EMILIAN DAMIAN POPOVICI
MARIANA ANGHEL
RADU PAVEL

LUMINIŢA MIRELA BĂDIŢOIU
SORINA MARIA DENISA LAITIN
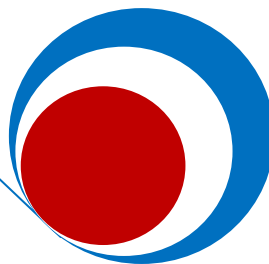
PRACTICAL EPIDEMIOLOGY
for students and residents

TABLE OF CONTENTS

IMMUNOPROPHYLAXIS OF INFECTIOUS DISEASES

DEFINITIONS

Immunization represents the active induction or temporary conferral process of immunity, by administering various immunobiological products.

It can be:

1. **Active** – which determines the induction of own antibodies production, by vaccine and anatoxin administration, unlike the
2. **Passive** – which temporarily transfers immunity by high-tech antibodies administration, as total immunoglobulin, specific or specific serums.

The immunization and vaccination terms are not synonyms. While immunization describes an immunity induction or supply process by active or passive means, vaccination strictly refers to the administration of a vaccine or anatoxin. Consequently, vaccination does not guarantee immunization!

ACTIVE IMMUNOPROPHYLAXIS

It represents the insertion into the human body of non-toxic and avirulent antigens, which can stimulate and induce an immune response similar to the one produced naturally.

Active immunisation generally gives long term immunity, for a period of months or years (e.g.: after diphtheria-tetanus vaccination, the protection persists for approx. 10 years). However, there are exceptions as well, like the flu vaccine which, due to its viral agent particularities, needs to be repeated annually.

Post-vaccine immunity is only installed after a certain period of latency, weeks to months, needed to produce own antibodies in protective titre. In immediate risk situations, this interval was left unprotected, it can be covered by the administration of passive immunization agents – immunoglobulins or specific serums.

The practice uses several classifications of vaccines. The most usual are:

A. Depending on the nature of the antigen:

1. **antiviral vaccine –** for example, the flu, measles, rubella, poliomyelitis, hepatitis viral A/B, rabies vaccines;
2. **antibacterial vaccines –** like the tuberculosis vaccine (BCG), diphtheria, tetanus, typhoid, pneumococcal vaccines;
3. **mycosis vaccines –** with a small scale of use compared to the first two categories (e.g. candida vaccine);
4. **protozoal vaccines –** like the vaccine candidates against malaria with sporozoites, merozoites or gametocyte antigens, each with a limited efficiency. Worldwide, great efforts are made in order to make such vaccines effective, by obtaining multivalent formulas, with a concomitant action on the various stages of the parasite agent.

B. In relation to the number of antigenic components:

1. **monovalent vaccines**, where the antigen comes from one single microbial species – for example the BCG only contains strains of *Mycobacterium tuberculosis bovis*;
2. **complex vaccines,** which contain several types of strains of the same species, like in the flu vaccine (with 2 strains of flu virus A and one or 2 type B) or in the poliomyelitis vaccine (with strains of 1 and 3 polio virus serotypes);
3. **combined vaccines,** which combine several antigens coming from different species, in order to simplify vaccination programs. Now, there are in Romania:
    - bivalent product – diphtheria-tetanus for adults (dT);
    - trivalent – acellular diphtheria-tetanus-pertussis (DTPa), measles-rubella-mumps;
    - tetravalent – DTPa + inactive polio vaccine (Tetraxim- Sanofi Pasteur);
    - pentavalent - DTP/DTPa + inactivated polio + *Haemophilus influenzae* type b (PentAct-Hib-Sanofi Pasteur/Pentaxim-Sanofi Pasteur)
    - and hexavalent - the 5 previous components are also added the recombined HBsAg DNA (Infanrix Hexa – GlaxoSmithKline/ Hexacima-Sanofi Pasteur).

C. **According to the method to prepare the vaccine**:

1. **Live corpuscular vaccine, attenuated or super-attenuated** – which include living microorganisms, with low virulence by repeatedly passing culture media, by passages at various animal hosts or by genetic mutations. It awards a persistent protection, like the natural post-infectious one, however it can generate multiple and severe post-vaccine reactions. Such products are the BCG and most of the antiviral vaccines – poliomyelitis with live strains, measles, rubella. Due to their high reactogenicity, contraindicated to persons with immunosuppressants of various etiologies. As well, the teratogenic risk imposes their exclusion from the vaccination programme for pregnant women.

2. **Inactivated corpuscular vaccines** – which include full inactivated (killed) bacterial/viral particles by heat or formalin. Induced post-vaccine immunity is smaller compared to that determined by previous products, however, adverse reactions are lower. The same category includes as well cellular pertussis vaccines, hepatitis A or inactivated poliomyelitis vaccine.

3. **Bacterial anatoxins** – are products from the exotoxins of microorganisms by the neutralization of toxigenesis, but keeping the immunogenic capacity. The initially obtained native anatoxin is optimized by purification and absorption on mineral support, and purified and absorbed anatoxin will come out. Such procedure will increase the efficiency of immune stimulation. The most used anatoxins are tetanus – ATPA and diphtheria – ADPA.

4. **Subunit vaccines, with antigenic fragments** – contain an antigen or an antigenic function with the main role in triggering the production of antibodies. The removal of various cellular protein components, of nucleic acids, without major importance for immunogenicity, leads to a significant decrease of adverse reactions. Frequently used are the flu vaccines with surface antigens type hemagglutinin and neuraminidase (Influvac-Solvay Pharmaceuticals, Fluarix-GlaxoSmithKline) or those with fragments and from the virion (Vaxigrip-Sanofi Pasteur). Usual as well are the vaccines obtained by molecular recombination, like hepatitis B - 2nd generation (Engerix B-GlaxoSmithKline; Euvax B-Sanofi Pasteur; Recombivax HB-Merck&Co) or 3rd generation, which contain recombined HBsAg DNA.

5. **Idiotype vaccines** – their conformation is similar to that of the initial antigenic determinant. These products were applied on various epidemiological trials, in the attempt to obtain an HIV_1 vaccine. Like

the vaccine candidates with covering proteins – idiotype gp120 or idiotype vaccines CD4.[1] In the field of HIV/AIDS infection, the researches in the field of vaccinology bump into multiple deontological, ethical and social problems. Although the manifestation of the epidemiologic process of the HIV/AIDS infection is presently influenced, by the antiretroviral treatment, we hope, for the future, to obtain as well effective vaccine products.

6. **Vaccines with stable non-pathogenic mutants** – they offer an protective immune response in diseases where the cell-mediated immunity is important. Although during the evaluation/application phase, they proved their superiority to certain classical vaccines, less immunogenic and much more reactogenic. Same situation is that of the typhoid vaccine with stable non-pathogenic mutants, but there is the possibility to develop other dysenteric, cholera, malaria, rotavirus vaccine candidates.

7. **DNA vaccines** – still under study, determine the stimulation of cell immunity by inserting a foreign DNA in the genome of the host cell.[1] They stimulate the immune cell response, compared to most of the vaccines addressed to humoral immunity. This type of vaccine product is a hope for the effective prophylaxis of VHC viral hepatitis or of the pathology caused by high antigenic variability viruses (the flu virus or HIV). Although advantageous by the fact that the human body contact to a live vaccine strain is avoided, there are as well some potential oncogenic risks, by incorporating the DNA in the host cell chromosomes or by inhibiting the tumour suppressor genes.[2]

   D. **From the point of view of vaccine compulsoriness**:

1. **Compulsory vaccines** – administered to the entire population according to the vaccine calendar, periodically updated within the National Immunization Programs. These programs can vary from one country to another, depending on the epidemiological situation, the geographic area and the material resources of the healthcare system. Harmonization has been a tendency lately, especially European, according to the requirements of the Worldwide Health Organization. In Romania, the **National Immunization Program 2019** includes the following compulsory vaccines:
   ➢ Tuberculosis – with live attenuated vaccine BCG;
   ➢ poliomyelitis – with IPV inactivated vaccine;
   ➢ diphtheria- tetanus- pertussis – with non-cell DTP within the hexavalent product (DTPa-IPV-Hib-HBsAg recombined DNA) or tetravalent (DTPa-IPV), followed by dTpa from 14 years old;

> - *Haemophilus influenzae* type b – concomitantly to the 2 previous vaccines, within the hexavalent product (DTPa-IPV-Hib-HBsAg recombined DNA);
> - hepatitis B with HBsAg recombined DNA (mono- or hexavalent vaccine);
> - measles-rubella-mumps – trivalent vaccine with MMR live attenuated strains;
> - Pneumococcal Conjugate Vaccine.

Table no. I National Vaccine Calendar – Romania 2019 – in force [3]

Recommended age	Vaccine	Comments
The first 24 hours	HEP B	In maternity
2 – 7 days	BCG	
2 months	DTPa-IPV-Hib-HEP B* Pneumococcal Conjugate Vaccine	General Practitioner
4 months	DTPa-IPV-Hib-HEP B* Pneumococcal Conjugate Vaccine	General Practitioner
11 months	DTPa-IPV-Hib-HEP B* Pneumococcal Conjugate Vaccine	General Practitioner
12 months	MMR	General Practitioner
5 years	MMR	General Practitioner
6 years	DTPa-IPV	General Practitioner
14 years	DTPa	General Practitioner

* hexavalent vaccine.

2. **Vaccines applied in cases with high epidemiologic risk** – indicated to the tourists who visit various endemic areas, in case of natural calamities, floods, earthquakes, wars, with massive disorganization of social life or in other cases of major epidemic potential. This category also includes the vaccine for hepatitis A, typhoid, meningococcal, yellow fever etc. There were in 2005 reported some measles outbreaks at the children from the survival camps in the Aceh region, after the tsunami which had devastated the coasts of the Indian Ocean. Under such conditions, The WHO organized a campaign of vaccination, in order to put an end to the development of a possible epidemics. [4]

3. Vaccines applied under high individual risk conditions – addressed to the individuals who can contact more frequently a certain infectious disease or who can develop more severe clinical forms, due to personal risk factors. They can be:

➤ Professional – the healthcare staff has a firm indication for the vaccine for hepatitis B, but also for flue or rubella immunisation. The teachers, public employees and other categories with special social importance can be vaccinated flu. The teachers or military staff will be object of additional immunizations, in compliance with the epidemic potential of the mission.

➤ Age older than 65 years old is considered an endangered factor, so that these persons have an indication for flu and pneumococcal vaccination, in order to limit complications and post-infectious mortality.

➤ Pre-existing pathology (chronic diseases, malignant haematological affections, oncologic pathology, immunosuppression) interferes with the practice of active immunoprophylaxis. Therefore, the patients with chronic obstructive bronchopneumopathy have firmer indication for pneumococcal and influenza vaccine, and immunodepression leads to the replacement of live vaccines with inactivate/ subunitary products, but as well to the administration of pneumococcal vaccine, chickenpox or *Haemophilus influenzae* b vaccination.

VACCINATION PRINCIPLES

The following principles need to be met in an action of vaccine planning:

1. The age group with maximum receptivity or with the highest risk to develop severe disease types will be protected. Therefore, the pertussis component vaccine starts at the age of 2 months, because maternal pertussis antibodies are not protective for the child.

2. The seasonality of certain infectious diseases is to be met, by organizing the vaccination campaign before the epidemic season. For example, influenza vaccine is applied in Octobers and Novembers, before a possible epidemics during winter-spring time (for the regions with temperate climate).

3. One also needs to meet the immunisation schemes, established for each and every vaccine. To be taken into account the minimum age

for the vaccine, the route of administration proper to each and every product, the number and size of the doses, the rhythm of boosters as well as the intervals between various immunisations. Between the live vaccine products, to generally meet an interval of minimum 30 days, while in case of inactivated vaccines or of vaccines with antigenic fragments, such pause it not to be applied.

4. One needs to meet the permanent or temporary indications and contraindications.

5. Before the vaccine campaign, the following need to be assured: the material basis represented by various biological products, the medical instrumentation needed as well as the compulsory refrigerating conditions during the transportation and storage of vaccines (in order to provide a constant temperature range of 4-8°C, monitoring by way of a chart of temperature).

6. The medical staff needs to be trained related to the correct vaccination technique, to the indications, contraindications, and possible post-vaccine side effects.

7. The records for the immunisations needs to be rigorous. They shall be registered in the individual sheet of the patient, in the special vaccination registry, and in the vaccination book. The newly born receive in maternities a vaccination booklet, which shall include all the subsequent immunisations during the period of their childhood up to 14 years of age. For teenagers, adults or persons from the extra-territory, vaccination certificates are to be issued. They shall mention: the trade name of the product, the producer, the series and number of the batch, the date of administration, validity, size of the dose, number of the booster, route of administration, signature of the medical staff in charge with the vaccination and possibly, some remarks related to immediate effects. Starting with 2011, the physicians have had the obligation to account for the dates related to vaccines (compulsory or optional) and on-line in the National Electronic Immunisation Registry (*https://www.renv.ro/renv/login.php*).

8. The patients left behind need to recover as soon as possible.

9. In case of major events, the Vaccine Adverse Event Reporting System (Sistemul de Supraveghere al Reacţiilor Adverse Postvaccinale Indezirabile – RAPI) is to be immediately notified.

VACCINE CONTRAINDICATIONS

In order to prevent such post-vaccine effects and to avoid ineffective immunisation, one needs to know and meet any and all the contraindications of vaccines. They can be:

1. **permanent contraindications** – their existence compels to give up the administration of the vaccine, indefinitely. The following fall under such category:
   - anaphylactic personal antecedents, to the vaccine or its components – for example anaphylaxis to the egg protein leads to giving up vaccines prepared based on chick-embryos (influenza, measles);
   - live attenuated vaccines cannot be administered to pregnant women and to patients suffering from congenital immunodepressions, acquired or iatrogenic. Vaccination, for such children, will use inactivated products or products with antigenic fragments;
   - the revaccination with cell/non-cell DTP is not indicated for children with encephalopathy developed in the first 7 days after pertussis vaccine. The infants with neurologic pathology need to be evaluated rigorously but the risks after the administration of DTPa are small.
2. **temporary contraindications** – impose the postponement of the vaccinations for the entire period of such pathological condition. Subsequently, after his or her recovery, the child needs to be vaccinated in accordance to his or her age and vaccination schedule. They are represented by:
   - acute diseases with moderate or severe evolution, with or without fever, up to the end of the disease process;
   - febrile conditions with temperature exceeding 37.5°C;
   - administration of blood or immunoglobulin products – which is prerequisite to temporize vaccination by 2 weeks before and 3 months after administration;
   - the immunosuppressive, chemical treatment and/or radio-therapy, systemic corticotherapy (with doses of at least 2 mg/kg/day, for at least 2 weeks) lead to the postponement of immunisations with live antigens, for the whole period of the treatment plus 3 more months after completion.[2]
3. **Precautions** –are mainly related to the administration of products which contain pertussis components (DTPc/DTPa), if the following occurred during a previous dose:

- ➢ hyperpyrexia ≥ 40,5°C;
- ➢ strong crying of more than 3 h, in the first 48 h;
- ➢ hypotonic-hyporeactive status, in the first 48 h;
- ➢ febrile/afebrile seizures, in the first 3 days after vaccination.

In such cases, vaccination is only indicated if its benefits outweigh potential risks.

THE FOLLOWING ARE NOT CONTRAINDICATIONS TO IMMUNISATION:

- ➢ slight or moderate local reactions;
- ➢ slight breathing intercurrences, in afebrile status;
- ➢ slight or average diarrhea;
- ➢ treatment by antibiotics;
- ➢ convalescence after acute diseases;
- ➢ severe post-vaccine antecedents in family;
- ➢ prematurity (the BCG is temporized up to 2 months, as for the rest the doses and the administration schedule are similar to those used for the full-term new-born children);
- ➢ the natural alimentation of the infant (only for the oral poliomyelitis vaccine, a pause of 3 hours before and after administration is a must);
- ➢ allergic antecedents to penicillin or other non-specific allergies;
- ➢ contact with a pregnant woman (children with pregnant moms will be vaccinated according to the ordinary schedule).

POST-VACCINE SIDE EFFECTS

Post-vaccine side effect is considered a medical accident arisen at most 1 month after vaccination and which can be or not be caused by a vaccine or vaccination. [5] Only in the case of the BCG, certain side effects can last a period of 12-16 months after vaccination.

The tolerance of the population to side effects is minimum, as vaccination addressed to healthy persons and, it sometimes has compulsory character.

The following post-vaccine side effects are declared by telephone, in the following 24 h from identification, to the epidemiologist with jurisdiction on the concerned territory:

1. **Severe local reactions** - lymphangitis, lymphadenitis, abscess at the place of inoculation (consecutive especially for BCG vaccination),

erythema/swelling extended to the nearby joint or lasting for more than 3 days, or which lead to hospitalization;

2. **Reactions from the CNS** – acute paralysis (motor neurone disease) consecutive to poliomyelitis vaccination; the Guillain-Barre syndrome; encephalopathies; encephalitis; meningitis; febrile/ afebrile seizures;

3. **Other severe side effects** which need hospitalization (anaphylactic reactions, toxic-septic syndrome, collapse, hyperpyrexia, arthralgia, very strong myalgia, with serious alteration of the general condition) or which lead to the death of the vaccinated person.

Depending on the cause, the post-vaccine side effects can be classified in:

1. **Reactions induced by the vaccine** – represented by certain particular reactions of a person at a certain vaccine product, effects which would not appear in the absence of vaccination;

2. **Reactions enhanced by the vaccine** - which can occur as well in other situations at susceptible persons, but which are precipitated by the vaccination;

3. **Coincident reactions** - which would have appeared even if the person had not been vaccinated, without a causal relation to the immunisation product;

4. **Reactions related to the vaccination schedule** – due to production faults, manipulation or administration errors, deficiencies in preserving the vaccine;

5. **Post-vaccine reactions due to unknown cause** – when not falling under any of the previous categories.[2]

VACCINATION EFFICIENCY

The immunisation obtained after the vaccine depends on various factors:

1. **Depending on the vaccine** – type, quality of the antigenic stimulus used, route of inoculation, size of the dose, route of administration;

2. **Depending on the vaccinated person** – age, nutrition status, existence of infections and congenital/acquired/iatrogenic immunodepressions, stress;

3. **Depending on the person which makes the vaccine** – related to the competence and responsibility of the physician.

The vaccine efficiency is checked by 2 criteria:

A. **Epidemiological criterion** – multi-annual morbidity is studied in order to mark out the decrease of the incidence and the loss of the

seasonal character of the disease, for all vaccinated persons compared to unvaccinated persons or in the vaccination period compared to the period before the vaccination. For the poliomyelitis vaccine, one shall follow the exclusion from circulation of the savage pathogenic agent and its replacement to vaccine strains.

B. **Immunologic criterion** - the specific antibodies are titrated at vaccinated individuals, with the determination of the seroconversion frequency and of the average level touched by the antibodies titre. At the level of the population, we organize collective serologic surveys. In case of other vaccinations, we test post-vaccine allergy (for example, the IDR to tuberculin).

The vaccine effectiveness needs to be researched in the case the incidence of the disease does not decrease concomitantly to the increase of the vaccination coverage or in case infectious pathology occurs at several patients, with proper vaccine antecedents.

Worldwide researches in the field of vaccinology are pretty active nowadays, in order to:

➢ establish an effective prophylaxis against emerging diseases (HIV, hemorrhagic fever, the Lyme disease);

➢ fight against re-emerging diseases (TB, malaria, convulsive cough, diphtheria) partly determined by the growth of chemotherapy resistance of the pathogenic agents, by the decrease of the protective antibodies at adult age or by the lack of effective vaccines;

➢ to obtain real vaccines against germs with high antigenic variability (the influenza virus);

➢ as well as to obtain prophylactic and therapeutic conduct, in certain chronic diseases with infectious competence: - gastro-duodenal ulcer (by the etiological involvement of the *Helicobacter pylori*),

- neoplasia (cervical – for which 3 papilloma vaccines are already used: tetravalent Gardasil/ Silgard - Merck Sharp & Dohme, bivalent Cervarix -GlaxoSmithKline) and the latest appeared, Gardasil 9, with antigens from 9 HPV subtypes - 6, 11, 16, 18, 31, 33, 45, 52 and 58.

- prion diseases – spongiform encephalitis and the Creutzfeldt-Jakob disease,

- multiple sclerosis,

- cardiovascular diseases (by the association with *Chlamydi pneumoniae*).

Most of the vaccines presently used are just a little reactogenic, due to the new production technologies, focused on antigenic subunits (proteins purified or polysaccharides), on genetic engineering or on live vectors. During the new millennium, they have licensed new vaccines amongst which:

> - the pneumococcal vaccine conjugated by 13 components;
> - the attenuated live influenza vaccine, cold adapted;
> - the tetravalent meningococcal vaccine conjugated polysaccharides;
> - zoster vaccine;
> - human papillomavirus vaccine with 9 components;
> - and the pentavalent rotavirus resorted vaccine,

which proves the quick dynamics of researches in vaccinology.[6]

VACCINES INCLUDED IN THE NATIONAL IMMUNISATION PROGRAM

1. TB VACCINATION

Tuberculosis is presently considered a re-emerging disease, with universal spread, both due to classical risk factors (poor social-economical conditions, disadvantaged minorities, alcoholism, malnutrition), and due to the HIV pandemics, with high susceptibility amongst immunodepressants or due to the occurrence of antituberculostatics multi-resistant strains.

