revealed by CFR and **E2**, **E1** antigens; revealing of them is by neutralization reaction. E1 possesses hemaglutination activity. Antibodies against E2 antigens have expressed protective action.

This virus is sensitive to environmental factors. It is inactivated by ether, chloroform, formaldehyde. It is destroyed by heating at 56ºC, but survives for several years at 60ºC.

Cultivation: The virus can been grown in many primary cell cultures and continuous cell lines and they cause cytopathic changes. Experimental infection can be produced in animal organisms. A suitable experimental model for the teratogenic effects of Rubella is the pregnant rabbit in which the virus infects the fetus transplancentally, leading to congenital malformations.

Rubella is primarily a mild childhood fever. It may be **acquired congenitally** or **ostnatally.**

A. Postnatal Rubella

Rubella virus is excreted in oropharyngeal secretions and infection is acquired by inhalation. Virus multiplies locally in the upper respiratory tract and in the cervical lymph nodes, followed by dissemination throughout the body by the way of the blood stream.

Incubation period is 2-3weeks. After an incubation period a brief prodromal period with fever and malaise is followed by a generalized fine, pink, maculopapular rash development, which starts on the face and progresses downward to involve the extremities. The rash is generally discrete and ordinarily disappears by the third day. Fever is usually inconspicuous, but a characteristic feature is that postauricular, suboccipital and posterior cervical lymph nodes are enlarged and tender from in a very early stage of illness. The illness is of short duration and recovery usually complete, within 3-4 days after the appearance of the rash. Mild polyarthritis, usually involving hands, occurs in about 60% adult women caused by immune complexes. Complications like thrombocytopenic purpura, postinfectious encephalopathy and rubella progressive panencephalitis are sometimes observed.

B. Congenital Rubella

The significance of rubella virus is not as a cause of mild childhood disease but as a teratogen. Rubella virus can cross the placental barrier in early pregnancy, and infect the fetus where it disseminates and grows in every fetal organ. It may result in a large variety of congenital abnormalities or death of fetus. Congenital annormalities may include total or partial neurosensory deafness, cataract, glaucoma, microphthalmia or retinopathy leading to blindness, congenital heart disease especially patent ductus arteriosus, sometimes accompanied by septal defects and pulmonary artery stenosis, microcephaly with mental retardation, thrombocytopenic pupura and hepatosplenomegaly. This is known as congenital rubella syndrome(CRS).

About 20% of all infants infected in utero during the first trimester of pregnancy are born with severe and usually multiple congenital abnormalities and many of the remainder have milder defects. Severe congenital abnormalities may lead to intrauterine death with abortion or stillbirth. If rubella occurs in the fourth month of pregnancy, the risk reduces to approximately 5% and only abnormally likely to be seen in neurosensory deafness. If infection occurs after the fourth month of pregnancy, congenital abnormalities are very infrequent.

The rubella virus is present in all excretions of congenitally infected infants. About third of them continue to shed the virus for six months, and a few for a year or more. The virus may persist in tissues such as cataractous lenses for several years, infected babies constitute an important source of infection to the staff in nurseries.

After postnatal Rubella acquired, stable, life long immunity is formed, which depends on hemaglutinin and virus neutralization immunoglobulins. After congenital Rubella immunity is not stable.

Prophylaxis: Alive attenuated **MMR vaccine** is recommended for all infants in the second year life (15months). The vaccine is effective and long lasting (10years) and causes few side effects. Because it is alive vaccine, it should not be given to immunocompromised patients or to pregnant women.

Laboratory diagnosis: 1.Virological: the virus may be isolated from blood during the early stages or more successfully from throat swabs in rabbit kidney or vero cells. 2.Serological: ELISA for IgM and IgG antibodies gives valuable information. A finding of IgM alone, without IgG, means current acute infection. IgG antibody alone, without IgM means past infection or vaccination and denotes immunity.