The BCG vaccine is prepared from a live strain of *Mycobacterium tuberculosis bovis* with attenuated virulence, derived from the Calmette-Guerin original strain, which was obtained by successive passages for 13 years old on glycerinated potatoes, with ox bile.

Form of presentation: the BCG is delivered in brown glass vials, with live bacterial mass, lyophilised, with appearance of white powder, non-adherent on the walls. The Sauton's medium is used as solvent, which is a clear, colourless fluid, delivered in separate vials. After the suspension, the vaccine comes homogenous, slightly opalescent and needs to be administered in maximum 1 h after the preparation. To be kept in the dark and at 2-8°C.

Administration: to be inoculated strictly id. 0.1 ml suspension, in the posterior external area of the left arm, after previous disinfection of teguments with alcohol. The correct injection leads to the formation of a papule with the diameter of 5-6 mm, with appearance of an orange peel which shall persist for 30 minutes. In 1-3 weeks, a small erythematous shiny nodule shall appear at the injection site, which turns into a pustule, with or without subsequent fistulisation and the occurrence of central crust. After the removal of the crust, a depigmented slightly bumpy scar (marker of the previous vaccination) appears, but it can actually miss, especially at infants.[7] An ordinary post-vaccine reaction lasts for a period of 3 months, while ulcerated is extended up to 4-6 months.

Post-vaccine allergy is installed in 6-8 weeks, it can be marked out by the IDR at tuberculin and decreases step by step, lasting for approx. 20 years.[7] The BCG vaccination is efficient for the prophylaxis of the disseminated tuberculosis or of the tuberculous meningitis, especially amongst children below 5 years old, preventing massive lymphatic and hematogene dissemination, but it cannot fight against primo-infection or the reactivation of latent infection. As well, it offers

good protection against *Mycobacterium leprae* and some effects against other non-tuberculous micobacteria.

The vaccine coverage (the relation between the number of vaccinated individuals / number of vaccinable individuals ×100) needs to be higher than 95%, in order for the protective effect to be obtained at the level of the population.

The vaccination schedule includes:

- ➢ **BCG primary course** administered in maternity, in the first 2-7 days of life or up to the age of 2 months (in case of infants with body weight below 2500g);
- ➢ **Reading the post-vaccine scar** at all infants between 5 and 10 months. The dose will not be repeated to the persons without post-vaccine scar or with a diameter below 3 mm, but only the children not vaccinated BCG are to be recovered.

Contraindications:

- ➢ **Indefinite** – represented by the symptomatic HIV infection, congenital immunodeficiencies, leukemia, lymphomas, generalized oncologic pathology, immunosuppressive treatments, pregnancy and positive reactions to tuberculin. In developed countries, the infants infected by HIV are not vaccinated with the BCG, but the WHO recommends it for the people living in areas with a high risk of tuberculosis;
- ➢ **Temporary-** represented by the body weight below 2500 g at birth, the acute febrile diseases, the period of infectious diseases, dystrophias, malnutrition, transitory immune deficits. 6 weeks after vaccination, avoid immunosuppressive treatments which can prevent the installation of tuberculin allergy.

Post-vaccine complications consist of:

- ➢ Abscesses at the spot of the inoculation, regional lymphadenopathies, lymphangitis (determined by the deeper inoculation of the vaccine, arisen at approx. 1% of the vaccinated individuals);
- ➢ Osteitis /osteomielitis in the first 8-16 months after vaccination or
- ➢ Disseminated infection with *M. tuberculosis bovis* vaccine strain, manifested 1-12 months from vaccination. These generalized manifestations are rare at immunocompetent individuals, with more frequent occurrence at immunodepressed individuals.

The BCG vaccination strategy is very much different from one country to another, depending on the current epidemiological situation. In the strongly developed countries, (USA, Western Europe etc.) selective vaccination of the risk groups is performed – children exposed to multi-

resistant bacillus strains, TB contacts not vaccinated with IDR at negative tuberculin, without being applied to the entire population.

There are no certain serologic tests for quantifying the protective immunity after the tuberculous infection or BCG vaccination.[7]

Despite all this, **IDR at tuberculin (Mantoux test)** has been used for a long time, in order to mark out the post-vaccine response, in order to detect the *M. tuberculosis* infection at symptomatic or asymptomatic persons, as well as for the evaluation of cellular mediated immunity.

Testing in Romania is performed by 2 units/0.1 ml PPD, purified protean fraction from tuberculin (the filtrate of a 6 week human tuberculous bacillus culture, in glycerinated medium). The PPD is delivered as vials of 1 ml products and is used in average for 6 tests. It is administered strict id. in the medial third part of the forearm. The correct inoculation is followed by the appearance of a papule of approx. 5 mm, which persists for 10 min. The reading of the reaction is to be done 72 h afterwards, writing down the transversal diameter of the area, as well as its appearance in case of blistering, necroses, ulcerations, without taking into account simple erythemaatoase reactions.

The reaction is negative when the diameter of the papule falls between 0-9 mm.

Positive reactions are interpreted differently, depending on the age and existence of the vaccine scar.

Therefore:
- ➢ In the absence of the vaccine scar, the positive reaction (≥ 10 mm) represents bacillus infection and needs chemoprophylaxis or the tuberculostatic treatment;
- ➢ In the presence of the vaccine scar:

  - children between 0-5 years of age are checked by radiographies in case of tuberculin reactions of 10-14 mm, with tough induration or with the appearance of blistering, necrosis, general reactions, either in case of induration over 15 mm, with ordinary appearance;

  - children over 5 years of age and youngsters are checked b radiographies in case of reactions of 10-19 mm, with tough induration or with the appearance of blistering, necrosis, general effects, or in case of induration larger than 20 mm, with ordinary appearance.

There can be also falsely positive effects, resulted from erroneous interpretations (reading the erythema and not the induration), by the appearance of cellulites or of the allergic phenomena Arthus. Falsely negative reactions can be due to anergy or to the lack of epidermis elasticity.

2. POLIO VACCINE

Poliomyelitis is an infectious disease rarely manifested clinically (between 4-8% of the cases), which can lead to an affection of the Central Nervous System (less than 1% of the cases). After an acute period, 10-15% of the patients with paralytic form will suffer from permanent damage, as paralysis and muscle atrophy.[2]

The national polio vaccination programs had a decisive contribution to the reduction of the disease incidence and even to its eradication in large geographical areas. The number of polio cases have decreased by more than 99% since 1988 (with an estimate number of 350,000 cases in more than 125 endemic countries), getting to be 33 cases reported in 2018. Only regions from 3 countries (Afghanistan, Nigeria and Pakistan) persisted to be endemic – which represents the smallest geographical area from the millenary history of the disease.[8] Outbreaks appeared in 2013 in some non-endemic countries as well (Somalia, Ethiopia, Cameroon and Syria). The WHO is presently striving to liquidate the residual epidemiologic outbreaks, in order to eradicate it globally.

The last case, in Romania, of savage virus polio was recorded in 1992, and starting with 2002, all the European countries (Romania included) were declared free from savage viral strains. But the possibility of import cases, with the reestablishment of transmitting it to populations with lo vaccine coverage, is a permanent threat. In the epidemics from Tajikistan, in the first half of 2010, with 293 cases of flaccid acute paralysis, out of which 83 confirmed with savage polio virus type 1, some cases reached as well the territory of Russia, polio-free country for at least 8 years.[9]

Syria has reported 24 cases of poliomyelitis (up to March 4, 2014), mostly children younger than 2 years old, due to the dramatic reduction of vaccination coverage due to the military conflict in the region.[10] Additionally, the WPV1 virus was isolated from the samples collected for the routine surveillance of residual waters, in 2 towns in South Israel (Beer Sheva and Rahat), especially in the areas inhabited by Bedouins or Arab-Jews communities.[11] This circulation has also been marked out in 2014.[12] The presence of outbreaks in the Middle East leads to high risks, both for Europe, and for the rest of the world, as each day Syria is left by around 6,000 refugees, most of them to Turkey and Egypt, main tourist areas for Europeans. [13] Plus the fact that some parents refuse to vaccinate their children, considering that poliomyelitis is a disease of the past – therefore vaccination coverage is low in countries like Bosnia/Herzegovina, Ukraine, Austria, insufficient in order to prevent its transmission in case of re-entry.

In such a context, the specific prophylaxis is maintained in the immediate actuality, by polio vaccination with Salk parenteral inactivated vaccine, or Sabin live attenuated vaccine, with oral administration.

A. The inactivated poliomyelitis vaccine – IPV, prepared for the first time by J.Salk, contains strains of 1,2,3 polio virus serotypes inactivated with formalin, able to produce specific seroneutralized antibodies. The vaccine was brought again in our daily lives after the cases of paralytic polio after the vaccination, with attenuated live product. Although less immunogenic, IPV does not present such a risk, and therefore it has also been reintroduced in Romania as well in 2008, in the compulsory vaccination of infants / children, but also of the persons with various immunosuppresions (HIV included), of their family contactants or of adults with a risk (lab personnel, medical staff getting in touch with savage polio virus excretory, travellers to endemic and epidemic areas for this pathology). It has been reintroduced for more than 10 or 20 years in the countries with high economical standards in Europe, North America.

The commercial products contain either just the IPV (Imovax polio – Sanofi Pasteur), or they are combined vaccines which include as well DTP/DTPa (Tetraxim-Sanofi Pasteur), *Haemophilus influenzae* type b (Pentaxim- Sanofi Pasteur, with DTPa+IPV+Hib) and hepatitis B (Infanrix Hexa – GlaxoSmithKline with DTPa+IPV+Hib+ HBV).

The IPV efficiency is of 90-96% in the prevention of paralytic poliomyelitis and of 100% in the occurrence of seroneutralized antibodies, after the administration of 3 doses of vaccine. The immune response persists on long term (10-18 years), for more than 95% of the vaccinated individuals by 3 or 4 doses. Ig A occur at the level of the mucosa, but are 3-4 times less versus post-vaccination with live attenuated component.[2]

B. Trivalent attenuated live poliomyelitis vaccine - VPO, prepared by A.Sabin, includes live strains from 1,2,3 polio virus serotypes, attenuated by genetic mutations. It protects by the induction of humoral immunity, with the production of circulating antibodies, as well as by local immunity, with formation of intestinal Ig A and at the level of the oropharyngeal mucosa. This local immunity assures protection against re-infection, opposing to the multiplication of the savage strains and diminishing their circulation. The elimination of vaccine viruses by faecal may lead to occult immunisation of the persons in the entourage of the vaccinated individual ("oil stain" coverage). Such repeated passages may select neurovirulent mutants, able to determine paralytic poliomyelitis with vaccine virus.

The VPO is administered easily orally, has a low price, a good compliance at the population level, but presents the disadvantage to generate paralytic accidents (peripheral motor neuron syndrome), at the vaccinated individuals or their contacts. It still remains the product most used in poor countries or in countries under development.

Its efficiency is of approx. 95% in developed countries and smaller in the others (70-90%).[14] The antibodies stay present for a long time, which explains the absence of the disease at the vaccinated adults in antecedents.

Starting with April 2016, WHO recommended the introduction of inactive polio or live **bivalent** strains vaccine **(1+3),** as consequence of the worldwide eradication of the viral 2 subtype.

Form of presentation:

➢ The inactivated vaccine (component in the tetra- or hexavalent product) is delivered as syringe preloaded with 0.5 ml opalescent solvent (+/- 1 lyophilized vial in case of hexavalent vaccine). To be stored at 2-8°C and the freezing needs to be avoided;

➢ Sabin's vaccine is found in uni- or mult-dose plastic vials, which contain a light rose clear fluid. They are not allowed to sour or change colour. Stored in freezing conditions -10/-20°C, for 1-2 years. After defrost, to be kept at 2-8°C, and can be used in maximum 1 month time.

In Romania, the **current vaccination schedule** includes:

➢ The administration of 3 doses of IPV, by intramuscular route, at 2, 4, 11 months, as component within the hexavalent product– 0.5 ml;

➢ Revaccination is practiced at the age of 6 years old, by 1 dose of IPV+DTPa (tetravalent). [3]

The administration of the inactivated product is intramuscular, at infants and small children in the region of the hip, at the joint of superior 1/3 to the medium, 2-3 cm laterally from the medial line or in deltoid at older children and adults.

The live strains vaccine (still used in various regions of the globe) is administered per bone, in dose of 0.2 ml (2 drops), according to a schedule similar to that presented above. The general rules for the administration of the VPO are:

➢ Appearance of regurgitation or vomit in the first 5-10 min. after administration, leads to the vaccination being repeated within the same session;

➢ The maternal milk consumption is to be avoided for minimum 3 h before and after the administration of the vaccine. Immediately after vaccination, the infant can receive tea;

> Intramuscular injections, surgical interventions which can be temporized or dental extractions are contraindicated for 30 days after the vaccine (any parenteral treatment is IV administered);
> Intercurrent febrile conditions will be fight against with antipyretic therapy, in the first 6 weeks from vaccination.

In endemic areas, in the areas with circulation of savage viruses (in the previous 3 years), where vaccine coverage is below 80%, with great migrations of the population or close to endemic territory, additional vaccinations like "the national immunisation days", which is the "mopping-up" are organized. Children below 5 years receive 2 more doses of VPO, at interval of 1 month, irrespective of their vaccine antecedents.

Contraindications and side effects for each and every type of vaccine, are presented comparatively in table no. II :

Table no. II Contraindications and side effects in case of IPV versus VPO 23vaccination

INACTIVE POLIO VACCINE	ATTENUATED LIVE POLIO VACCINE
Permanent contraindications	
Represented by anaphylactic reactions at a previous dose of IPV or at vaccine components - streptomycin, neomycin, polymyxin B.	Not indicated for the vaccination of children infected with HIV*, with congenital immunodeficiencies or a different etiology (hematologic diseases, solid tumours, long term immunosuppressive therapy), as well as for their family contacts.
	Precautions for pregnant women or adults (more than 18 years).
Temporary contraindications	
Represented by the febrile acute diseases.	Represented by febrile acute diseases, medium clinical and severe forms of diarrhoeal disease.
Side effects	
Rare, especially local - erythema, swelling and pain, 2-3 days after the vaccination.	generally minor – discreet pharyngitis, 1-2 stools of low consistency; febrile condition.
	Rarely, post-vaccine acute flaccid paralysis can occur, with persistent neurologic deficit of more than 60 days, in an interval of 4-30 days from administration, at the vaccinated person and of 4-75 days, at the contacts of the individual vaccinated. The risk of post-vaccine poliomyelitis is of approx. 1 case every 790,000 doses for primary course and of 1 every 2.6 million doses, for boosters. This risk is very high at the immunodepressed individuals.[14]

* In developing countries, where the condition of HIV infected is not known at birth and IPV vaccination is not available, the WHO recommends the administration of the VPO even to these infants, as immunodeficiency can occur later.[14]

The VPO vaccination needs to be continued in the countries with persistent savage viral strains circulation, while in those with interrupted circulation or large vaccine coverage, one can go to sequential schedules or only IPV schedules.[15]

Post-vaccine flaccid acute paralysis has had in Romania a high incidence compared to other geographic areas, mainly due to the intramuscular injections administered quite frequently to the small child.[14,15]   In order to avoid such cases, VPO vaccination was dropped for IPV vaccination, enacting in 2008 a combined schedule (IPV primary course and VPO boosters), and starting with 2010, IPV has been administered exclusively.

3. DIPHTHERIA VACCINATION

After the introduction of the population's vaccination against diphtheria (in Europe around 1930), the disease changed its character from endemic-epidemic in sporadically.[2] But around 1990, it became re-emerging in the states of the former Soviet Union, on the background of vaccine relaxation especially at adult age, with consecutive accumulation of susceptible persons, in parallel with the growth of the number of carriers of *Corynebacterium diphteriae* or of the atypical clinical cases, selected especially from the disadvantage groups of population (alcoholic, drug consumers etc.). The seriousness of the disease is determined by the toxic syndrome, occurred as consequence of the diffusion of diphtheria exotoxin diffusion in the body. Vaccination prevents this toxic syndrome, by inducing seroneutralized antibodies to B fragment of diphtheria anatoxin, preventing the penetration of the toxin in the cell.

The diphtheria vaccine component is performed usually concomitantly to the tetanus and pertussis vaccine, by the use of various related products. There are multiple such products:

1. **Diphtheria-tetanus-pertussis DTP vaccine** – contains diphtheria anatoxin obtained from the exotoxin of the Parck-Williams no.8 strain of *Corynebacterium diphteriae*, with marked toxigenesis; tetanus anatoxin obtained by the detoxification of the toxin in the *Clostridium tetani* 21D strain and inactivated corpuscular pertussis vaccine. Determines the simultaneous immunisation to the 3 pathogenic agents, in order to simplify the vaccination calendar.
2. **Trivalent vaccines with acellular pertussis component – DTPa** (e.g.: Infanrix-GlaxoSmithKline, Daptacel-Sanofi Pasteur). They

contain the pertussis toxoid next to one or more antigenic structures: filamentous hemagglutinin, agglutinogens, pertactin. The vaccine is less immunogenic but even less reactogenic compared to corpuscular vaccines.

3. There presently are **tetravalent, pentavalent and hexavalent vaccines** which contain DTPa – Tetraxim (DTPa+IPV, from Sanofi Pasteur), Pentaxim (DTPa + IPV + conjugated polysaccharide of *Haemophilus influenzae* type b, from Sanofi Pasteur) or Infanrix Hexa (DTPa+IPV+Hib+HEP B – from GlaxoSmithKline).

4. **The diphtheria-tetanus bivaccine** – An adult type vaccine is used (dT), with a content 5 times more reduced of diphtheria anatoxin compared to the paediatric product (DT), for the vaccination of persons older than 14 years. We recommend today the administration of DTP a (Adacel-Sanofi Pasteur), where the pertussis component is as well adult type.

5. **The monovalent vaccine** – the adsorbed diphtheria vaccine (VDA) contains purified diphtheria and adsorbed anatoxin, paediatric or adult type. It has restricted indications at unvaccinated persons for diphtheria, at the bacillus carriers or in the diphtheria outbursts.

Form of presentation: The commercial vaccines which include DTPa, are found as syringes preloaded with opalescent suspension, 5 ml/1 dose and need to be homogenized before administration. To be kept in dark and between 4-8° C.

The Administration is intramuscular, deeply, at the infant, in the thigh region, upon the joint of the upper 1/3 to the medial, 2-3 cm laterally from the medial line or in the deltoid at teenagers and adults.

Vaccination schedule:

➢ Primary course contains 3 doses each of 0.5 ml DTPa im., at 2,4,11 months concomitantly with the IPV, *Haemophilus influenzae* type b and Hepatitis B (hexavalent vaccine);

➢ Revaccination is to be performed at 6 years old, with a dose of 0.5 ml DTPa im. Combined with IPV (tetravalent vaccine)

➢ And at 14 years old, a dose of 0.5 ml dTpa;

➢ Subsequently, every 10 years, boosters with 0.5 ml dT or dTpa im., in order to preserve the protecting antibodies.

In case a booster is delayed, from various reasons, the vaccination schedule is to be continued, without being taken over from the beginning.

The vaccine efficiency after at least 3 doses, is more than 95% at children below 15 years old and in average of 70% at adult.[16] The protecting level of serum antibodies is considered to be 0.1 UI/ml, while long term protection is related to values of 1 UI/ml.[16] The post-vaccine protection does not prevent local colonisation with strains, especially

nontoxigenic, of *Corynebacterium diphteriae,* but the disease cases are rare and with much more benign evolution. The vaccine coverage needs to be more than 90% at children and more than 80% for adult age, in order to prevent a possible epidemic.

Contraindications:

➢ **temporary** are represented by the febrile conditions, the status period of acute infectious diseases, TB or evolutional chronic diseases. At the persons with blood coagulation disorders, the vaccine will be administered im., in the moment risks are minimum, and post-vaccine local compression needs to be extended (minimum 2 minutes).[16]

➢ **permanent** are related especially to the pertussis component in the related vaccines. Encephalopathy with debut in the first 7 days after the first dose, contraindicates the administration of the pertussis component. Another permanent contraindication is the anaphylaxis after a previous dose.

➢ After the administration of the vaccines with pertussis component, the following can occur: hyperpyrexia (fever higher or equal to 40.5°C), collapse/shock, persistent crying (more than 3 h) in the first 48 hours after vaccination, convulsions in the first 3 days after immunisation. In such situations, the continuation of the schedule with the diphtheria and tetanus vaccine is preferred. The introduction of the acellular pertussis vaccine reduced a lot the incidence of such secondary reactions.

Post-vaccine side effects:

➢ Local reactions may occur: erythema, nodule, pain at the place of inoculation, with favourable evolution 2-3 days afterwards or general, represented by transitory conditions;

➢ The diphtheria anatoxin can determine, especially to adults, delayed hypersensitivity (Arthus phenomenon). Therefore, vaccines for the use of adults contain a smaller quantity of diphtheria anatoxin;

➢ Tetanus anatoxin can determine brachial plexus neuropathy (1 case/200,000 doses), algodystrophy syndrome, Guillain-Barre syndrome (0.4 cases/1 million doses), anaphylaxis (1 case/100,000 doses);

➢ The classical pertussis component can generate persistent crying (3.15%), convulsions (1 case/1750 vaccinated individuals), hypotonia-hyporeflexia syndrome (0.06%), exceptionally post-vaccine encephalopathy (1 case/2.4 million vaccinations). [1,17] In order to reduce such reactogenicity, the acellular pertussis vaccine entered the market instead of the corpuscular.

4. PERTUSSIS VACCINE

We estimate worldwide that there appear annually 40 million cases of convulsive cough, out of which care 360,000 evolve towards death, and 50,000 remain with long term neurologic sequela, especially in developing countries.[17] There are 2 types of pertussis vaccine:

1. **The inactivated corpuscular vaccine** – contains chemically inactivated *Bordetella pertussis* bacillus. To be used only in association to the diphtheria and tetanus anatoxin, eventually with inactivated polio, *Haemophilus influenzae* type b or hepatitis B vaccines. To be administered to children younger than 3 years old, after such an age, there is a high risk of side effects. After 4-5 doses, the protecting antibodies appear at more than 80-90% of the vaccinated individuals, the length of its effect persisting for 6-12 years.[2] The contraindications, precautions upon vaccination and the side effects were presented in the previous chapter. Due to the higher reactogenicity, such a component was excluded from the calendar of compulsory vaccinations in some developed countries (England, in the 70s), which determined the re-emergence of convulsive cough and of epidemic phenomena. Efforts have been subsequently made in order to obtain an effective vaccine, with lower reactogenicity, and the acellular product entered the market.

2. **Acellular vaccine** – contains pertussis toxoid, next to one or more antigenic structures: filamentous hemagglutinin, agglutinogens, pertactin. It has been initially used in Japan, in order to replace the cellular component afterwards from the related vaccines, in most of the developed countries (in the 90s). This product was introduced in Romania in the National Immunisation Program at the end of 2008. The pertussis toxin is chemically or genetically inactivated, and then included in the vaccine as toxoid. It can also be administered at ages older than 3 years, the in force administration schedule being presented at the diphtheria component. Real contraindications are the anaphylaxis and encephalopathy with debut after 7 days from the administration of a previous dose of pertussis component. The children with pre-existing neurologic pathology can have an indication of vaccination with the product, taking into account the lower risks. For the individuals with febrile convulsions in their antecedents, pyretic medication will be administered before the immunisation and in the first 24 h. Following the vaccination, the immune response appears at 90% of the individuals. An adult type trivalent vaccine dTpa (Adacel-Sanofi Pasteur) has recently appeared, with a lower quantity of pertussis antigens, and used as booster, every 10 years, for teenagers and adults (especially in the persons getting in touch with infants - parents, family members, medical staff, care staff).

5. SIMPLE TETANUS VACCINE

The tetanus, fatal in 40-50% of the cases, is determined by the *Clostridium tetani* bacillus, manifested by an infection localized at the level of the admission gate and by neuromuscular hyperexcitability consecutive to the diffusion of bacterial exotoxin (tetanospasmin) in the body. Neonatal tetanus is even more serious, with fatality between 50-90%, and the survivals can develop severe neurological sequelae and development retard.

By vaccination and proper post-exposure conduct, this pathology can be managed up to removal. In order to touch such a target, vaccine coverage for the tetanus component needs to be more than 95%.

The exposure to a high infected dose (plague with tetanigenic potential, birth with the possibility of the umbilical stump infection, in default of an aseptic conduct), can exceed the immunity granted by the previous vaccinations, so that additional preventive measures are to be taken.

Presently, besides combined vaccines, simple tetanus vaccination is performed only with **purified and adsorbed tetanus anatoxin (VTA).** For adults, we recommend the dT bivaccine, eventually with dTpa three-vaccines (e.g.: Adacel-Sanofi Pasteur), in order to grant concomitant protection for other 1-2 pathologies.

The ATPA is produced by detoxification due to formalin fixation of the exotoxin of the *Clostridium tetani* 21D strain, aluminium phosphate (or calcium) purification and adsorption. Delivered as monodose vials / preloaded syringes of 0.5 ml, with a whitish milky liquid which needs to be homogenized before the administration (Tetavax-Sanofi Pasteur). To be kept in the dark and in a temperature range of 2 to 8°C, avoiding freezing. The inoculation is intramuscular in the tight up to 3 years of age or in the deltoid muscle for the adult and big child.

Simple tetanus vaccination with VTA has the following **indications:**

1. **Primary prophylactic immunisation of the persons without tetanus vaccine or of the adults with tetanus antibodies below the protective level** – involves a primary course with 2 doses of 0.5 ml VTA im. 30 days interval, followed by a booster with a dose of 0.5 ml VTA one year after the primary course and another identical booster 5 years from revaccination I.

2. **Revaccination of pregnant women** with proper tetanus vaccination antecedents, in the month 7 ½ of the first pregnancy, with a dose of 0.5 ml VTA im. The unvaccinated primigravidas or those with incomplete vaccine antecedents will be subject of a 2 doses schedule at an interval of 30 days, starting with the 7th and a half pregnancy

month, with revaccinations 1 year after the administration of the 2nd dose and 5 years after. As related to a new pregnancy, the ATPA revaccination is to be performed only if more than 10 passed from the latest tetanus revaccination.

3. **Emergency revaccination of complete first vaccinated persons or revaccinated persons with tetanus in antecedents**, **with the occurrence of a tetanigenic wound** –0.5 ml VTA or dT to be administered im., optimum in maximum 24 hours from the occurrence of the wound (for major wounds and contaminated – if the individual did not receive tetanus anatoxin in the latest 5 years/for minor and uncontaminated wounds – if the person failed to receive tetanus anatoxin in the latest 10 years). In case of superficial wounds, only the ATPA is administered. Only in case of serious multiple traumas with massive blood losses, multiple open fractures, the patients infected with HIV, will be associated 3.000-20.000 UAI of tetanus serum or 200 - 500 UAI tetanus specific immunoglobulin, concomitantly to the first vaccine dose, but in a different anatomic region.[2]

The category of tetanigenic wounds includes the following: wounds from burs, nails, chips, wounds with conditions of anaerobiosis, muddy wounds, dusty, soiled with animal defections (come from the agricultural, zootechnical, gardening sector, road accidents included), bites (animal or human), wounds with retention of foreign bodies, with anfractuous edges, devitalized tissues, open comminuted fractures, infected varicose ulcers, 2nd and 3rd degree burns, frostbites, birth or abortion without the observance of asepsis.

Under such conditions, besides the administration of the active/passive prophylaxis, the surgical drainage of the wound with large debridement is a must, as well as the removal of foreign bodies, the excision of the devitalized tissues, the hemostasis, aseptization of the wound with hydrogen peroxide 3% and antibio-prophylaxis with Penicilin or Erythromycin (for allergic individuals), for 7-10 days.

4. **Accelerated vaccination of non-vaccinated individuals or of individuals with unknown/uncertain/incomplete vaccination antecedents, with the occurrence of a tetanigenic wound** – to administer 3 doses of ATPA (or dT) each of 0.5 ml at an interval of 14 days, plus 1 booster 1 year after. In case of severe wounds, to combine active to passive immunisation, by tetanus seroprophylaxis (3.000-20.000 UI) or tetanus specific immunoglobulin (250 UI or if the tetanigenic wound is older than 12 h, at the persons weighting more than 90 kg and/or in the presence of massive contamination - 500 UI).

5. **In order to treat the tetanus** – to administer ATPA as well, as naturally going through the disease does not determine protective immunisation.

The minimum protecting titre of antitoxin antibodies is considered to values of more than 0.01 UAI/ml, measured by the method of in vivo neutralization. After complete vaccination, to appreciate that antitoxic immunity is efficient for approx. 10-15 years and develops at 98% of the individuals. In order to keep up the protective level at adult age, boosters are necessary with 0.5 ml dT im., administered at an interval of 10 years.[18]

Contraindications:
- The anaphylaxis or appearance of neurologic diseases after a previous dose of tetanus vaccine contraindicates the repetition of the doses;
- But there are no contraindications for emergency vaccination with VTA, in case of potentially tetanigenic wound;
- As well, it is not contraindicated to immunosupressed individuals (HIV infected, with hematologic, oncologic pathology, transplanted etc.).

Side effects:
- Local reactions prevail – erythema, oedema, swelling, sometimes even affecting the neighbouring joint. Subcutaneous administration can favour the growth of the incidence of such local reactions or the evolution towards an abscess;
- Pyrexia can also appear, brachial plexus neuropathy (1 case/ 200,000 doses), algodistrofic syndrome, Guillain-Barre syndrome (0.4 cases/1 million doses), anaphylaxis (1 case/100,000 doses), presented as well at the diphtheria vaccination with combined vaccines.

The preventive conduct of neonatal tetanus applies in case of birth under improper conditions, when asepsis cannot be assured – home birth, birth in nature, with dusty, soiled umbilical wound, or when the umbilical cord was sectioned with non-sterile instruments. The measures include the umbilical wound dressing, excision of the infected parts, antisepsis, sterile ligation of the umbilical cord, applying a sterile dressing, chemoprophylaxis with Penicilin for 7-10 days and the administration of a dose of antitetanic serum 500 UI or specific immunoglobulin 200 UI.[2]

There still existed in September 2018, 14 more countries where removal of the disease has not been validated yet, which is the threshold of below 1 case to 1000 living infants, on the entire territory.

6. *HAEMOPHILUS INFLUENZAE* TYPE B VACCINE

Haemophilus influenzae is responsible for multiple infections of the respiratory tract (ear infection, sinusitis, epiglottitis, pneumonia), for osteoarticular diseases (septic arthritis, osteomyelitis), for facial/orbital cellulitis, but also for invasive pathological entities (meningitis, sepsis), especially at children aged below 5 years. The top of the incidence is between 6-7 months and 18 months of life. It is estimated at worldwide level to annually appear 400,000 deaths assignable to this pathogenic agent.[19]

Haemophilus influenzae type b is involved in 95% of the infections with *Haemophilus influenzae*. In Eastern Europe, an incidence of meningitis with such etiology was marked out, amongst children below 5 years old, of $6.1\%_{000}$ in Bulgaria, $3.1\%_{000}$ in Poland, $7.6\%_{000}$ in Romania of $17.3\%_{000}$ in Slovakia.[2]

As consequence of the frequency of the diseases with such a germ but also of its seriousness, the vaccine *Haemophilus influenzae* type b was decided to enter the Romanian vaccination calendar. Economically developed countries previously included this product in their National Immunisation Programs (91 countries in 2009) and after more than 10 years of use, reduced the incidence of invasive forms by 90%.[2]

The Hib vaccine assures protection to invasive clinical forms, but fails to prevent illnesses with strains of *Haemophilus influenzae* of other types and meningitis with a different etiology. It refers to children aged 2 months to 59 months, but also to immune-supressed (suffering from HIV, immunodepression following chemotherapy, congenital immunodeficiencies, splenectomy, bone marrow transplant).

Types of vaccines: Hib vaccines contain capsular polysaccharide of *Haemophilus influenzae* type b (polyribosylribitol-phosphate - PRP), conjugated with a carrying protein - diphtheria or tetanus anatoxin. The following vaccines exist:

> ➢ **Monovalent** – only with Hib component (Act-Hib-Sanofi Pasteur) or
>
> ➢ **Combined –** together with the DTP vaccine, DTPa (tetravalent), with the DTPa+IPV (pentavalent - Pentaxim-Sanofi Pasteur) or DTPa+IPV+HEP B (hexavalent - Infanrix Hexa-GlaxoSmithKline). For them, tetanus anatoxin will be used for the conjugation.

Form of presentation: box with 1 vial including powder lyophilized vaccine, accompanied by syringe preloaded by 0.5 ml solvent (which includes the DTPa, IPV, +/- HEP B components), for the making of the

suspension. After reestablishment, to be administered in maximum 30 minutes. To be kept in the fridge, between 2 ⁰C and 8 ⁰C.

Route of administration is deeply intramuscular, in the region of the hip, for the infant and in the deltoid for the adult.

The vaccination schedule used in Romania is partially similar to that of the DTPa and IPV components:

- ➢ The primary course includes 3 doses at 2,4,11 months, as component in the hexavalent product;
- ➢ If the child is older than 1 year and has not been vaccinated with Hib component, one single dose of Hib monovalent vaccine will be administered;
- ➢ The children with a full Hib vaccination series but following to be splenectomised, need to be revaccinated 7-10 days before surgery;[19]
- ➢ 2-3 more doses of monovalent vaccine can also be administered 12 months after bone marrow transplant.

The immune response appears at most of the vaccinated individuals, with titres of more than 0.15 μg/ml (considered protective). The effectiveness was of 97.5% after the administration of one dose and of 98.8% after 3 doses of DTPa+IPV+Hib.[19]

Contraindications:

- ➢ **Temporary** – represented by fever, status period of acute infectious diseases;
- ➢ **Permanent –** include anaphylaxis at the vaccine components (at a previous Hib vaccine component or at the tetanus anatoxin), hypersensitivity to neomycin, streptomycin, polymyxin B;[20]
- ➢ Plus the contraindications of the other components– DTPa, IPV and HEP B.

The side effects after the vaccine include:

- ➢ Rare local reactions - erythema, swelling, pain at the place of inoculation in the first 48 h;
- ➢ Systemic reactions - fever, irritability, sleep disorders, urticaria, cutaneous eruptions, in the same interval;
- ➢ Oedematous reactions accompanied by cyanosis or transient purpura, at the level of the lower limb where the vaccine was administered, but possibly contralaterally as well, with appearance in the first hours after vaccination, short duration (a few hours) and spontaneous remission with no traumas; [20]
- ➢ Anaphylactic reactions, Quincke oedema, are exceptional.

<div style="text-align:center">**7. MEASLES VACCINATION**</div>

Measles is an infectious disease with general responsiveness, highly contagious (95%) and high fatality 0.1-0.2% presently/3.5% before vaccination, by complications like pneumonia (at the little child) and acute encephalitis (at the big child and adults). It can also induce subacute sclerosing panencephalitis, with late debut (4-17 years old) after a measles episode and lethal evolution in 6-36 months.[2] In such a context, the vaccination of the population against the measles virus was a step forward, determining important modifications in the manifestation of the epidemiologic process, especially by reducing the incidence and mortality due to such disease. Recently, however, due to the stronger and stronger anti-vaccination movement, Romania, and the European or American space as well, face the re-emergence of such pathology.

Types of products: The products imposed, with a good proof of effectiveness after their entry at large scale, contain live virus strains, with supraattenuated virulence. The most frequently used strain is named Schwartz. The following types of measles vaccine exist:

➢ **Monovalent** - lyophilised live vaccine, prepared from the hyper-attenuated Schwartz strain on chicken embryo fibroblasts (e.g.: Rouvax- Sanofi Pasteur). Seroconversion is higher than 95%, but there is the risk, in case of infants younger than 9 months, of its interference with maternal antibodies, and the consecutive reduction of post-vaccination immunity. Because morbidity by measles is preserved high at children younger than 9 months, there is an interest for the development of new vaccines adapted to the young age.

➢ **Bivalent** – obtained by the combination of the measles to the rubella components.

➢ **Trivalent** – many states are presently practicing concomitant vaccination with trivalent vaccine measles rubella-mumps. Such vaccines (M-M-R II-Merck Sharp& Dohme, Trimovax-Sanofi Pasteur, Priorix-GlaxoSmithKline), are found in the vaccination schedules of several industrialized states (USA, Canada, Australia, Western Europe etc.), and starting with 2004 they have also been included in the Romanian compulsory immunization calendar. The measles component (attenuated strain, cultivated on chicken embryo fibroblasts) is added the rubella component (attenuated live strain, cultivated on human diploid cells) and a mumps component (live attenuated strain, obtained in embryonated eggs).

➢ **Tetravalent** – a 4 components vaccine was licensed in 2005 (the three being added the varicella component), used between the age of 12 months and 12 years.[21]

Form of presentation: The commercial products are delivered as preloaded syringe by 0.5 ml solvent, with the white-yellowish powder vaccine vial. There are also multi-dose products (with 10 doses). After the reestablishment, the liquid is clear, pale yellow, pink yellow or orange-yellow in colour. The administration needs to be performed right after the dissolution. To be kept in the dark, frozen -10°C/-20°C (in case of the monovalent vaccine) or between 2°C and 8°C (three-vaccine). After defrost, the measles vaccine can be preserved for 14 days at temperatures between 2°C and 8°C.

Route of inoculation is mainly subcutaneous in the deltoid region for the monovalent product and intramuscular/subcutaneous for the three-vaccine.

The vaccination schedule in Romania includes:
➢ O primary course by 0.5 ml prepared trivalent measles-rubella-mumps, inoculated im./sc. at the age of 12 months;
➢ A booster, as well by 0.5 ml prepared trivalent, inoculated im./sc. at the age of 5 years.

The administration of the infecting post-contact measles vaccine can prevent the disease or modify its evolution, if made in the first 72 hours. In case of measles epidemics, the measles vaccine can be administered as well to the infants between 6-11 months old, following for the second dose to be administered at 12 months of age (with trivalent product).

Vaccination determines an hummoral and cellular immune response, but compared to the natural passing through the disease, the quantity of antibodies produced is lower. The protective effect will appear at 60-70% of the infants younger than 9 months old and at 95-98% of the children vaccinated with an age range between 12-15 months, and is installed for a period of 15-20 years.[21]

Contraindications can be:
➢ **Permanent** - in case of anaphylactic antecedents at the egg protein or neomycin, congenital or acquired immunodeficiencies (leukemia, neoplasia), pregnancy. At the HIV positive patients, severe immunosuppression with a level of CD^{4+} lymphocytes below $200/mm^3$ at an age older than 5 years old, below $500/mm^3$ at age between 1 and 5 years old and below $750/mm^3$ at infants, contraindicate the administration of the vaccine.[2,17]
➢ **Temporary** – represented by febrile acute diseases, evolving neurological pathology, chronic diseases of the breathing

apparatus in activation period, immunoglobulin therapy or blood derivates therapy (the vaccine can be administered 2 weeks before or 3 months after the blood product), chemo/radio/cartico-therapy (the immunisation is possible after at least 1 months from the end of the therapy). However, vaccination is possible in those taking topical corticoids, for substitutive purpose or in small doses (immunosuppression is considered to appear after a total dose of 20 mg/day of Prednisone, administered on general route, daily or alternatively, for minimum 14 days). As well, children with light infections of their upper airways, average ear infection or diarrhoea can also be vaccinated. [21]

The side effects can appear 5-12 after vaccination, with a maximum in the 6th and 7th day.

> - Minor side effects are represented by febrility (5-15% of the vaccinated individuals), nasopharyngeal catarrh, symptomatology of respiratory disorder, light ear infection, digestive disorders (nausea, vomiting), transitory exanthema (5%), arthralgia, passenger arthritis. The joint symptomatology is due especially to the rubella component.

> - Major reactions are rare: seizures (especially at children with personal antecedents or family history of seizures), thrombocytopenia (1 case/25,000 doses), post-vaccine encephalopathy (0.4-1 case/1 million of vaccinated individuals), mumps or unilateral deafness (due to the mumps component), anaphylaxis (1-3.5 case/1 million doses). The incidence of encephalitis after vaccination (causality connection of which is uncertain) is 1,000 times smaller compared to that determined by the natural measles infection (1 case in 1,000-2,000 persons sick with measles). Neither after prolonged seizures nor after encephalitis, no significant permanent neurological traumas have been found.[2,21] No association has been found between autism and the MMR vaccine, although many scientific studies have been performed after the launch of the controversy in 1998, in the UK.

8. RUBELLA VACCINE

Active immunoprophylaxis in case of rubella is important both for the reduction of the disease incidence, and most often, for preventing the congenital rubella syndrome (CRS).

The Australian ophthalmologist, Norman McAlister Gregg, identified in 1941 the connection between rubella infection and congenital cataract at infants. Subsequently, the teratogenic potential of the rubella virus was marked out, and it can lead to ocular malformations (cataract, congenital glaucoma, retinopathy, microphthalmia), hearing malformations (deafness), cardiovascular (Ventricular septal defect, arterial channel persistence, coarctation of the aorta, pulmonary stenosis), affections of the CNS (microcephaly, mental retardation), but also bone malformations, hepatosplenomegaly etc. More frequently at the big child with the syndrome of congenital rubella, diabetes mellitus type I or encephalopathy like subacute sclerosing panencephalitis, named progressive panencephalitis. The conception product will be as much affected as maternal infection appears at younger gestational ages. Therefore, the consequences of rubella arisen in the first quarter of pregnancy are foetus death, spontaneous abortion, premature birth /stillbirth or multiple foetal malformations. After the 4th month, unique malformations prevail, especially deafness.

In order to control / remove rubella and the congenital rubella syndrome, many countries have adopted a vaccination strategy by 2 compulsory rubella doses for children and women.