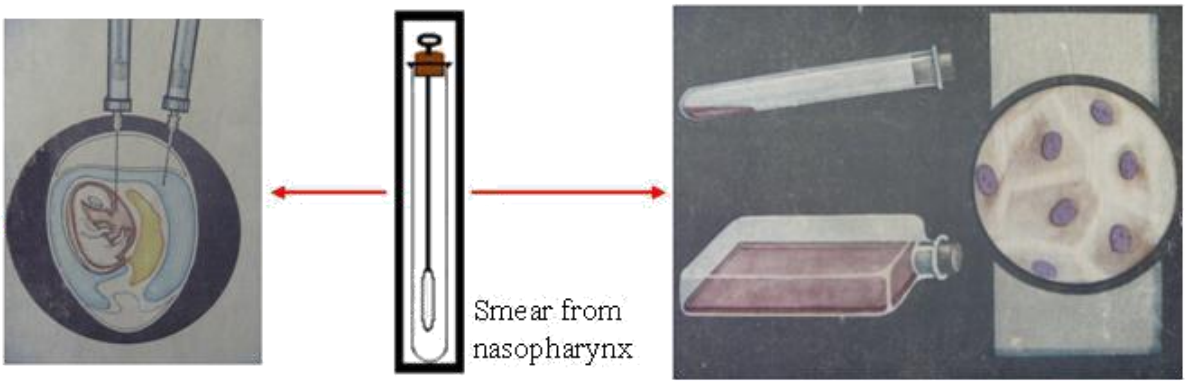
In congenital rubella, the virus may be isolated from variety of sources such as urine, throat swabs, leucocytes, bone marrow or cerebrospinal fluid. In a new born baby, demonstration of Rubella IgM antibodies are diagnostic of congenital rubella as IgM antibodies do not cross the placenta. However, many babies have rubella IgG antibodies, acquired transplacentally. Radioimmune assay, heamagglutination inhibition test are used too.

LABORATORY DIAGNOSIS OF ARD

BCAUSATIVE AGENTS	METHODS
Influenza virus, Parainfluenza virus, RS-, adeno-, rhino-, reo-, entero-, corono viruses	Immunofluorescence Virological Serological (CFR, HAIR, BNR)
Pneumococci, Klebsiella, Bordetella, Streptococci, Staphylococci, Mycoplasma, Chlamydia, Rickettsia	Bacterioscopic Bacteriological Serological

VIROLOGICAL METHOD

Isolation of viruses

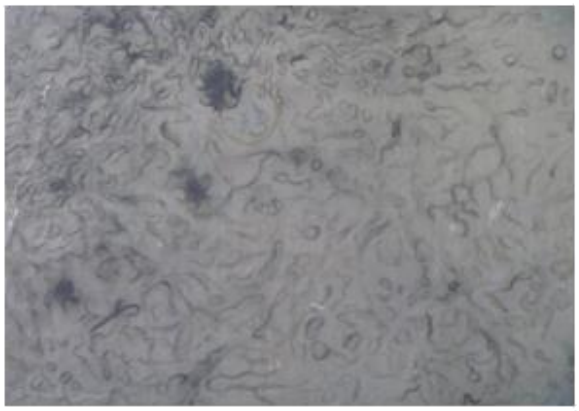


IDENTIFICATION

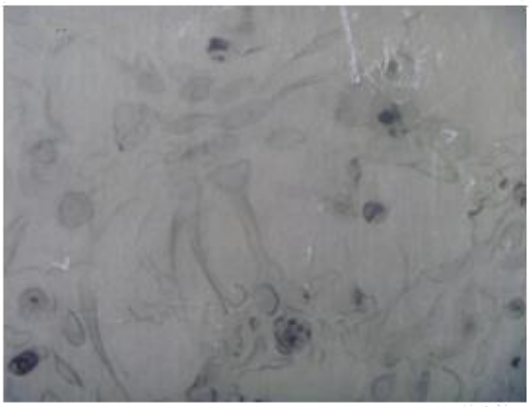
HAR

IDENTIFICATION (CFR, HAIR, BNR, IFR)