The following **vaccine products** are possible**:**
- ➢ **Monovalent** – they contain live virus strain, attenuated RA 27/3, obtained by the cultivation on human diploid cells WI-38 or MRC5 (e.g.: Rudivax-Sanofi Pasteur, Ervevax-GlaxoSmithKline) [22]
- ➢ Or **combined** – where the rubella component is combined with the measles, mumps or varicella component, in bivalent, trivalent, tetravalent products, described in the previous chapter.

  The forms of presentation, the storage conditions, the administration schedule are similar to those presented for the measles vaccine. The vaccination triggers humoral and cellular immunity response, but the concentration of post-vaccine antibodies is smaller compared to that triggered by the natural infection. Seroconversion appears at more than 95% of the vaccinated individuals, with the persistence of the protective effect for 10-15 years afterwards. The titre of specific antibodies considered protective is of 10 UI/ml.[2, 22]

The vaccine is indicated to children older than 12 months, but as well to the children in schools, students campus, girls at puberty or women, before procreation. The following can also be vaccinated: susceptible adults (especially those around pregnant women, the medical staff or children's care staff) or seronegative women, after birth. It can also be administered after the contact with the wild virus, in the first 24 h. The identification of the individuals susceptible of rubella can only be made based on known personal antecedents, as there are multiple clinical cases, not diagnosed as rubella.

Contraindications are similar to those presented for the measles vaccine, with the modification that the individuals with egg anaphylaxis can be vaccinated with the monovalent product, manufactured on human cells. As well, sexually active women are not allowed to get pregnant for minimum 1 month after the rubella vaccine administration. If however it is administered during pregnancy, it is not considered as an indication for abortion.[22] Children with seizures in their antecedents will be vaccinated later, usually after they turn 2 years old. The administration of Rho(D) human immunoglobulins does not represent a contraindication for postpartum rubella vaccination.

Local **side effects** are minor. The general side effects include fever, pharyngitis, skin eruptions, cephalea, arthralgias, arthritis, thrombocytopenia or very rarely – transitory polyneuropathies. The vaccines the more reactogenic as the age of the concerned individual is older. At adult women, arthralgias are typical (appeared at 25-50% of the women vaccinated aged more than 20 years) and arthritis (at 15%), however transitory.

There can appear clinical re-infections at the vaccinated individuals, just like in the case of the naturally immunized people, even of pregnant women, but the risk of foetal affection is below 5% in the first pregnancy term, compared to 80% in primary infection.[2,22]

Both rubella and the syndrome of congenital rubella can be eradicated, and the universal implementation of rubella immunisation creates the premises for the disappearance of the CRS 100 years after its description.[22]

9. MUMPS VACCINATION

The mumps infection raises people issues by the high morbidity (in communities of children, soldiers) or by serious complications: encephalitis arisen in less than 0.3% of the mumps infections, which is responsible for most of the fatal cases; the orchitis which complicates more than 37% of the cases of disease for post-puberty men (with a risk of oligospermia, hypofertility, rarely sterility) or permanent deafness, usually unilateral, approx. 1 case every 20,000 patients. Although pancreatitis occurs in 4 % of the cases, the combination with diabetes mellitus is not scientifically proved.[23]

The mumps component was added in the compulsory Romanian vaccination calendar in 2004. The current vaccine products contain live strains, with attenuated virulence. The most frequent are represented by the Jeryl Lynn strain, attenuated by passages in embryo eggs and cellular cultures of chicken embryos, the RIT 4385 strain, a clone of it and the Urabe Am9 strain, existing in some Japanese or European products. These strains are included in monovalent vaccines (Imovax mumps - Sanofi Pasteur), bivalent, trivalent or tetravalent, presented as well in the previous chapters, with the indications for their storage, form of presentation, in force vaccination schedule.

After the vaccination with the standard Jeryl Lynn strain, the seroconversion occurs at 94-98% of the vaccinated individuals, after the RIT 4385 strain, the immune response occurs in 92-96% of the cases and after the Urabe strain, in 95%. Protection is on for 10 years.[23]

Vaccination is recommended to children older than 12 months, to bigger children and unvaccinated teenagers, to adults susceptible of mumps (especially students, soldiers, healthcare staff) or family members of immunosuppressed patients.

Contraindications are similar to that described for the measles vaccine, when the MMR vaccine was also included, with the remark that positive HIV children are to be vaccinated only if asymptomatic or do not suffer from severe immunosuppression. They will be vaccinated at 12 months old, in order to prevent the reduction of the immune response as the HIV infection evolves. The family history of diabetes mellitus is not considered contraindication for the vaccine.

The most frequent side effects are febrile condition and parotiditis occurred 10-14 days from vaccination. There have been rare accounts on allergies, skin eruptions, pruritus, purpura, febrile seizures, orchitis, perception hypoaccusis (1 case/1 million doses) or encephalitis (0.4 cases/1 million doses). Aseptic meningitis following vaccination has a variable incidence, from approx. 1 case/ 800,000 doses for the Jeryl Lynn

strain, to 1 case/ 11,000 doses, for the Urabe strain. Despite all this, Urabe seems to assure a higher protective level, at a lower cost, but with higher reactogenicity.[23]

Under such conditions, the products which contain the Urabe strain are preferred in the areas with wild mumps virus and with low vaccine coverage, in order to pass, following people's immunity development, to Jeryl Lynn strain vaccines.[2]
Re-infections with a different mumps virus genotype are possible, but rare. [23]

10. HEPATITIS B VACCINATION

Viral hepatitis B is an important public health problem by the risk of persistence (in 20 % of the cases), by its evolution towards chronic hepatopathies and primary hepatocarcinoma, with death in an interval of 15-20 years, but also with a risk of vertical transmission, especially in the perinatal period (infectivity 70-90% if AgHBe is present or 5-20% if absent).[24] We estimate at worldwide level, 2 billion people have passed such infection, amongst which 257 million have persistent infection, and there appear annually approx. 887,000 deaths caused by HBV.[2, 25]

The specific prevention, both active by using hepatitis B vaccines, and passive, by administering specific HBs antibodies, has a very high efficiency, laying the foundations for the long term eradication of the hepatitis B virus infection. One needs to notice as well that the HBV vaccine is the first product with preventive effect against an oncologic pathology (hepatocarcinoma).

The vaccination strategies are different depending on the endemicity of the area. In the counties with high and intermediary endemicity, the WHO recommended starting with 1995 the vaccination of the infants, in parallel with the vaccinations of children, that of teenagers or adults with risk. In the countries with low endemicity, prevention is focused on the immunisation of the high risk groups, of the infants with mothers carrying HBV markers, and starting with 1997 and on generalized vaccination at birth.

The risk groups for the viral infection with the hepatitis B virus are:
  ➢ The middle, upper and auxiliary healthcare and medical staff;
  ➢ The military staff, fire fighters and policemen;
  ➢ Persons with hematologic, renal, politransfused/ dialyzed affections or persons with parenchymal organ transplant;
  ➢ Staff and residents in the social work facilities for children, prisons or institutions working with psychic patients;

> Persons with sexual conducts generating risks: homosexuals, bisexuals, prostitutes, persons with multiple patients, patients with a different sexually transmitted pathology;
> Drug addicts using drugs with iv administration, adepts of tattoos, piercing;
> Persons originating from areas with high endemicity;
> Infants with mothers carrying HBV markers;
> The family members or those in around HBV infected persons;
> Travellers in endemic areas, if they are going to stay there for more than 6 months, a sexual contact with a local or getting in touch with the medical industry, in his or her capacity of patient of medical staff. [24, 26]

The immunisation can be made with the following **types of products:**
> **2nd generation vaccines,** obtained by genetic recombination. They contain recombined HBsAg DNA, obtained on cultures of *Saccharomyces cerevisiae,* where the S synthesis gene of the HBsAg is included using a plasmid. The commercial products are named Engerix B-GlaxoSmithKline; Euvax B- Sanofi Pasteur; Recombivax HB-Merck&Co, usually used in Romania as well.
> **3rd generation vaccines**, obtained as well by genetic engineering, on cultures of animal cells, which contain major protein or major and middle protein of the HBsAg. Vaccines of such type are available on the external market (GenHevac B-Sanofi Pasteur, Gen H- B-vax-Sanofi Pasteur).
> **combined** bivalent vaccines (Twinrix**-**GlaxoSmithKline combine an inactivated virus hepatitis A and one hepatitis B recombined DNA); tetravalent (Infanrix HepB- GlaxoSmithKline, with DTPa + Recombined DNA HBsAg), pentavaccines plus the IPV component or hexavaccines (including with Hib).

Form of presentation: the commercial products are delivered as vials (Euvax B) or preloaded syringes (Engerix B), with 1 paediatric use dose (10 µg/0.5 ml) or adult type (20 µg/1 ml). The suspension is slightly opalescent and needs to be shaken before use. To be stored at a temperature of 2-8 °C.

Administration is intramuscular in the anterior-external region of the hip (at newborn, infants and small children) or in the deltoid at older children and adults.

The vaccination strategy applied in Romania combines nowadays the vaccination of the newborn in maternity, with the school immunisation campaigns of teenagers, previously unvaccinated and with the vaccination of adults in the risk groups:

1. **the vaccination of the newborn** – The first dose of 0.5 ml is administered im. in the first 24 h after birth and the following 3 doses at 2, 4 and 11 months old, within hexavalent vaccination. The newborn come from mothers carrying the HBV markers are vaccinated at 0, 1, 2, 11 months old and concomitantly with the first dose of vaccine, but in a different place, to be administered intramuscular 0.5 ml specific hepatitis B immunoglobulin. Testing for HBsAg and the HBs antibodies will be performed at 12-15 months old.[26] The combination of the vaccination with passive prophylaxis by administering HBIg, prevents perinatal transmission in 85-95% of the cases and offers long term protection. In case of premature weighting below 2000 g, newborns from negative HBsAg mothers, the first vaccine dose will be delayed up to 1 month or up to hospital discharge.[24]

2. **Pre-exposure prophylactic vaccination** – to be eventually addressed to students in the medical education system, previously unvaccinated (medical colleges, Medical faculties, Dental Medicine etc.). Vaccination is applied as well to adults with a risk, previously unvaccinated. To be inoculated intramuscular 3 dose of 0.5 ml (at children aged 15 years and younger) or 1 ml (over such age) at 0, 1 and 6 months from the beginning of the vaccination. The dose doubles in case of heavy smokers and of overweight persons. The immunodepressed patients (HIV, chronic hemodialysis) receive double doses of 2 ml, at 0,1,2 and 6 months. The HBs antibodies appear in 1-3 months after vaccination, considering to be protective titre for more than 100 mUI/ml. The effective seroconversion is installed at more than 95% of the immune-competent infants, children and youngsters. The vaccination risk without immunization is higher amongst persons over 40 years old (immune response at approx. 75% of the individuals older than 60 years old), of smokers, obese individuals, immunosupressing or after an accelerated scheme (0, 7, 21 days and 12 months from the first dose). We recommend serologic testing of HBs antibodies 1-2 months following the last vaccine dose, only for medical staff, patients with a risk of failure to be immunised (HIV, hemodialysis etc.) and sexual contacts of the positive HBsAg individuals. Protection lays on a length of minimum 15 years related to the HBV and DHV, but not for other hepatic viruses as well or other pathogens with hepatic tropism. Even after the diminution of the titre below 10 mUI/ml, there is protection against HBV, due to the immunologic memory able to determine an anamnesis response. Therefore, we do not recommend the revaccination of the general immune-competent population, but only of the risk categories.

3. **Post-exposure prophylactic vaccination** – Addressed to the medical staff with major professional risk or sexually contacted individuals of persons with HBV acute or chronic hepatitis. In case of accidental exposure, during the use of sharp/cutting materials or by the contact of mucosa with infecting biologic fluids, we administer specific hepatitis B immunoglobulin, 0.06 ml/kg body, intramuscular in the first 24 h from the infecting contact. Concomitantly, we begin the quick vaccination schedule with 4 dose at 0 (the first 72 h form the infecting contact), 1, 2, 12 months. The previously vaccinated persons against HBV will be inoculated HBs antibodies. In case of protective levels exceeding 100 mUI/ml, only immediate sepsis prophylactic measures will be applied. Medical staff with antibodies between 10 and 100 mUI/ml will be protected by a booster. Those with titre below 10 mUI/ml will be repeated the vaccination schedule and administered specific immunoglobulins. The HBIg efficiency is of approx. 75% in preventing clinical hepatitis or the chronic infection, if used in the first 7 days after exposure (ideally in the first 24 hours).[24]

Table no. III Preventive conduct after exposure of skin / mucosa to blood – adapted afterwards [2, 26]

EXPOSED PERSON	RECOMMENDATIONS IF THE SOURCE IS		
	HBsAtg +	**Unknown / untested**	**HBsAtg -**
Unvaccinated	HBIg + vaccination (3 doses)	Vaccination (3 doses)	Vaccination (3 doses)
Previously vaccinated, with protective titre	No measures of specific prophylaxis	No measures of specific prophylaxis	No measures of specific prophylaxis
Previously vaccinated, with nonprotective titre	HBIg + vaccination (3 doses) Or HBIg 2 times at one month interval	If the source belongs to the risk groups, proceed as in case of a positive source	No measures of specific prophylaxis
Unknown HBs titre	Testing the person exposed for Atc HBs: ➢ Protective titre – no measures of specific prophylaxis ➢ Nonprotective titre - HBIg + one dose booster	Testing the person exposed for Atc HBs: ➢ Protective titre – no measures of specific prophylaxis ➢ Nonprotective titre – booster with one dose + retesting in 1-2 months	No measures of specific prophylaxis

Table no. IV Preventive conduct for sexual contact or family contact – adapted afterwards [26]

TYPE OF EXPOSURE	IMMUNOPROPHYLAXIS
Sexual contact with a person with HBV acute infection	HBIg + vaccination (3 doses)
Sexual contact with a person – chronic carrier of HBsAg	Vaccination (3 doses)
Family contact with a person with acute infection HBV	HBIg + vaccination (3 doses) if there is a sexual contact or risk of parenteral transmission
Family contact with a person – chronic carrier of HBsAg	Vaccination (3 doses)

The contraindications for the vaccination are reduced. The vaccination is to be postponed in case of severe acute febrile conditions and the immunisation of the individuals with anaphylaxis antecedents at brewer's yeast or after a previous vaccine dose is to be avoided. Pregnancy is no contraindication.

Secondary effects are minor and transitory.

- ➢ **Local reactions** can occur - erythema, induration, pain at the place of inoculation or
- ➢ **General** - subfebrility, cephalea, myalgia, arthralgia, fatigability, digestive disorders (nausea, abdominal pains), allergic manifestations (pruritus, urticaria). They are lighter and self-limited;
- ➢ Neurologic disorders have been seldom accounted for - encephalitis, paralysis, neuritis or chronic diseases (chronic tiresome syndrome, diabetes mellitus type I, autoimmune pathology), without a causal connection with the vaccination;
- ➢ No enough data are available in order to backup a causality relation between recombined vaccines and the Guillain-Barre syndrome or demyelinised diseases (multiple sclerosis).
- ➢ And anaphylaxis is very rare.[24]

11. PNEUMOCOCCAL VACCINATION

At worldwide level, approx. 1.6 million people die annually due to invasive pathology determined by *Streptococcus pneumoniae*, including one million children younger than five years old. This etiologic agent can lead to severe affections– meningitis, endocarditis, pneumonias/ empyema, bacteremias, septicemia but also non-invasive affections like middle ear infections and upper respiratory infection.[2]

Morbidity and mortality are significantly high at little children, old people, persons with comorbidity (cardio-vascular chronic pathology, pulmonary pathology, diabetes mellitus, cirrhosis, alcoholism) or immunosupressed individuals (congenital immunodeficiencies, infected with HIV, anatomic or functional spleen absence, Hodgkin disease, lymphomas, multiple myeloma, transplants of organ, renal failure or nephritic syndrome).

Available, several **types of anti-pneumococcal products:**

➢ **a polysaccharide product** with 23 serotypes which more than 90% of the types involved in invasive pathology, usable at adults, old people and children older than two years (PNEUMO23 – Sanofi Pasteur), destined especially to the individuals with chronic pathology, to institutionalized persons, splenectomised patients, immunosupressed people;

➢ **three conjugated products** for infants including 7, 10 or 13 serotypes (PCV7, PCV10 and PCV13) covering 65-80% of types involved in invasive diseases in infants/toddlers, but have lower prevention of non-invasive pathology (otitis media, pneumococcal pneumonia). Capsular antigens are conjugated to a carrier protein (diphtheria or tetanus anatoxin, or meningococcal outer membrane protein complex, etc.) and absorbed onto aluminium phosphate.

Form of presentation: The polysaccharide solution is delivered as a 0.5 ml single-dose prefilled syringe – a clear colourless liquid. The conjugated vaccine is delivered as a 0.5 ml single-dose vials – an opalescent liquid. Both to be stored at temperatures between 2 and 8°C, avoiding freezing.

Administration is intramuscular - in infants, in the hip region, and in teenagers and adults, in the deltoid.

The vaccination strategy: in Romania, the **pneumococcal conjugated vaccine** introduced in 2013 in the National Immunisation Programme (within the limits of funds available) is destined to prevent invasive diseases, pneumonia and acute otitis media determined by *Streptococcus pneumoniae* mainly in infants and children between 6 months and 5 years old.

- ➢ Primary course includes 3 doses of 0.5 ml each administered at 2, 4, 11 months old;
- ➢ Children older than 12 months who previously have not been vaccinated get 2 doses within minimum 2 months' time;
- ➢ Immunocompetent children aged between 2 and 5 are administered one single dose, while the immunosuppressed/with chronic pathology are vaccinated with 2 doses within minimum 2 months' time.

Polysaccharide vaccine - VPP23 with IM administration in deltoid (or SC administration in patients with hemostasis issues), one single dose of 0.5 ml. VPP23 revaccinations are not recommended in immunocompetents except for patients classified in invasive pathology risk groups.

Contraindications of the vaccination:
- ➢ The vaccination is postponed **temporarily** in case of upper airways infections, febrile state;
- ➢ **The vaccination is not to be performed** in patients who were hypersensitive to the previous dose.[2] The polysaccharide vaccine is not to be performed in patients who were administered a dose in the last 5 years, however vaccination is allowed in pregnant women in the third trimester.

Side effects:
- ➢ **Locally** erythema, swelling or pain may appear, or
- ➢ **General reactions** - subfebrility, irritability, persistent crying, hypotonia-hyporeflexia syndrome, skin rashes and anaphylaxis is rare.[2]   Other side effects of the polysaccharide vaccine: headache, muscle aches, fatigue.

The introduction of heptavalent conjugate vaccine PCV7 in infants diminished the rate of nasopharyngeal carriage and bacterial transmission, thus appearing the collective immunity, and decreasing the morbidity/mortality rate as a consequence of invasive streptococcus infections. But emerging serotypes that were not included in PCV7 had determined over the years the increase in carriage and in non-vaccine serotypes, which imposed the extension of number of serotypes in future vaccine solutions. In most European states, PCV10/PCV13 and PPV23 are included in national vaccination programmes. However, this remains an issue even after replacing PCV13 vaccine, which means more monitoring and research activities in order to develop new biological products.

VACCINES USED IN CASES OF EPIDEMIOLOGICAL RISK

1. INFLUENZA VACCINE

Influenza is at all times monitored by Public Health, because of its special epidemic and pandemic potential, as the 1918 influenza pandemic known as "Spanish flu" caused over 20.000.000 deaths globally, the 1957 "Asian flu" caused around 70.000 deaths, and the 1968 Hong Kong flu pandemic killed 30.000 people. [27,28] The latest global phenomenon from 2009 killed around 17.000 people, amounting to 4.763 in WHO Europe (up to April 2010) and 1-2% total mortality.[28,29]

Under these circumstances, vaccination remains the most important way of preventing/combating, also with an impact on reducing medical, economic and social costs.

Influenza vaccination has to overcome numerous challenges related to:

> ➢ The existence of 3 influenza viruses - A, B, C of numerous subtypes and variants determining specific immunity;
> ➢ The large variability of the pathogen allowing permanent changes in the antigenic structure (antigenic drift and antigenic shift);
> ➢ As well as, the existence of the extrahuman reservoir of flu viruses.

Under these circumstances, the effectiveness of vaccination shall correlate directly with the compatibility between the circulating virus strain and the strain existing in the vaccine. In case of major change in variability, the seasonal influenza vaccine with actual circulating strains will not be effective. This was the case back in season 2009/2010, when simultaneously there existed a monovalent vaccine for A/H_1N_1 pandemic strain, as well as a seasonal trivalent vaccine. Another drawback is represented by the short-term post-vaccine immunity – 1 year maximum. Despite all these limitations, influenza vaccine has big benefits for populations classified in risk groups, by reducing the morbidity, and especially further complications, and mortality combined with flu.

Nowadays, the vaccine composition is established annually, following a forecast of strains circulating in the next epidemic season, forecast which is performed by the WHO Collaborating Centre for Reference and Research on Influenza. Thus, the vaccine formulation is established in spring, it is produced in summer, and in autumn, between October and November, vaccination campaigns are carried out for population with major risk, before the beginning of the season with epidemic potential in the next winter-spring.

There may be **monovalent influenza vaccines** (with pandemic strain) or **trivalent / tetravalent seasonal influenza vaccines.** The latter, regardless of its preparation, contain 2 strains of A influenza strain, with pandemic potential, and 1 or 2 strains of B influenza virus B, with regional epidemic potential. For the 2018/2019 season, in the Northern hemisphere, the influenza vaccine contains 1 A/Michigan/45/2015 (H1N1)pdm09 strain, 1 A/Singapore/INFIMH-16-0019/2016 (H3N2) strain, one similar to B/Phuket/3073/2013 (Yamagata/16/88 line) strain and one B/Colorado/06/2017 (B/Victoria/ 2/87 line) strain.

The main **types of influenza vaccine** available on the global market are:

➢ **Live attenuated influenza vaccine** adapted to cold, administered as a nasal spray, obtained by genetic recombination (used on the external market);

➢ **Inactivated influenza vaccine**, with good immunogenicity but higher reactogenicity;

➢ Nowadays, **trivalent / tetravalent subunit and fragmented vaccines** lead the market, containing surface antigen only, or including also nucleoprotein, protein M (e.g.: Influvac/Influvac Tetra - Abbott; Fluarix/Fluarix Tetra - GlaxoSmithKline, Vaxigrip/Vaxigrip Pediatric/Vaxigrip Tetra - Sanofi Pasteur).

Most vaccines are obtained by cultivation in the allantoidal cavity of embryonated chicken eggs, formalin inactivated and chemical fragmentation.

The immune response through anti-hemagglutinin and anti-neuraminidase antibodies becomes protective in 2 weeks after administration of the preparation and persists up to 6 months or 1 year. The effectiveness in clinical disease prevention is about 70-90% in healthy persons, and about 50-60% in the elderly, when the vaccine formulation corresponds to the circulating influenza strains. Plus, it prevents up to 80% of death cases caused by influenza, in the elderly.

Risk groups: Any person older than 6 months can be vaccinated against influenza viruses however, active prophylaxis is particularly intended for the following groups:

➢ The elderly over 65;

➢ Long-term residents of retirement homes or other medical and social institutions;

➢ Institutionalised children;

➢ Children and adults suffering from chronic cardiopulmonary diseases (including asthma), metabolic disorders (diabetes mellitus), kidney disease, haemoglobinopathy, fatigue, born with or acquired immunosuppression (including neoplasia, HIV);

- Children and adults chronically treated with aspirin that might develop Reye's Syndrome after catching the flu;
- Medical staff, teachers, the army, firefighters, public servants, etc.;
- The elderly or people with risk factors, who travel in the southern hemisphere, during April-September or in the tropics (year-round).

Firm indication for this vaccination targets also the professional caregivers of high-risk persons or family members of these patients.

Form of presentation: The influenza vaccines widely used nowadays, that is fragmented vaccines, are delivered in 0.5 ml or 0.25 prefilled single-dose syringes (those for paediatric use). The liquid looks clear and colourless. To be stored at temperatures between 2 and 8°C. Other vaccine preparations are delivered in vials or sprayers similar to a syringe, for nasal administration.

Administration of common influenza vaccines:
- 2 doses of 0.5 ml each, deep IM or SC, 30 days apart, for children under 8 years old, previously unvaccinated;
- 1 single dose of 0.5 ml, for older children;
- for children between 6 months and 3 years old, 2 doses of 0.25 ml each, if previously unvaccinated;
- Inoculation in deltoid, in adult/teenager patients or in anterolateral thigh region, in children under 2;
- In patients with haemostasis disorder, SC administration is recommended. Lately, there has been a new product with 0.1 ml dose intradermal injection (IDflu-Sanofi Pasteur), available for adults (18-64 years of age), including for those with coagulation disorders.

Contraindications:
- **Definitive contraindications** – Represented by anaphylactic reactions to egg or other components of the vaccine (egg protein, neomycin, formaldehyde).[30] Persons with immediate hypersensitivity to eggs, or other types of allergies following exposure to egg protein, must ne rigorously assessed in order to decide whether to be vaccinated;
- **Temporary contraindications** – in case of acute infectious diseases, febrile state, etc.

Side effects are generally mild:
- Local side effects - pain, oedema, swelling, in the first 2 days following vaccination;

- Systemic side effects – moderate fever, headache, shivering, myalgia, arthralgia (so-called influenza-like syndrome). Present within 6 to 12 hours following vaccination and persist up to 2 days;
- Immediate hypersensitivity reactions include hives, angioedema, asthma crisis, anaphylaxis and are combined with egg allergy;
- Exceptionally, there may appear neuritis, seizure, transient thrombocytopenia, encephalomyelitis or Guillain-Baree Syndrome;
- Generally, corpuscular vaccines have a more obvious reactogenicity.

The table herein illustrates the differences between live influenza vaccines and fragmented inactivated vaccines:

Table no. V Live attenuated v inactivated influenza vaccines

CHARACTERISTICS	LIVE VACCINE	INACTIVATED VACCINE
Administration	Nasal	IM/SC
Vaccine excipients	No thimerosal	Some vaccines contain thimerosal
Vaccination recommendations	Persons aged between 2 and 49, with no contraindications of administration, prioritising: ➢ Persons aged between 25 and 49 living together with/carrying for infants under 6 months; ➢ Adults aged between 25 and 49 who are members of the medical staff and emergency services.	Persons older than 6 months, prioritising: ➢ The elderly aged over 65; ➢ institutionalised children and youngsters; ➢ children/adults with chronic comorbidity (including immunosuppressed); ➢ the medical staff, emergency services, caregivers of high-risk patients; ➢ persons living/carrying for infants under 6 months; ➢ pregnant women with risky pregnancies (second, third trimester).

Contraindications	➤ Severe egg allergy or severe allergies to other components of the vaccine; ➤ Under 2 or over 50 years old; ➤ Immunosuppression, pre-existent chronic pathologies – respiratory, kidney, cardiovascular, cardiorenal, hepatic, hematologic disease, diabetes, asthma, immunodeficiency; ➤ Children under 5 years old with asthma or one episode of wheezing in the previous year; ➤ Nervous system pathology with breathing and swallowing troubles; ➤ Children/teenagers with Aspirin long-term treatment; ➤ Any persons in close contact with an immunosuppressant; ➤ Pregnant women; ➤ Guillain-Barre Syndrome history[31].	➤ History of severe influenza post-vaccination side effects; ➤ Moderate and severe respiratory pathology delays the vaccination.
Side effects	➤ Rhinorrhoea, nasal congestion, dysphagia, fever, cough; ➤ Gastrointestinal disorders; ➤ Fatigue.	The most frequent side effect is local erythema at the inoculation site.

PASSIVE IMMUNISATION

DEFINITION

It represents the temporary transfer of immunity by administering ready-made antibodies, such as total or specific immunoglobulin or specific serums.

Passive immunisation is generally used in non-immune persons, in immediate epidemiologic risk cases or in persons with contraindications for certain vaccine formulas. It is possible to combine active and passive immunoprophylaxis, in order to cover the timeframe between vaccine administration and reaching protective antibody titre.

SPECIFIC SERUMS (ANTITOXINS)

They are antibody solutions obtained from the animal serum (mainly horses), immunized with specific antigens. Immunity is immediately installed, which allows their administration for both therapeutic purposes and post-exposure prophylaxis. They may generate very serious anaphylactic reactions, which is why, if possible it is recommended to be replaced by specific human immunoglobulin.

In Romania, anti-anthrax, antibotulinic and anti-diphtheria serums are used for therapeutic purposes while anti-rabies and anti-tetanus serums are also used for post-exposure prophylaxis.

There are certain specificities when administering serums, as follows:
- ➢ The dose is calculated according to the target (curative or prophylaxis), to age and body weight;
- ➢ It is administered as single dose, in order to increase its effectiveness and decrease the risk for side effects;
- ➢ To be administered in the shortest time possible as serums can only neutralize circulating toxins, without neutralizing toxins fixed on cells;
- ➢ IM administration, in the thigh region. In case of anaphylactic reaction, a tourniquet may be applied to the closest extremity of that limb, thus performing temporary stagnation of the serum spread and shock treatment. For curative purposes only, serums may be IV administered, subject to multiple precautions;
- ➢ Patients submitted to passive immunisation via serums must be carefully monitored from the first minutes (for emergency intervention in case of anaphylactic shock), as well as in the next 7-10 days in order to trace any possible late reaction.

Patients with high risk to allergic accidents are identified following the next steps:

- ➢ Targeted anamnesis, based on their personal history of allergies, and
- ➢ Mandatory serum sensitivity testing – via conjunctival instillation, scarification of the tegument or intradermal injection of serum dilution (generally 1/100 or 1/10). The development, 30 minutes later, of conjunctival congestion or local erythema, between 2 and 10 mm in diameter, possibly accompanied by an oedema, translates a hypersensitivity condition.[1]

Minimal desensitization scheme is applied in persons with negative testing, where IM administration of the serum is performed. From a 1/10 dilution in physiological serum, 0.25 ml is injected subcutaneously and then wait for 30 minutes. If there is no reaction, 0.25 ml of non-diluted serum is injected subcutaneously too. After another 30 minutes, dose is increased up to 1 ml of non-diluted serum. If again, once the 30 minutes have passed, there is still no reaction, the remaining of the calculated quantity of serum is administered intramuscularly.[1]

In case of hypersensitivity, slow desensitization is applied, meaning successive administration, subcutaneously, every 30 minutes, of small doses of serum, starting with minimal nonreactive testing dilution and then increasing progressively the concentrations up to non-diluted serum. The development of some allergic reactions makes it obligatory to administer, 30 minutes later, the previous dose when the allergic reaction had developed. The scheme is longer or shorter depending on how sensitive the patient is. If desensitization is unsuccessful, serum is to be replaced by specific immunoglobulin if available.

Possible **side effects** develop because of the equine protein content which is strongly allergenic. The frequency and the intensity of serum reactions depend on the body's sensitivity, on the administered serum dose and on the history of similar inoculations.

There may be **immediate reactions**, as follows:

1. **Nonspecific febrile reaction** manifested by shivering, fever, nervousness, pain and warmth on the injection site, within the first hour after administration. It is the consequence of nonspecific pyrogens and ceases after local anti-thermal treatment.[1]
2. **Anaphylactic shock** which appears immediately after serum administration, following the intervention of Ig E reaginic antibodies. The symptomatology is characterized by hives rash, glottic oedema, bronchospasm, filiform pulse and low blood pressure. Emergency demeanour entails applying a tourniquet on

the root of the limb where injection was performed and, depending on the gravity of the clinical situation, antihistamines, adrenalin, hydrocortisone hemisuccinate are to be administered, and, if necessary, CPR procedure should be performed.

Late reactions, as follows:

1. **Arthus phenomenon** – is a local sensitivity reaction, following the intervention of circulating immune complexes, developed as a consequence of serum reinjection on the same site, at short intervals. It develops as a local congestion sometimes evolving into a necrosis and gangrene.
2. **Accelerated serum reactions –** appear between 2 and 5 days after serotherapy, with a symptomatology similar to the one of serum sickness;
3. **Serum sickness –** occurs within 6 to 12 days after serum administration, developing low grade fever, skin rashes, facial swelling, glottic oedema, arthralgia, neuritis, as a consequence of serum antibodies and antigen – antibody immune complexes formation. The main line of treatment includes antipyretic, antihistamines and analgesics, and in case of severe reactions, corticosteroids are prescribed.[1]

TOTAL IMMUNOGLOBULINS

They may be administered in cases of immediate or substitutive epidemiologic risk, in cases of certain immunodeficiency diseases. They are sterile solutions containing human immunoglobulins G, covering the entire spectrum of infections and immunizations underwent by the donor individuals. Plasma is collected from tens or hundreds of donors, who must test negative for hepatitis B or C virus or HIV.[32]

In prophylaxis for measles, intramuscular administration is recommended of 0.2-0.4 ml/kg body weight dose, during the first 3-4 days after infectious contact, only to receptive persons below vaccine age or having contraindications related to measles vaccine (hypersensitivity to egg protein, immunodeficiency). Immunity is immediate and lasts for 3-4 weeks. [1,21]

In prophylaxis for hepatitis A virus, intramuscular doses of 0.02-0.06 ml/kg body weight are administered in the first 2 weeks after infectious contact (to family members, institutionalized persons with mental deficiencies). Effective protection is installed 3 to 5 months, in 70-85% of the cases. Total immunoglobulins may also be administered

prior to exposure, in prophylaxis for receptive tourists who intend to travel for more than two weeks in VHA-endemic areas (especially in infants, the elderly, immunosuppressant persons, individuals with chronic liver disease or other chronic comorbidities). Persons with IgA deficiency have contraindication to these immunoglobulins, as there is the risk of developing anaphylactic shock.[32]

Monomeric Ig G immunoglobulins with IV administration are used in substitution therapy in immunodeficient patients (congenital hypogammaglobulinemia, HIV infection), in autoimmune diseases (ITP idiopathic thrombocytopenic purpura) or when treating some serious diseases with intrainfectious immunosuppression (sepsis, meningitis, pneumonia). Dose of 200-400 mg/kg body weight, in low perfusion.[1] Octgam (Octapharma) is such a preparation.

SPECIFIC IMMUNOGLOBULINS

They contain specific human antibodies against a certain microorganism or a certain antigenic determinant. They are free from anaphylactic reactions.

Generally, anti-tetanus (Tetagam P / TETIG – CSL Behring/ TEVA Pharm. Works) and anti-rabies (Berirab - CSL Behring) immunoglobulins are used, the remaining being advised in prophylaxis and treatment for infectious diseases in patients with high risk:

➢ Varicella-zoster virus immunoglobulins are administered to prevent post-exposure disease in immunosuppressant children – see table no. VI;

➢ Cytomegalovirus immunoglobulins are used in the prophylaxis and treatment of the cytomegalovirus disease in recipients of transplant organ (kidney, liver, bone marrow);

➢ Hepatitis B immunoglobulins are recommended post-exposure in new-borns delivered by HBV-infected mothers, as well as in individuals, post mucous/parenteral contact with infectious biological fluids (medical staff, sexual contacts), in association with hepatitis B vaccination. The usual dose of 0.5 ml is intramuscularly administered within the first hours after birth, and of 0.06 ml/kg body weight in adults. The injectable solution ImmunoGam 312 UI/ml is an intramuscular preparation. There are also preparations that are administered in infusions such as Hepatect CP 50UI/ml (BioTest Pharma), available in Romania too. The active and passive immunoprophylaxis combination

prevents in 85-95% of the cases, both acute infection and chronic carriage development via vertical transmission (review chapter on hepatitis B vaccination).

Indications regarding immunoglobulin administration are summarized in table no. VI.

Immunoprophylaxis in immunocompetent persons is primarily performed through vaccination, while immunosuppressant persons mainly receive specific immunoglobulins.[1]

Table no.VI Indications regarding immunoglobulin administration – adapted from [26]

INFECTION	INDICATION	TYPE OF IMMUNOGLOBULIN
Tetanus	Significant exposure Non-immune subject Infection clinically manifested	Specific immunoglobulin
Rabies	Exposure to rabies/to infected animal	Specific immunoglobulin
Cytomegalovirus	Infection treatment and prophylaxis in transplant patients	Specific immunoglobulin
Varicella-zoster virus	Immunosuppressant children (with leukaemia, lymphoma, HIV/AIDS) with no varicella history New-borns whose mothers developed varicella 5 days prior to giving birth or within 2 days following the delivery Premature babies <28 weeks or with birth weight <1000g and hospitalized premature babies, with no varicella history	Specific immunoglobulin
Hepatitis A	Family contact Travelers to developing countries Outbreaks in day-care centres or among institutionalized persons	Total immunoglobulin
Hepatitis B	Percutaneous/mucous exposure to HBV Sexual contact with a person having positive HBV markers New-borns delivered by mother with positive HBV markers Prevent HBV reinfection, after a liver transplant performed following liver failure induced by HBV hepatitis	Specific immunoglobulin

EPIDEMIOLOGICAL INVESTIGATION

DEFINITION

It is a method for studying and researching determinant and contributory epidemiological factors, involved in the emergence and spreading of (non) communicable diseases in the population.

It is a critical method used for intervention in communicable disease outbreaks, and sometimes it is also used for investigation purposes, in diseases where the etiopathogenesis and/or epidemiology are still insufficiently known.

In communicable diseases, the epidemiological investigation is executed both in sporadic cases and in family outbreaks or diverse epidemic scale. The investigation may also be targeting the endemic evolution of a chronic disease, presence or emergence of risk factors, prognosis for the disease normal evolution, update of disease prevention and control programmes, and assessment of the prophylactic measures efficiency. [33]

The final purpose in cases of infectious diseases is to establish and monitor the application of measures for outbreak clearance, as well as of measure in order to protect the community.[34]

CLASSIFICATION

There may be:

> **Operational epidemiological investigations** – for prevention or combating (emergency). The latter is performed by specialized personnel (epidemiologists, infectiologists, microbiologists), in order to identity the epidemiologic process and to stop disease dissemination. It is also the most widely used type of investigation in day-to-day operation.

> **Epidemiological investigations for research purposes** – that are actually different types of studies: descriptive, analytical, retrospective, prospective, longitudinal, transversal, seroepidemiological, eco-social-epidemiological studies or cost-benefit analysis. They may target the incidence, the prevalence, the mortality, the causality, the assessment of contributory risk factors or the assessment of economic effectiveness of prevention and combating actions.[33]

EPIDEMIOLOGICAL INVESTIGATION FOR COMBATING PURPOSES (EMERGENCY)

It is mainly performed in communicable diseases, in order to limit and stop the spreading of the emerging outbreak. There are two steps:
- ➢ Individual (preliminary) epidemiological investigation, and
- ➢ Collective or definitive epidemiological investigation of the outbreak.

INDIVIDUAL EPIDEMIOLOGICAL INVESTIGATION (IEI)

It is primarily intended for the ill, but also for other categories impacted by the outbreak:
- ➢ Suspects;
- ➢ Known germ-carriers;
- ➢ Contacts, many of whom will selected as new cases;
- ➢ Former patients in recovery;
- ➢ Deceased, if the case.

    It is executed by a practitioner or possibly, by people with specific training under the guidance of the practitioner. Pre-existing templates may be used however, a tailored investigation is preferred, free from the rigidity of standard forms and adapted to each and every case specificity.

For a detailed anamnesis (with the help of the ill or next of kin), the following steps must be taken:

1. **Identify the individual through personal data collection** – family name, first name, age, sex, address, occupation, employer;
2. **Define and describe the moment of infection,** namely:
    - ➢ Define the actual (date when first symptoms/indications of the disease appeared) and the seeming onset of the disease (date when the first medical exam was requested);
    - ➢ Define retrospectively the incubation period (minimum to maximum extreme);
    - ➢ During this timeframe, the moment of infection is to be established (sole or multiple) together with its circumstances (when/ where/ how did it happen);
    - ➢ The purpose behind this is to try to identify and neutralize the source of infection which contaminated that person, as well as to stop its dissemination. There may also result inconclusive epidemiological investigations, when this objective cannot be achieved unless further investigations, including laboratory analysis, are performed;

➢ During this stage, multiple data is collected: about the first disease manifestations and its progress, about personal history (the Apgar score, natural nutrition, vaccination history, etc.), about infectious/non-infectious pathological history and family history, about travelling for pleasure or work in endemic/epidemic areas, within the upper limits of the incubation period;

➢ Also, information about living and working conditions, food shopping, food storage, water source status, subject's level of health education (personal hygiene, home hygiene), presence of pets, synanthrope and insect vectors indoor, etc., in order to identify transmission channels.

3. **Select and register contacts** – is an important stage as it allows to take prophylaxis measures in contacts, to limit infection sources and to avoid the extended evolution of the outbreak. Fill in a spreadsheet the following data: family name, first name, age, address, occupation, employer, date/type of infectious contact, applied prophylaxis measures (vaccination, passive immunoprophylaxis, chemoprophylaxis). In order to establish contacts, the following elements are to be taken into account:

➢ Presumptive diagnosis;
➢ Maximum duration of incubation;
➢ Period of contagiousness;
➢ Transmission channels of suspected infectious agent.

Overlooking some contacts leads to extension of the epidemiological outbreak.

4. **Establish a list of contaminated items surrounding the ill** (in order to disrupt the transmission of the pathogen) – during this stage, laboratory investigations come into place. Elements to be taken into account: the period of contagiousness (from the onset until isolation), all possible means to eliminate the germ and its level of resistance to the external environment. Search for contaminated items surrounding the ill both at their home and office /environment frequented by the ill during their contagiousness. Immediate and urgent confinement measures are taken for the ill/suspects (in the hospital or at home, in case of less serious pathologies, when the epidemiological context allows it). The contacts receive post-exposure prophylaxis and are monitored both clinically and through lab investigations. Depending on the pathogen and its transmission channel, various decontamination, disinfection and deratization (DDD) measures shall be applied.

Following drafting of the individual file, upper medical forums shall be notified about the current epidemiological situation and adopted preliminary measures.