CYTOPLASMATIC ACTION OF VIRUSE (CPE)



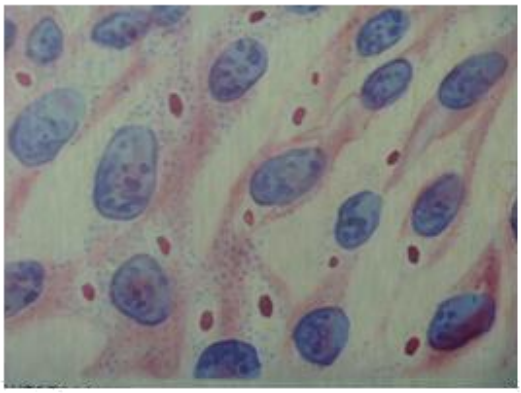
Normal cells



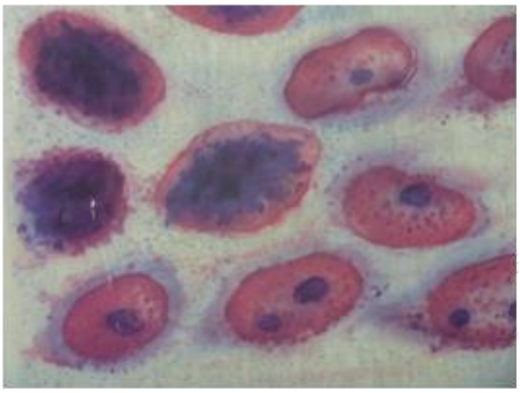
CPE

CULTURE OF FIBROBLASTS OF CHICKEN EMBRYO

Viral inclusions

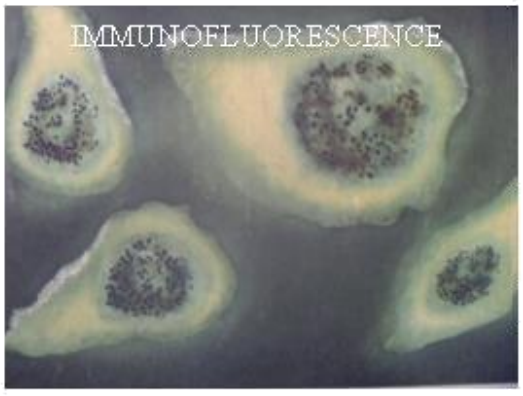


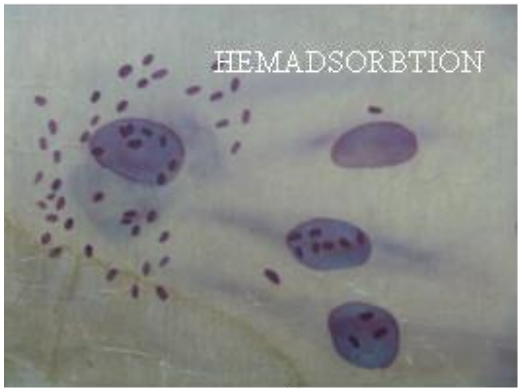
Cytoplasmatic inclusions in smallpox

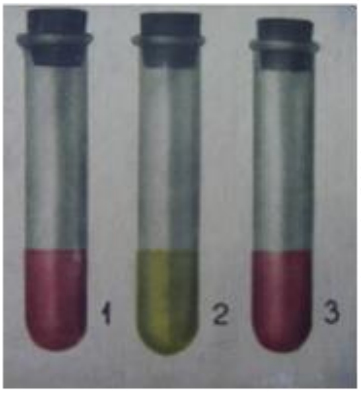


Intranuclear inclusions in adenoviral infections

PATCHES FORMATION







INHIBITION OF METABOLISM (color test)

1. Initial color of nutrient media
2. Changes of initial color
3. Saving of the initial color due to viral reproduction