EPIDEMIOLOGICAL INVESTIGATION OF THE OUTBREAK (COLLECTIVE/DEFINITIVE)

It starts with the data provided by the individual epidemiological investigation which are then verified and completed by laboratory investigations and by other general information on the outbreak. It ends when the epidemiological outbreak is resolved, and entails the following stages:

1. Collecting general data about the outbreak;
2. Processing of obtained data;
3. Establishing control measures;
4. Applying measures in resolving the outbreak and monitoring their effectiveness.

It is performed by epidemiologists, in collaboration with general practitioners, infectious disease doctors, microbiologists and other specialists, depending on the specificity of each epidemiological process, as well as on how it manifests itself. [33]

1. **Collecting general data about the outbreak** – Data may be collected via anamnesis, interview or epidemiological observations. Data is completed by a doubtless diagnosis of infectious disease and by laboratory investigations. The investigation starts with the clinical and epidemiological anamnesis, without waiting for the lab results, which will later on validate or not the clinical and epidemiological presumption. A pharyngeal or nasal exudate, urine sample, blood sample, other pathological products, water samples, food samples, etc. may be collected, depending on the germ and on the disease progression stage. Therefore, the pathogen may be identified in products coming from the ill/suspect/carrier/contact or in the environment, a significant immunologic answer may be captured by increasing the dynamics of the specific antibody titre, or the pathological values of certain samples indirectly plead for a certain pathology. Establishing the particularities of the epidemiological process entails identifying:
   - The source of infection (human, animal);
   - The transmission channels and possible ways of spreading the etiological agent;
   - Population's receptivity, by knowing data about the population broken down by gender, age, density,

morbidity, mortality, birth rate, travelling of the population, predominant occupations within the impact range. Focus on populations classified in risk groups (extreme ages, different professional categories) or on the existence of closed groups, with high risk of dissemination.

Also monitor secondary factors, favouring the epidemiological process:

  ➢ Natural/environmental factors – by collecting information on the climate, the geographical setting, the weather in the last three months, water sources used by the population, etc.

  ➢ And also, economic and social factors – regarding the economic, social, cultural, and hygiene/health levels, the availability of medical assistance, communications status, food supply, water supply, waste disposal, vector existence, etc.

Also collect data on the evolution of communicable diseases in recent years, in a given area. Thus, if there was an epidemic of measles in the last two or three years, the risk of a new epidemic of the same disease is significantly reduced, as it offers long-term immunity and needs a longer period of time in order to accumulate a new cohort of receptive people. On the other hand, an epidemic of bacillary dysentery in the last two or three years increases the risk of a new current epidemic, because of the high number in *Shigella* excretors among the population.

This data helps to forecast the spreading potential of a current epidemiological process, to identify methods in order to stop its transmission, and to protect the population against an epidemic.

2. **Processing of obtained data** – All information is then systematized, selected and processed as follows:

  ➢ **Establish a timeline of cases breaking out –** under the form of table with former and current patients, chronologically, starting with the actual onset, then stating the family name, surname, age, gender, address, occupation, employer/frequented groups, screening data, diagnosis data, isolation, recovery or death. The table may also contain information on the clinical manifestation of the disease, progress, comments of case specificity. In the same table, but separately, contacts and germ carriers are specified.[34] Based on this table, indexes may be calculated on the effectiveness of medical staff involved

in monitoring infectious diseases: Screening index – as the number of days passed between the illness and the detection, including the first day of illness and detection day; or diagnosis index / isolation index where the computation is similar, but it is related to the detection day. Therefore, this table may underline the infectious moment or the transmission channel of a pathogen.

➢ **Drafting a topographical map of the illness –** on the topographical map of the town/building impacted by the outbreak, all events of illness are marked using fraction circles. The numerator represents the sequence number of the case allotted in the chronological table, while the denominator represents the time of the actual onset of the illness (date and month). E.g.: 1/18.06. Each event is registered next to the domicile or workplace, rarely other criteria are being used. A large concentration of cases in a particular area may lead to the drafting of a possible way of transmission.

➢ **Drafting a graphic representation of the evolution of illnesses –** through linear charts or histograms where on the abscissa, time units are represented (days, weeks, months, years), while on the ordinate, the number of cases are represented. Also via charts, cases are distributed based on multiple criteria (gender, age, occupation, etc.). These representations aim at illustrating the evolution of the outbreak, the existence of common infectious moments and other aspects that are more difficult to notice.

➢ **Drafting a lineage chart –** in order to graphically represent links between illness instances, within the maximum incubation period. The transmission of the illness is followed from a primary case to two or more secondary cases, filiations. Whenever the secondary case passes on the disease, that case becomes the primary one for the subsequent cases. It is then obtained a family tree of illnesses of the outbreak, as shown below:
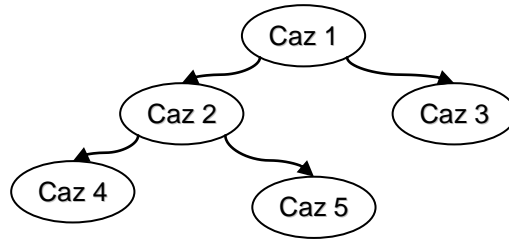
Figure number 1 Example of lineage

3. **Establishing control measures** – based on the epidemiological diagnosis that needs to identify: the pathogen, the source of infection, the ways of transmission, level of sensitivity/resistance in the population, favouring secondary factors, and also to include elements of epidemiological prognosis. The countering plan shall aim to:

   ➢ **Neutralise any infection source** – by early detection of ill patients, by mandatory isolation in the hospital or at home, and by reporting the event via a unique reporting file (see Annex I).[35] Demeanour towards the suspects is identical with demeanour towards the ill, but with separate isolation until the case is confirmed or ruled out. The contacts must be isolated at home or in collectivity (moral isolation), actively monitored – both clinically and via lab investigations, eventually being temporarily suspended from a workplace presenting epidemiological risk. As post-contact prophylaxis, active (vaccinations) or passive (serums/immunoglobulin administration) measures or chemoprophylaxis are being used. Measures to be used in germ carriers include active detection, regular medical check-up – clinical and lab, applying sterilisation treatment, temporary or permanent exclusion from risk sectors (central water distribution network/public food service/collectivities with children). In particular instances, extrahuman sources of germs must also be neutralised.

   ➢ **Disrupt the transmission in the outbreak** – actual and terminal decontamination has been in practice, together with destruction of germs from the patient's excretions, as well as environmental germs (air, items, surfaces, water, foods). In particular cases, one may use disinfection, in order to destroy any insects acting as disease vectors (flies, fleas, lice, mosquitos, ticks, cockroaches) or use deratization, in order to destroy rondents. It may come to

closing of public food service places, to setting quarantines in communities or hospital facilities. Plus, sanitary education should be focused on amongst people from the outbreak, in order to observe individual and collective hygiene rules. A theoretical training should be delivered on ways of disease transmission, onset symptomatology, and prevention measures, and recommendations should be made on adopting anti-epidemic demeanours and attitudes.

- ➢ **Lower the population's sensitivity** – by applying non-specific measures (increase in the overall resistance via an adequate diet, rich in vitamins, avoid infectious contacts, avoid physical and psychological overload, obey resting hours, individual/collective hygiene rules) or specific measure (active/passive immunoprophylaxis, chemoprophylaxis).
- ➢ **Eliminate negative influences exerted by secondary factors**.

All measures should be effective and include direct responsibilities and deadlines.

4. **Applying measures in resolving the outbreak and monitoring their effectiveness** - after the outbreak liquidation plan is drawn up, the correct application and efficiency of the planned measures are monitored. The duration of the outbreak supervision, the date and the person declaring the extinction of the outbreak and the end of the process of analysing the measures taken are established. The absence of new cases, after a period that exceeds the maximum incubation of the disease, starting from the date of isolation of the last case from the outbreak, certifies the correctness of the implementation and application of the control plan.

An outbreak can be declared extinguished if there are no germ carriers in the affected area and no new cases have emerged.

The chronological table of the diseases, the topographic representation, the lineage of the cases, charts regarding the evolution of the outbreak, the list of contacts, the results of the laboratory investigations and any other useful information regarding the respective outbreak are attached to the final file of the investigation.

DECONTAMINATION/STERILISATION

DEFINITIONS

Cleaning is a preliminary method of decontamination, which ensures the removal of microorganisms from surfaces and objects, with the removal of dust and organic substances.

Decontamination is the process by which 90 to 99.9% of the microorganisms existing on inert objects (except for bacterial spores) are destroyed. The old term "disinfection", still widely used, is considered unfit for inanimate carriers that cannot be infected.

Antisepsis it is the process by which most of the germs stationed on living tissues (whole or damaged skin/mucosa) are temporarily destroyed.

Biocide is a broad spectrum chemical agent that inactivates microorganisms on both living tissues and inert items. The terms that have the suffix "-cide" mean an action of destruction (e.g.: a germicide is a substance that kills microorganisms). The term of germicide includes both antiseptics and decontaminants.[36]

Virucides, fungicides, bactericides, sporicides and tuberculocides are biocides that kill the type of microorganism identified by their prefix.

Antiseptics are germicides that are applied to living tissues and skin, while **decontaminants** are applied only to lifeless objects, being harmful to the skin or tissues.

Sterilisation is a method by which all microorganisms, both vegetative and sporulated, are eliminated/destroyed.

DECONTAMINATION BY MEANS OF MECHANICAL TREATMENT – CLEANING

Sanitation begins with the cleaning stage. Deposits of organic matter (oils, fats, carbohydrates, proteins) represent not only places of accumulation and development of germs, but at the same time hinder the physical contact between the decontaminant and the surface to be decontaminated/sterilized. For effective cleaning, it is useful to know the type of substance to be removed, as it plays an important role when effectively choosing the cleaning agent to be used.

In the cleaning process, the substances must first be solubilized or suspended in solution and then removed. Thus, the sugar and some of the

inorganic substances can be dissolved and removed with water. Also, most food particles can be suspended and removed with water jets. Warm water is used, with a temperature between 35 and 45°C, temperature at which it has a higher emulsifying and dissolving power. Above 55°C, proteins coagulate and adhere to the support item. Substances that are not soluble in water will remain on surfaces in the form of thin biofilms or deposits.[37] Thus surfaces containing oils and fats will require highly emulsifying cleaning agents (detergents, soaps) and those containing proteins will require chlorinating or strongly alkaline agents (e.g.: sodium hypochlorite).

The following factors also play an important role in the cleaning process: temperature, water hardness, pH, contact time of the cleaning agent with the surface.

The main techniques used in this stage are:

1. Washing with water - Warm water, detergent or soap solutions are used, depending on the nature of the substances that are to be removed. It can also be associated with some mechanical processes (dusting, scrubbing). It is important to observe the times of soaking, washing and rinsing.

2. Wiping with wet cloths – Using detergent/decontaminant cloths.

3. Vacuuming – In the hospital environment, only wet vacuuming is suitable.

4. Other techniques - ventilation, wet sweeping, brushing, dusting (the latest not being suitable in busy or crowded places).

Cleaning is done manually or automatically. Manually, brushing or scrubbing is done, followed by flushing with water, with or without pressure. Mechanical or automatic cleaning is performed using ultrasonic devices, decontamination machines.

In the case of ultrasonic cleaning, by propagating the ultrasounds in the aqueous solution, it is determined the rupture of the links that hold the particles attached to the surface.

For cleaning instruments, detergent solutions with approximately neutral pH value of 7 are used because they do not attack metals or other materials in the composition of medical instruments (e.g.: flexible endoscopes). [37]

The materials used are:

> ➢ soaps;
> ➢ cationic detergents;
> ➢ anionic detergents;
> ➢ neutral detergents.

Detergents are synthetic organic chemicals that have a hydrophilic component and a hydrophobic component, acting as a surfactant and as emulsifying agent of fat in aqueous solutions.

The hydrophobic nature, usually a long carbon chain of alkyl or alkyl aryl type, produces hydrophobic interactions (Van der Waals) with oil or fat particles from the surfaces to be cleaned. The hydrophilic nature, charged electrically or with polar groups, produces polar interactions with water molecules. When shaken, the detergent-linked fat particles detach from the surface to be cleaned and pass into the solution as emulsion, which can be easily removed. Detergents can be foaming, emulsifying, dispersing or dispersion stabilising agents. One of their important actions is the destruction of lipophilic membranes of microorganisms, action in which simultaneously enzymes located in membranes are denatured and inactivated. Also, ionic detergents attach to electrically charged proteins, denaturing them.[38]

These two combined actions of destroying and denaturing cell and protein membranes underlie the germicidal action of detergents. This is secondary to the main action of cleaning.

In order to increase the cleaning capacity of detergents, additives are added in the composition, such as:
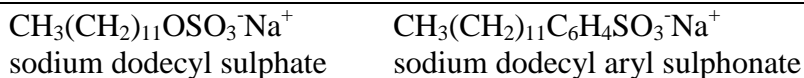
> carboxymethylcellulose - forms a protective layer on the cleaned surfaces, preventing dirt redeposits;
> pyrophosphates, tripolyphosphates, silicates - used in the case of hard water, because they form soluble salts with Ca^{2+} and Mg^{2+}, thus preventing the precipitation and foaming processes;
> abrasives – for surface smoothing;
> foaming agents – for foam generation and stabilisation;
> oxidising agents – for whitening (for instance perborates);
> enzymes (peptides, amylases, lipases) - for the hydrolysis of proteins, carbohydrates and fats;
> pH modifying agents - to generate a pH suitable for the removal of a particular type of substance;
> odour neutralising agents.

One of the most important properties of detergents is their biodegradability, on a mandatory basis promoting biodegradable detergents as close as 100%.[39]

Anionic detergents - are Na or K salts of alkyl or alkyl sulphonic acids where the hydrophilic component is negatively charged $–OSO_3^-$ (sulphate) or $-SO_3^-$ (sulphonate). Unlike soaps, detergents in this class can

be used in hard water acidic solution because calcium and magnesium sulphonates are water soluble.
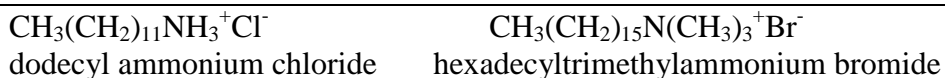
The most commonly used anionic detergents are:

$CH_3(CH_2)_{11}OSO_3^-Na^+$	$CH_3(CH_2)_{11}C_6H_4SO_3^-Na^+$
sodium dodecyl sulphate	sodium dodecyl aryl sulphonate

They act as an emulsifying agent, by engaging superficial fats/cells and colonising bacteria on the surfaces to be cleaned, and by eliminating them along with the foam. Protein detritus and acid pH reduce their effectiveness. They are bactericides just like *Staphylococcus* spp. and *Streptococcus pneumoniae.* They are used for cleaning of floors, toilets and glassware.

Cationic detergents - are quaternary ammonium salts where the hydrophilic component is positively charged being represented by the ammonium ion $-NH_3^+$, $-N(CH_3)_3^+$.

The most commonly used cationic detergents are:

$CH_3(CH_2)_{11}NH_3^+Cl^-$	$CH_3(CH_2)_{15}N(CH_3)_3^+Br^-$
dodecyl ammonium chloride	hexadecyltrimethylammonium bromide

In addition to the emulsifying action, it also has an important germicidal action - bactericidal, fungicidal and partially virucidal. The spectrum of action is selective, mainly on the gram-positive germs and less on the gram-negative ones. The presence of organic substances does not affect their effectiveness, however an anionic detergent inhibits it.

Non-ionic (neutral) detergents - are ethers or esters of higher fatty acids or alkyl acids, containing one or more OH groups as hydrophilic components, wherein the hydrophilic component has no electrical charge and therefore the detergent action of these compounds is independent of the pH of the solution or the presence of other ions. For instance:

$CH_3(CH_2)_{14}COOCH_2C(CH_2OH)_3$
pentaerythrityl palmitate

They are used for cleaning of floors, furniture and tableware.

Important to note:
- ➢ The cleaning products are not to be mixed and should be distributed to different departments in their original packaging, label included;

- Products are stored in central spaces with easy to clean floors/walls, with natural ventilation, optimum humidity, in neat conditions;
- The chief nurse together with CPCIN staff establish, monitor and verify the decontamination schedule of the department.[40,41]

DECONTAMINATION BY MEANS OF PHYSICAL TREATMENT

I. Dry heat:
- **Flaming** of the bacterial culture loop. It does **NOT** apply to medical and surgical instruments, needles, etc.;
- **Incineration** – for wastes, pieces of anatomy, laboratory animal carcass.

II. Humid heat:
- **Pasteurisation** of liquids at temperatures between 55-95°C - 90-95% of the pathogenic microorganisms are destroyed;
- **Washing** at 60-95°C – for linen, tableware and laboratory glassware;
- **Boiling** using 100°C water, and destroying vegetative forms in 10-20 min., as well as less resistant sporulated forms. This treatment is applied for food, water, linen, cutlery and tableware;
- **Steam ironing** of linen and clothes – destroys vegetative forms in 5-10 seconds, while spores are destroyed in 50 seconds.

III. UV radiations:
- The wavelength range of UV radiation is between 210 and 328 nm and the maximum bactericidal effect is between 240 and 280 nm. Mercury-vapour UV lamps emit radiations having λ = 253.7 nm, which is within the optimum bactericidal range. This effect is due to DNA destruction following the formation of thymine dimers;
- They are used in the decontamination of drinking water, titanium implants, and contact lenses. Also, UV lamps are used for the decontamination of air micro flora or smooth surfaces in laboratories, operating rooms, insulators, sterile rooms (through direct or indirect radiation lamps);

> Gamma and ultrasound radiations are useful in industrial disinfection/sterilisation of medicines and foods.

DECONTAMINATION BY MEANS OF CHEMICAL TREATMENT

The decontamination process must create conditions that prevent the survival and multiplication of bacteria. Bacteria multiply very quickly and at the same time adapt (become resistant) to the action of some chemicals. Thus, the bacteria that survives a chemical agent will quickly give birth to new generations resistant to the action of that chemical agent, which in time will make the chemical agent inefficient in that area. For this reason, the efficiency of bactericidal impregnation methods of clothing or surfaces in medical facilities is questionable.[39]

The factors that influence chemical decontamination are:
  - ➢ The disinfectant's spectrum and germicidal power;
  - ➢ Initial number of bacteria on the treated surface;
  - ➢ Amount of organic material on the surface (biofilm existence);
  - ➢ The nature of support item;
  - ➢ Concentration of the decontaminant;
  - ➢ Contact time, temperature;
  - ➢ pH
    - can have optimal activity at acidic pH (in the case of phenols, halogens);
    - can have optimal activity at alkaline pH (in the case of glutaraldehyde, quaternary ammonium salts);
    - the activity is optimal at neutral pH (in the case of chlorhexidine);
  - ➢ The product stability over time (sodium hypochlorite is unstable);
  - ➢ Corrosivity – hypochlorites corrode metals;
  - ➢ Toxicity - formaldehyde and glutaraldehyde are toxic.[40]

The chemical decontaminant is chosen according to the following criteria:
  - ➢ To be effective, with high bactericidal capacity;
  - ➢ Not to be neutralised by protein detritus;
  - ➢ To result in stables mixtures;
  - ➢ Easy to prepare, apply, store/transport;
  - ➢ Not to be corrosive and not to cause destructive effects;
  - ➢ As little toxic as possible under in-use conditions;
  - ➢ Not to have persistent odour;
  - ➢ To be biodegradable. [40]

Table no. VII. The classification of decontamination by means of chemical treatment– adapted from [40]

Level of decontamination	Properties
High level decontamination	- Destroys all vegetative forms and a number of bacterial spores up to 10^{-4}; - Contact time - 20 min. – 1h; - glutaraldehyde 2%, hydrogen peroxide 6%, peracetic acid, sodium hypochlorite 5,25%.
Medium level decontamination	- Destroys *M. tuberculosis*, bacterial vegetative forms, most viruses and fungi, except bacterial spores; - Contact time – at least 10 min.; - Phenols, iodophores, alcohols, chlorine generators.
Low level decontamination	- Destroys most bacterial vegetative forms, some viruses, fungi, with the exception of bacterial spores, mycobacteria, mould and viruses without envelopes; - Contact time - under 10 min.; - Phenols, iodophores, alcohols, sodium hypochlorite 5,25%.

Table no. VIII. The classification or medical instruments in terms of the type of decontamination required – adapted from [40,42]

Type of medical equipment	Method of decontamination/sterilisation
Critical medical equipment	It has a high risk of infectivity. These instruments must be perfectly sterile as they enter into sterile tissues and into the vascular system. This category includes: surgical instruments, cardiac and urinary catheters, implants and probes used in the sterile cavities of the body, etc. Reuse of these instruments requires **decontamination followed by sterilisation.** In the case of temperature sensitive objects, it is recommended to use ethylene oxide sterilisation, hydrogen peroxide gas plasma sterilisation, or sterilising chemical agents such as: solutions with $\geq 2.4\%$ glutaraldehyde, solutions with 0.95% glutaraldehyde and 1.64% phenol, 7.5% hydrogen peroxide, 7.35% hydrogen peroxide with 0.23% peracetic acid, 0.2%, peracetic acid, 0.08% peracetic acid with 1.0% hydrogen peroxide.
Semi-critical medical equipment	It includes instruments that come into contact with the mucous membranes (except for the periodontal mucosa) or with skin continuity solutions - endoscopes, laryngoscopes, cystoscopes, endotracheal tubes, anaesthesia/assisted breathing equipment. These devices should not contain microorganisms, only a small number of bacterial spores being acceptable. This type of instruments requires **chemical sterilisation or at least high level decontamination**, using chemical disinfectants such as: glutaraldehyde, hydrogen peroxide, ortho-phthalaldehyde, peracetic acid with hydrogen peroxide. Oral / rectal thermometers, hydrotherapy baths require **medium level decontamination**.

| Non-critical medical equipment and environment | It includes devices that come in contact with intact skin, which is an effective barrier for most microorganisms.

They fall into two categories:
  ➢ items used in patient caring– stethoscopes, bedpans,
  ➢ surfaces – floors, headboard, handrails, furniture, etc.
The type of decontamination used: **medium or low level decontamination**. The advantage of this type of items is that they can be decontaminated on the spot, using the following disinfectants: 70-90% ethyl or isopropyl alcohol, sodium hypochlorite (5.25-6.15%) diluted 1:500, phenol detergent solution, iodophor detergent solution, detergent solution with quaternary ammonium salts.

These disinfectants are effective in destroying vegetative forms of bacteria (e.g.: *Listeria*, *Escherichia coli*, *Salmonella*, vancomycin-resistant enterococci, *Staphylococcus aureus* methicillin-resistant), fungi (e.g.: *Candida*), mycobacteria (e.g.: *Mycobacterium tuberculosis*) and viruses (e.g.: poliovirus), at an exposure time of 30–60 seconds.
In the case of decontamination of surfaces using mops or wipes, if they are not cleaned properly and the decontaminant solution is not changed in time (after a maximum of 3-4 rooms or every 60 minutes), then this process can lead to a strong microbial contamination throughout the entire medical unit. |

Table no. IX The classification of the hospital environment in terms of contamination risk and decontamination method used – adapted from [40]

Risk	Decontamination method
Minimum risk	- walls, floors, ceilings, sinks, drains, bed fittings, cabinets, - CLEANING + low, medium or high level DECONTAMINATION, if biological products are present.
Low risk	- Items that come in contact with intact skin – stethoscopes, blood pressure cuff, dishware, other sanitary items - CLEANING + low, medium or high DECONTAMINATION if biological products are present.
Medium risk	- Items that come in contact with mucous membranes – thermometer, endoscopes, assisted-breathing equipment - Contaminated items - Items used in immunosuppressant patients - High level DECONTAMINATION, CHEMICAL STERILISATION
High risk	- Items that come in contact with skin/mucosal continuity solutions, or that are introduced in sterile areas – needles, catheters, surgical equipment, implants - STERILISATION

RULES OF PRACTICE FOR THE DECONTAMINATION:

- ➢ It does not replace cleaning or sterilisation!
- ➢ **Decontamination comes before cleaning** in the outbreak or when organic matter is present;
- ➢ The alternation of substances is recommended in order to prevent resistance;
- ➢ Effective concentration and action time must be complied with;
- ➢ Fresh solutions, within the validity period, are used;
- ➢ Chemical and microbiological control of the disinfectant solutions used is randomly performed;
- ➢ Labour protection rules must be complied with.[40]

There may be:

- ➢ **Prophylactic** decontamination
- ➢ **Outbreak** decontamination (when only high level decontaminants are used), with two entities: current and terminal.

Prophylactic decontamination aims to prevent the occurrence and spread of communicable diseases in the population (decontamination of drinking water, residues, public transport, etc.)

Decontamination in the outbreak, the **current** form - is done in the space where the confirmed or suspected case of communicable disease is present, during the whole period of contagiousness, as well as around the contacts and carriers, during the germ elimination period. The process concerns the pathological biological products eliminated by the patient or carrier, as well as the environment in the patient's room, including all items in the related space.

Decontamination in the outbreak, the **terminal** form – is done one time only, after the patient or the carrier has been removed from the outbreak.

It is used:

- ➢ in the sections of infectious and contagious diseases, according to the cases of nominally declared disease;
- ➢ in outbreaks of nosocomial infections/with multi-resistant germs;
- ➢ in immunosuppressed medical departments (neonatology, haematology, oncology, transplant, burn ward);
- ➢ in the operating blocks, birth rooms, ICU wards;
- ➢ emergency services, ambulances, etc.

Microorganisms that require terminal decontamination are:

- ➢ *Mycobacterium tuberculosis;*
- ➢ enterobacteriaceae or non-fermentative germs secreting β-lactamase;
- ➢ *Staphylococcus aureus* methicillin-resistant;
- ➢ Group A β-hemolytic streptococci;
- ➢ Hepatitis viruses, polio viruses, etc.

The main types of chemical disinfectants include: alcohols, chlorine and chlorinated compounds, aldehydes (formaldehyde, glutaraldehyde, *ortho*-phthalaldehyde), hydrogen peroxide, iodophors, peracetic acid, phenols, quaternary ammonium salts. [38] Most of them are used alone, but also in combination (e.g.: hydrogen peroxide and peracetic acid mixture). Personnel working with decontaminants, especially chlorine, formaldehyde, glutaraldehyde, should minimize contact time and use protective equipment (gloves, masks, ventilation).

1. ALCOHOLS

The alcohols used are: ethyl alcohol and isopropyl alcohol. Optimal bactericidal activity occurs at concentrations of 60%-90% solutions in water (volume/volume) and is abruptly reduced when concentrations fall below 50%. Bactericidal action is achieved by protein denaturation (dehydration with loss of conformation and aggregation). They have bactericidal, tuberculocidal, fungicidal, virucidal action. *Pseudomonas aeruginosa*, *Serratia marcescens, E. coli, Salmonella spp.*, *Staphylococcus aureus* and *Streptococcus pyogenes* are destroyed in 10 seconds. Ethyl alcohol inactivates all lipophilic viruses (herpes, vaccinia, influenza virus), as well as many hydrophilic viruses (adenovirus, enterovirus, rhinovirus, echovirus, astrovirus, HBV, HIV, rotavirus, except VHA or poliovirus).[32]

As disadvantages - they are flammable, evaporate easily, destroy rubber or other plastics.

They are used in decontamination of surfaces, instruments (oral, rectal thermometers, stethoscopes, laryngoscopes), skin antisepsis (of hands, inoculation sites of parenteral treatment).

2. SUBSTANCES RELEASING ACTIVE CHLORINE (HYPOCLORIC ACID)

The main compound that releases active chlorine is the hypochlorite. Other compounds are chlorine dioxide (freshly prepared by mixing two components), sodium dichloroisocyanurate, chloramine-T. The advantage of these compounds over hypochlorite is linked to a greater stability and a stronger bactericidal effect. They are cheap, effective, at low concentrations - they are non-toxic, have a wide range of use and act quickly.

Disadvantages include:
- Production of eye / oropharyngeal irritation;
- Corrosion for metals;

- ➢ Inactivation by organic detritus;
- ➢ Fades or bleaches the items;
- ➢ Relatively low stability.

Sodium hypochlorite - NaOCl with 12.5 % active Cl (hypochlorite)

Hypochlorite is the most widely used chlorine-based decontaminant, being available in liquid (sodium hypochlorite) or solid (calcium hypochlorite) form. The most common commercial product is sodium hypochlorite in aqueous solution of concentration of 5.25% - 6.15%, being also a household product (chlorine bleaching product). The compound is cheap, does not produce toxic residues, destroys microorganisms fixed on surfaces too, has a broad spectrum antimicrobial action (bactericidal, virucidal, fungicidal, tuberculocidal effect). The bactericidal action is due to the formation of hypochlorous acid (HOCl). Disadvantages include - produces eye, oropharyngeal and oesophageal irritation, gastric burns, has low stability, corrodes metals, fades the colour of items, releases free chlorine (toxic gas) in contact with an acid (HCl) or ammonia (NH_3). At acidic pH the solutions are unstable, with toxic effects at a concentration of 5% and corrosive at a concentration of 10%. It is used for the decontamination of surfaces - in concentration of 4%, of linen - 2%, of tableware - 0.5-1%, of glassware 10%.

Calcium hypochlorite - Ca(OCl)$_2$ with 25% active Cl (hypochlorite)

It is also known as chloride of lime (chlorinated lime). It has bactericidal, virucidal, and sporicidal action. Storage in sealed, dark-coloured containers. It is slightly irritating, and when below 15% concentration, it is no longer used. It decontaminates:
- ➢ surfaces – walls, baths, - at 20 g‰ concentration;
- ➢ bed linen, protection equipment - 40 g‰;
- ➢ waste collection containers – 50-100 g‰
- ➢ and also, it is used for treatment of contagious patients' faeces– 200-400 g/kg.

Chloramine-B (N-Chlorobenzenesulfonamide sodium salt) and Chloramine-T (N-Chlorotosylamide sodium salt) with 25-29% active Cl

They have bactericidal, virucidal, fungicidal action, and at high concentrations, tuberculocidal action too. They are used for decontamination of walls, floors in concentration of 2 g%; of plastic objects 1-2 g%; of bed linen; of protection equipment - 1-2 g%, for 1-2 h; of tableware - 0.5-1 g%, for 30-60 min.; of thermometers.

3. ALDEHYDEs
FORMALDEHYDE - CH_2O (37% formaldehyde solution is called FORMOL)

It is used as a decontaminant in both gaseous and liquid form. It has a wide range of bactericidal, fungicidal, virucidal, sporicidal, and tuberculocidal action. The effect is achieved by blocking the amino and sulfhydryl groups of the proteins, as well as the purine cycle of nucleic acids. The presence of organic material does not reduce the efficiency of the agent. It is of limited use, being a toxic agent, potentially mutagen, teratogen. It is used for decontamination of surfaces, linen, excretions and for the disinfection of rooms using formol (in areas with bacillary load with B.K.), but is not used in patient rooms, in the paediatric and neonatal wards, food offices. It is used by spraying technique using special devices, after sealing the rooms, 2-5%, during the exposure time 6-24 h, or by vaporizing technique, after lifting the mattresses/cushions, sealing the rooms, in a concentration of 3-10 g/m3. After 24 hours, ammonia neutralisation is performed (½ of the amount of formol used) for 3 hours and then ventilation for 2-4 hours. To decontaminate the linen, add 2% to the soaking solution.

GLUTARALDEHYDE - $CH_2(CH_2CHO)_2$ (Glutaric dialdehyde)

It is a high-level decontaminant or chemical sterilizer. The aqueous glutaraldehyde solution is acidic and does not destroy the spores. Activation is done using alkalizing agents, at pH value of 7.5-8.5 the glutaraldehyde solution becomes sporicidal. After activation, the solution can be used for about 14 days, after which it is inactivated as a result of a polymerization process. Newer formulations (glutaraldehyde-phenol-sodium oxide) stabilise glutaraldehyde for one month. It has broad spectrum bactericidal, fungicidal, tuberculocidal, virucidal, slow sporicidal (3h) action, so it is used as a high-level disinfectant or chemical sterilizer. Glutaraldehyde aqueous solution $\geq$2%, tapped at pH 7.5-8.5 with sodium bicarbonate, effectively destroys bacterial vegetative forms in less than 2 minutes, *M. tuberculosis*, fungi and viruses in less than 10 minutes, *Bacillus* spp. and *Clostridium* spp. spores in 3 hours. Micromycetes, however, are resistant to glutaraldehyde action.

The 1-1.5% concentration represents the minimum level for effective concentration. It is non-corrosive, retains its activity in the presence of protein detritus and is relatively inexpensive. It has irritating effects on skin, ocular, respiratory mucosal and possible teratogenic / mutagenic effects. It is useful in decontamination of heat-sensitive medical equipment (endoscopes, spirometers, anaesthesia/intensive care equipment, dialysis machines, laparoscopic trocars).

ORTHO-PHTHALALDEHYDE (OPA)

It is a high-level decontaminant that received clearance in 1999. It contains 0.55% 1,2-benzenedarboxaldehyde (OPA), being a clear, slightly bluish solution with 7.5 pH. It has a stronger germicidal action than glutaraldehyde, destroying glutaraldehyde-resistant mycobacteria as well as *B. atrophaeus* spores. After 2004, it began to replace glutaraldehyde in chemical sterilisation processes, because it is stable in the 3-9 pH range, does not irritate the eyes and nasal pathways, has a shorter action time. The main disadvantage is due to the colouring of proteins in grey, including the colouring of unprotected skin.

4. HYDROGEN PEROXIDE (OXYGENTATED WATER)

Hydrogen peroxide, in concentrations of 6% -25%, is considered a chemical sterilising agent. Its germicidal action is carried out by means of the hydroxyl radical, which attacks the unsaturated fatty acids in the composition of cell membranes, the nitrogenous bases in nucleic acids, and the proteins. A 7% stabilised hydrogen peroxide solution (0,85% phosphoric acid, to maintain a low pH) is sporicidal (within 6 hours of exposure), mycobactericide (20 minutes), fungicide (5 minutes), virucide (5 minutes) and bactericidal (3 minutes). Synergistic sporocidal effects are observed in combinations of hydrogen peroxide (5.9% -23.6%) and peracetic acid. It is marketed as a 3% antiseptic solution, but high-level decontamination requires a 7.5% solution for a 30-minute exposure at 20°C. It does not generate residues, odours or irritations, it does not coagulate the blood, it does not require activation, but by contact it may produce eye damage and it is not compatible with some materials (zinc, copper, nickel, silver).

5. IODOPHORS

An iodophor is a combination of iodine and a solubilizing agent or carrier. The best known iodophor is the iodine-povidone combination, where povidone (polyvinylpyrrolidone - a water soluble polymer) is the carrier. The combination has the germicidal activity of iodine, but it does not colour and has a low toxicity.

They have bactericidal, virucidal, tuberculocidal, weak sporicidal or fungicidal activity. They are used for the antisepsis of the hands, the teguments, of the operating block in concentrations of 7.5 and 10%. Betadine is a commercial product based on iodine-povidone (100 mg/ml). Iodine can cause skin, eye, gastric, irritations and allergies.

6. PERACETIC ACID - $CH_3C(O)OOH$ (PEROXYACETIC ACID)

It is a chemical sterilising agent with rapid action against all types of microorganisms. It does not produce toxic compounds upon decomposition, it is also active in the presence of organic matter, it acts against spores and at low temperatures (50-55ºC), it does not coagulate blood and does not leave residues. It is compatible with most materials and instruments. However, it corrodes copper, bronze, steel, galvanic sheet, it can only be used for immersive instruments, it is relatively expensive, it can damage the eyes or the skin. Stability decreases with increasing dilution. Automatic machines are used for chemical sterilisation of medical instruments, which use 0.2% peracetic acid, at 50ºC (for endoscopes, arthroscopes, surgical or dental instruments).

7. PHENOLIC DERIVATIVES

Phenol was the first germicidal agent used for decontamination in hospitals by J. Lister, in 1867. Subsequently, for the improvement of antiseptic properties, numerous phenol derivatives were synthesized by replacing a hydrogen atom in the aromatic ring with various radicals (alkyl, phenyl, benzyl, halogen). Two of these derivatives are commonly used as decontaminants in hospitals: ortho-phenylphenol and ortho-benzyl-para-chlorophenol (chlorophen).
They are stable in solution, inexpensive, have bactericidal (including for *Pseudomonas aeruginosa*), fungicide, tuberculocide, weak virucide (or null) but not sporocidal action. They are used only for the decontamination of the hospital environment - air, surfaces and sometimes for the anatomical-pathological equipment. They are corrosive to Al, Cu, Zn, they are not effective for parenterally transmitted viruses, they have caustic effects on the skin, eyes, respiratory or gastric mucosa, moderately toxic to CNS. Not used in food offices, patient rooms, in paediatric and neonatal wards, and for equipment that comes into contact with the mucous membranes.

Vesphene 1% (2-phenylphenol + p-thirds amylphenol + alkaline bases) destroys M. *tuberculosis* in 10 min. It is used for decontamination of surfaces (tables, beds, laboratory surfaces or non-critical medical objects), by wiping, spraying, in a concentration of 0.4% or for decontamination of the air, in a concentration of 10%.

8. QUATERNARY AMMONIUM COMPOUNDS

Chemically, they are organically substituted quaternary ammonium compounds, in which the nitrogen atom is bonded by 4 covalent radicals (R 1 -R 4) alkyl or heterocycles forming the ammonium ion. It binds ionically to an anion resulting in a quaternary ammonium

salt. The names of such compounds used as disinfectants are alkyl dimethyl benzyl ammonium chloride, alkyl dodecyl dimethyl ammonium chloride, dialkyl dimethyl ammonium chloride.

They have fungicidal, bactericidal, virucidal action against lipophilic viruses, but they have no sporicidal, tuberculocidal action against hydrophilic viruses. They are used for decontamination of non-critical surfaces such as floors, furniture, walls, as well as for decontamination of medical equipment that comes in contact with intact skin (blood pressure cuffs).

9. BIGUANIDE CHLORHEXIDINE

It has bactericidal (higher on gram-positive germs), fungicide, partially virucide, but not tuberculocide or sporicide action. The solutions are prepared with sterile distilled water or alcohol (not to be contaminated with *Pseudomonas aeruginosa*). It is used in pre- and post-operative antisepsis of the skin, at concentrations of 0.5-1% or of wounds. May cause eye irritation, contact dermatitis.

HEXACHLOROPHENE

It is rarely used, in nosocomial epidemics with *Staphylococcus aureus,* for hands antisepsis.

10. ANTISEPTICS

They are not sterilising agents but temporarily reduce the microorganisms on the skin and mucous membranes. In order to prevent their contamination, the date of first opening and the validity period by which they can be used (according to the manufacturer's instructions) are marked on the container, then the container is closed after each handling and the partially empty bottle is not to be filled with a new quantity of product. It is also forbidden to touch the mouth of the bottle; antiseptics should be used as such (not in solution); delivered in small vials. [40] Examples of antiseptics:

➢ Chlorhexidine gluconate 0,05-0,5%;
➢ Phenosept;
➢ Spitaderm;
➢ Cutisan;
➢ Betadine dermic;
➢ Tincture of iodine 5%;
➢ Hydrogen peroxide 3%.

As the hands of the medical staff are the most important germ vector in the nosocomial environment, the techniques established by WHO for hand washing and antisepsis are presented in Annexes V and VI.[43]

Sterilisation is a process that destroys or eliminates all forms of microbial life and is carried out in the sanitary units by means of physical methods (pressure steam sterilisation, dry heat sterilisation, filtration, irradiation) or chemical methods (ethylene oxide sterilisation, hydrogen peroxide gas plasma (with sterilising liquids). [39] The theoretical probability of the persistence of microorganisms must be lower than 10^{-6}.

General rules applied in sterilisation:
- ➢ All medical units must guarantee the sterility of the medical devices purchased from the market or sterilised in the hospital;
- ➢ All surgical instruments, textiles, items, solutions that penetrate into the sterile tissues or vascular system must be sterile;
- ➢ Disposable devices and materials will never be re-sterilised;
- ➢ The functional circuits of the sterile/non-sterile medical instruments must be observed;
- ➢ A prerequisite for the efficiency of the sterilisation method is the prior cleaning of the objects. Prior to sterilisation, the medical equipment is subjected to a thorough cleaning by soaking for 8-15 min. in cold water or with added disinfectant (e.g.: Ampholysine plus, Amphosept BV, Instruzyme, Sekulyse), then washed and rinsed;
- ➢ In the arrangement of medical kits and packages, avoid pressing on soft materials, avoid also degradation and possible contamination of the kit (maximum permissible eight is 5 kg).

I. Physical Methods of Sterilisation

A. Dry heat method – hot air ovens (Pupinel)
The method is used only if the materials subject to sterilisation can be damaged by wet heat (powders, oils, sharp instruments).

High heat in the presence of air, initiates processes of oxidation of cellular components, processes doubled by rapid and extended denaturation (loss of molecular conformation), especially proteins.

Dry heat sterilisation is non-toxic, does not affect the environment, is inexpensive, the used sterilisers (ovens) can be easily installed and are not corrosive to metal and sharp instruments. Disadvantages include lower and slower penetration; high temperatures are not suitable for all materials, and the long time required for microbial destruction makes it uneconomical.

The following temperature/time ratios are recommended for effective sterilisation:

> 180ºC/60 minutes,
> 160ºC/120 minutes
> respectively, 150ºC/150 minutes.

There are two types of procedures used:

> static - where the air, heated by the electrical resistances from the base of the steriliser, will rise by convection at the top of the chamber;
> forced-air circulation - there is also a fan that pushes the hot air at high speed through the steriliser chamber, allowing a faster transfer of energy from the air to the instrument.

The effectiveness of the sterilisation is assessed:

1. on each cycle – by the reached temperature and by the colour transfer of adhesive strips,
2. after one month – by performing biological tests
3. and after six months, by inspecting the steriliser.

In order to check the sterilisation process, *Bacillus atrophaeus* spores are used. This procedure is done for sterilising laboratory glassware, dental equipment, talc, paraffin, but not on aqueous solutions, items made out of rubber or fabric.

B. High-pressure steam - Autoclave

The method involves exposing the materials to contact with high-pressure steam (wet heat), being considered the most effective method of sterilisation.

An autoclave is used to sterilise surgical equipment, soft material (to 3 atm., 134ºC, 10-30 min.), rubber (to 2 atm., 121ºC, 30 min.), to decontaminate wastes/sterilise laboratory environment, infusion instruments. It destroys microorganisms by irreversible coagulation, denaturation of enzymes and structural proteins. Proper treatment by autoclaving will inactivate all microorganisms such as bacteria, fungi, viruses, as well as bacterial spores. In order to inactivate prions, the autoclaving duration must be doubled. For effective sterilisation, it is necessary to respect the sterilisation times:

> for pre-treatment (vacuuming) and pre-heating stage;
> for sterilisation stage;
> for post-treatment (post-vacuuming) stage – where humidity excess is eliminated, so that sterilised fabrics can have a weight gain of up to 1% (for new sterilisers).

The effectiveness of the sterilisation is assessed:

1. on each sterilisation cycle:

- ➢ Permanent recording of physical parameters - temperature/pressure chart;
- ➢ Transfer, only above a certain temperature, of the colour of the adhesive indicator strips, of the special wrapping papers, of the indicators placed in packages (if the transfer has not been realized, the material is non-sterile);

2. on a daily basis:

- ➢ Bio indicators like *Bacillus stearotermophylus* – a vial containing *Bacillus stearotermophylus* spores is subjected to sterilisation, then seeded on a suitable medium, containing an indicator that changes colour in the case of a metabolic activity of the bacillus. The Stearotest vials keep their purple colour at 120ºC (turning the colour into brown shades denotes reaching a higher temperature, and changing the pH indicator into yellow, a lower temperature);
- ➢ Checking the penetration of steam by the Bowie & Dick test (in the morning, before the first sterilisation) - a special paper is inserted in a textile package; uniform colour change of the geometric pattern means proper penetration. Otherwise, the steriliser is not used and requires a technical inspection.

3. on a quarterly basis, a regular inspection should be performed, then the following are verified:

- ➢ Temperature/pressure chart;
- ➢ Bowie & Dick test;
- ➢ Textile moisture control - sterilisation of a sample box with 20g folded gauze, weighed before and after sterilization.

The packages are labelled with the date, time, steriliser, the person who performed the sterilisation and are recorded in the sterilisation journal. The duration of the persistence of sterility is 24 h for boxes/packs, 1 month for materials packed in special paper, and 2 months for materials packed in plastic bags, provided the integrity of the packaging is maintained.[40]

C. Sterilising water used for surgical hand washing
It is done in autoclaves, at 1.5 bar, for 30 min.; the sterilised water is used only during the day it was sterilised.

D. Flash Sterilisation
It represents the rapid sterilisation of an unpackaged object at 132°C for 3 minutes at 2 atm., which allows the rapid penetration of steam. It is suitable for items/instruments that cannot be packed, sterilised and stored before use, or when there is not enough time for

classic autoclaving. It is not suitable for implantable devices nor as a routine sterilisation method, because the absence of protective packaging allows contamination during transport, the sterilisation parameters (time, temperature, pressure) are at minimum values and there are no biological indicators for monitoring the sterilisation process.

II. Chemical Methods of Sterilisation

The use of heat as a sterilising agent is not the only option, as it distorts a whole range of thermosensitive materials, such as: biological materials, optical fibre, electronics or plastic devices. [42] In this case, chemical sterilisation is used, which is carried out at relatively low temperatures (50-60ºC) and uses sterilising gases or liquids.

When using sterilising gases, the material subject to sterilisation is exposed at high concentrations (5-10% volume/volume) of highly reactive gases such as: ethylene oxide (alkylating agent), hydrogen peroxide, formaldehyde and ozone. Sterilising fluids, which are also high-level disinfectants, include hydrogen peroxide, peracetic acid, reactive aldehydes such as glutaraldehyde or ortho-phthalaldehyde.

A. Chemical sterilisation by immersion in sterilising fluids entails 3 stages:
> - Decontamination, at least medium level decontamination, followed by cleaning;
> - Immersion sterilisation;
> - Rinsing with sterile water.

It is suitable for the sterilisation of endoscopes, fiberscopes, instrumentation made out of composite thermosensitive materials. The chemical solution should not be used for more than 48 hours after preparation, in containers with a lid, and up to 24 hours in ultrasound devices. The maximum number of sterilisation cycles is 30.[40]

B. Ethylene oxide sterilisation

The method is suitable for thermosensitive objects or equipment - plastic material, composite materials, fragile materials. Ethylene oxide is a flammable gas, and at concentrations above 3%, it is toxic. In staff, it can cause contact dermatitis, respiratory mucosal irritation, CNS depression, and insufficient desorption can cause in patients, haemolytic phenomena, tracheal stenosis, cardiovascular collapse, allergies.

An entire cycle lasts:
> - 4-8 hours, with the actual sterilisation stage of 180 min. at 37ºC and subatmospheric pressure;

> ➢ or 2-5 hours, with the actual sterilisation stage of 60 min. at 55ºC and subatmospheric pressure.

The final stage of desorption requires a special, ventilated space, where stopping is forbidden for medical personnel.

C. Low-temperature formaldehyde sterilisation

Formaldehyde is brought into the gaseous state and introduced into the sterilisation chamber at a concentration of 8-16 mg/l. A complete sterilisation cycle lasts 3 to 5 hours, with the actual sterilisation stage of 10 minutes at 73°C or 80°C and sub-atmospheric pressure, or 30 minutes at 65°C and sub-atmospheric pressure. A disadvantage of the method is the mutagenic and carcinogenic potential of formaldehyde, which requires special measures for personnel protection.

D. Peracetic acid sterilisation

Peracetic acid is a strong oxidising agent, which also acts in the presence of impurities (for example in the case of sterilisation of endoscopes).

In 1988, an automatic chemical sterilisation system for medical, surgical and dental instruments was introduced, using 35% peracetic acid, together with an anti-corrosive agent. After introduction into the chamber, dilution of peracetic acid with distilled water is carried out up to a concentration of 0.2% and a temperature of 50ºC.

E. Hydrogen peroxide sterilisation

Hydrogen peroxide is a very powerful oxidising agent. In sterilisation, it is used at high concentrations of 35% to 90%. The greatest advantage of this agent is the short sterilisation cycle of 25-30 minutes long. In 1993, hydrogen peroxide plasma technology was introduced as a sterilisation method. The plasma state is obtained by going through the following steps:

> ➢ remove air from the chamber (vacuuming);
> ➢ introduce hydrogen peroxide of 6 mg/l in the chamber and vaporise it under the action of vacuum and temperature;
> ➢ obtain the plasma state, containing many free reactive radicals, by gas irradiation with microwaves or various radiofrequencies;
> ➢ sterilisation (destruction of microorganisms) through the action of free radicals against the proteins, fatty acids, nucleic acids from their composition;

> eliminate excess gas and introduce air up to atmospheric pressure.

If there is moisture in the chamber, the sterilisation process cannot take place.

SAMPLING, TRANSPORT, PRESERVATION OF THE MAIN BIOLOGICAL PRODUCTS IN EPIDEMIOLOGICAL PRACTICE

For a proper sample collection, an essential step in making a relevant microbiological diagnosis, it is necessary to know the following:

➢ which is the pathological product that may contain the suspected germs, based on the different evolution stages of the disease;
➢ which is the optimum sample collection time;
➢ if the biological product is normally sterile or contaminated with saprophytic flora;
➢ which is the correct sample collection technique, respecting asepsis rules;
➢ what is the right amount to be collected from the pathological product;
➢ requirements for packaging and transporting the sample;
➢ what is the optimum time to deliver it to the laboratory
➢ how to preserve the sample, when it cannot be immediately transported.[44]

For the bacteriological examination, it is preferred to take the pathological samples before starting the antibacterial chemotherapy, but if it has already started, it must be written on the delivery note to the laboratory, the preparation and the dose administered. The container will be labelled with the patient's name/surname, the pathological product and the requested examination, and the delivery note shall also indicate the number of the medical file, the presumptive diagnosis, the date/time of the sample collection, and possibly other significant data that may guide the microbiological analysis.

The optimum time for the sample to reach the laboratory is 1-2 hours, depending on the pathological product, but also on the suspected germs. For the transport of (potentially) infectious substances, the triple packaging system is used to protect the sample from external contamination, but also to protect the environment and the persons handling them. This system consists of:

➢ **Layer one** is represented by the primary sealed waterproof container, containing the sample; it is properly labelled (e.g.: top for stool testing, exudate container, urine container, etc.);
➢ **Layer two** is represented by a waterproof isothermal bag used in order to protect the primary container. Several primary containers can be placed in a single secondary packaging;
➢ **Layer three** is represented by special waterproof containers with rigid walls and a closing mechanism, easily transportable, made

of materials that allow cleaning and disinfection in order to avoid contamination of the person carrying the samples and/or the environment; it protects the secondary packaging against physical damage during transport (e.g.: isothermal bags).

The transport of samples between medical units is carried out with individualized vehicles, cleared by the Public Health Directorate. The forms containing the sample data accompany it throughout the entire itinerary, from the collection to the time of registration and identification.

Products collected in the epidemiological practice can be secretions, excretions, tissue fragments obtained by biopsy or autopsy, food items, water and air samples, etc. Most of the evidence comes from the sick, but convalescents, healthy carriers, contacts or dead patients are also investigated.

The bacteriological diagnosis includes isolation of germs, identification up to species level, determination of sensitivity to antibacterial preparations by carrying out the antibiogram and classification into resistance phenotypes. For research purposes or in high-tech laboratories, the investigation can also be completed with the study of bacterial strain clonality or with the determination of the genetic substrate of resistance.

Each biological/pathological product entails a particular collection technique, presented below:

I. Respiratory tract infections

a. Collection of the nasal exudate (in sinusitis, angina, diphtheria, germ carrying) - it is performed by wiping each vestibule of the nostrils with a sterile disposable tampon (one for each cavity). The patient is positioned with the head in extension and the tampon is gently inserted until it reaches the posterior wall, then gently rotate it until it loads with nasal secretion. The manoeuvre can be repeated to increase the amount of mucus collected. Subsequently, the swab is easily withdrawn, reinserted into the protective tube (with or without Amies/Stuart transport medium) and sent to the laboratory within a maximum of 2 hours.

b. Collection of ear discharge (otitis being related to the nasopharyngeal sphere) - is performed using general purpose disposable swab. Position the patient with the head slightly bent in the side, draw the ear lobe down and anteriorly in order to expose the external auditory meatus, gently insert the swab along the external ear canal, rotate it to collect existing secretions, then withdraw and it is inserted into the protective tube (with or without transport medium). After labelling, it is sent to the laboratory within a maximum of 2 hours.

c. Collection of pharyngeal exudate (in angina, diphtheria, scarlet fever, germ carrying) - it can be done in the morning, before brushing your teeth and before eating, or 3-4 hours after ingestion of food, teeth brushing or using oral antiseptics. The patient with their head extended will open the mouth cavity to the max and pronounce the vowel A. With a sterile (or disposable) spatula depress the back of the tongue and carefully insert the swab, without touching the palate, uvula or tongue. Wipe with a circular motion the posterior wall of the pharynx, the palatal tonsils, insisting on the inflamed or ulcerated area presenting purulent deposits. Carefully remove the swab (in order not to trigger the vomiting reflex), insert it back in the protective tube (with or without transport medium) and send it to the laboratory within a maximum of 2 hours.

d. Sputum collection (in lower respiratory tract infections) - the indirect method is performed in non-intubated, cooperating patients. In the morning, after a prior energetic rinsing of the oral cavity with physiological serum and a simple brushing of the teeth, the patient is invited to cough and expectorate in a sterile container, 2 ml in acute infections or the entire morning expectoration respectively expectoration discharged within 1-2 hours, in chronic coughers. If the sample consists mainly of saliva, the sampling is repeated until obtaining a properly qualitative sample. Sputum can also be collected by direct methods - bronchoscopy or tracheal puncture. In children, the collection method used is called gastric lavage. The obtained sample is sent within maximum 1 hour to the laboratory. Refrigeration decreases until cancellation, the possibility of isolation of some germs (*Neisseria meningitidis*, *Haemophilus influenzae*), so it cannot be used as a preservation method.[45]

d. Collection of bronchial aspirates (in lower respiratory tract infections) is performed:

- by lower tracheal aspiration, through the intubation probe, being the easiest method for patients requiring ventilator support. It is performed using a sterile probe inserted via the intubation probe. After aspiration, the probe with the collected secretions is inserted into a broth culture medium, sectioning in a sterile manner approximately 10 cm from it, and then send it to the laboratory urgently;

- another technique used is the bronchoscopic sampling, which is easier to perform in the case of an ICU patient due to the presence of the orotracheal intubation probe and the impaired state of consciousness. The technique involves aspiration of the bronchoscope canal, with or without bronchial irrigation and/or bronchial brushing, with brush protected in the telescopic cannula system with distal polyethylene glycol plug, which allows to avoid oropharyngeal contamination, but also to perform

transbronchial biopsy. The patient is placed in dorsal decubitus position at 45° or lateral decubitus position (in the case of the orotracheal intubation). The bronchoscope is directed towards the lesion, the catheter protecting the brush is inserted and slid into the internal canal until the polyethylene glycol plug is forced. This is non-toxic and will rapidly be absorbed into the mucosa. Under visual control, the brush is removed 1-2 cm and inserted directly into the lesion exudate, then withdrawn through the internal canal of the bronchoscope. The outside of the catheter is washed with alcohol and dried, while the brush, from which the catheter was withdrawn, is separated aseptically, and placed in a 1 ml vial of lactated Ringer solution, and sent to the laboratory for quantitative seeding, within a maximum of 2 hours.[45]

II. Bloodstream infections

a. Blood may be collected in order to perform biochemistry, immunology, haematology and bacteriology tests (for isolating the existing bacteria into bacteremias/septicaemias of a diverse aetiology, typhoid fever, endocarditises, etc.). Blood culture is carried out in case of a de novo occurrence of a shiver or if body temperature rises over 38.5ºC, by a new venous puncture, avoiding to draw blood from the pre-existing venous catheters. It is preferable to perform the blood culture test before starting the anti-microbial treatment. The optimum volume is of 20 ml of blood per sample/ 3-5 ml in the case of children. As most bacteria are intermittent, a single blood sampling ensures an 80% sensitivity, while three sampling over 24 hours, ensure a 100% sensitivity.[45] Venipuncture is performed in the hollow of the elbow or at the level of jugular veins (for new-borns or infants), after a thorough antisepsis with Betadine, then with rubbing alcohol, in order to leave the skin dry. The medical personnel performing this manoeuvre will wear sterile disposable gloves. After having applied the tourniquet, immobilize the most obvious vein using your left index finger, and puncture on the vein axis with your right hand, under a 30º angle, keeping the needle with the bevel up. When retracting the piston, the blood must enter the syringe, provided that the positioning is correct. Afterwards, remove the tourniquet, take the needle out of the vein and exert constant pressure, using a sterile swab, until hemostasis is complete.

After collection, the blood is distributed into the blood culture vials for the aerobic, anaerobic germs, and, possibly, for fungi (with the plugs previously disinfected), shaking gently for homogenization with the medium. These vials are sent to the laboratory within the shortest time possible (maximum 1 h) and they are introduced in automated systems (e.g.: BACTEC), where they are monitored for 10 days.

For typhoid fever, 5-10 ml of blood will be collected in the first week of illness and 25-30 ml later on, when the number of germs in the blood flow is more reduced. The culture medium used is either simple broth or ox bile broth, keeping the 1/10 proportion between the seeded blood and the volume of the medium.

In order to highlight the antigens/antibodies by serological reactions, draw 10 ml of whole blood, without anticoagulant, and wait for about 1 h at the room temperature, until plasma is separated from the blood clot. Plasma settles down in a container and it is centrifuged for 10 min. At 2500 rpm, in order to obtain the clear, non-haemolysed serum, necessary for the diagnosis.

Regardless of the Atg-Atb type of reaction, you should take into account the following aspects:

➢ In general, the correct interpretation is possible only on paired samples, collected in different stages of the disease, with or without emphasizing the titer rise in the dynamics;
➢ If only one sample is available, the result may be considered positive, if the titre exceeds by at least twice the specificity threshold and it is supported by the clinical aspects;
➢ A positive and a negative witness serum are used more frequently.

In current practice, such reactions are used for leptospirosis, brucellosis, Q fever, typhoid fever, titration of diphtheria and tetanus antitoxins, etc.

b. The intravenous insertion of the catheter, resected with the sterile scissors, is deposited in a sterile container and immediately sent to the laboratory.

III. Urinary tract infections

a. Urine collection for uroculture (in infections of the lower/upper urinary tract) is performed using a mid-steram urine sample in the case of non-catheterized patients, in a sterile container with a wide neck, identical for men and women, after a thorough local cleaning, with soap and water, of the external genitalia. It is preferable to take the sample from the first urine in the morning or at least 4 hours after the previous urination.

- In patients with prolonged catheterization (for urological or neurological causes), urine collection is performed after having decontaminated the distal end of the catheter, with 70% alcohol. 5 ml of urine are collected with a sterile syringe, and then the sample is transferred, under aseptic conditions, into the urine culture container. It is forbidden to take the sample directly from the drainage bag or to cultivate the catheter tip.

- The transcutaneous suprapubian puncture is reserved for carefully selected cases, being performed under surgical asepsis conditions. It is indicated for identifying infections with anaerobic bacteria, as it is very effective for avoiding the urethral contamination of the samples.

Samples should be processed within approximately 2 hours after collection, so as to prevent the multiplication of the microbial flora. If this condition cannot be met, the urine is stored at +4ºC, until the time of processing.

IV. Genital infections
a. Collecting the urethral discharge (in case of urethritis):
  ➢ in women - it is performed in the morning, before urination or one hour after the last urination, by the consultant. After putting the patient in the gynecological position, the external genitalia are cleaned with soap and water, followed by abundant rinsing, but without wiping. The exudate is removed from the urethral orifice by means of a sterile clamp and swabs, then, using another swab, the secretion from the urinary meatus is collected, while massaging the urethra through the vagina, or the urethral secretions are collected by inserting the swab over a distance of 2-4 cm and rotating it for 2 seconds. The rod is then inserted into the tube with the transport medium (Amies) and brought to the laboratory as soon as possible.
  ➢ in men – the collection is also performed by the consultant in the morning, before urination or one hour after the last urination, after an external cleaning. The spontaneous urethral discharge (in acute urethrites) or the secretions inside the urethra are collected by introducing a thin swab on a 1-2 cm distance, while rotating it for a few seconds. The rod is carefully extracted and it is introduced in the tube with the transport medium, it is labelled and brought to the laboratory as soon as possible. In the case of chronic urethritis, the prostate massage or the increase in the amount of secretions is practiced by consuming 2-3 glasses of beer, the night before, without urinating after 2 a.m.
b. Collecting vaginal discharge – the external genitalia shall be cleaned one evening before the collection, then, in the morning, the gynecologist will collect the discharge from the recto-uterine pouch using 1-2 sterile swabs, which will be then inserted into the protective tube containing the transport medium (Amies), labelled and quickly transported to the laboratory.

c. Collecting the endocervical fluid – this collection is performed by the consultant, after having viewed the cervix with a sterile speculum and after having removed the discharge or mucus in the cervix. The swab is firmly inserted into the cervix, it is rotated for 10 seconds, next, the protective tube containing a transport medium is introduced therein, and then labelled and quickly transported to the laboratory. The vaginal discharge and cervical fluid must be collected simultaneously, as some germs settle in the recto-uterine pouch (*Candida, Trichomonas vaginalis*), while the gonococcus or chlamydias multiply in the cervix.[44]

V. Skin infections
The following methods may be used:
a. From closed collections of pus (abscesses, phlegmons, boils, hydrosadenitis, etc.) – the sampling is performed by the surgeon when opening the collection or by aspiration puncture using a syringe with a fine needle, from the depth, after a prior antisepsis of the teguments. The product is transferred into a sterile anaerobic transport system and sent to the laboratory immediately.
b. From open, fistulized collections of pus - the intact skin around is cleaned with Betadine, and the exudate from the surface of the lesion is priorly wiped with sterile physiological serum. The sterile swab is introduced on the path of the fistula, scoop is performed as deep as possible, and then the swab is insterted into the tube containing Amies transport medium and immediately sent to the laboratory.
c. From wound drainage (surgical wounds, skin ulcers, burns) – after having cleaned the wound with sterile saline (Betadine for the circumscribed area), the tip of the swab is rotated for 5 seconds on a 1 cm^2 area, firmly enough to cause a slight bleeding, and then it is introduced into the tube and transported to the laboratory, within maximum 1 hour.

VI. Eye infections
a. Collecting the conjunctival discharge (in conjunctivitis) **-** the patient must be positioned with the neck in slight extension, with the eye open, the mucosa at the level of the inner corner of the eye is carefully removed and the discharge is collected from the tear sac without touching the skin, using a sterile disposable swab (one swab for each eye separately). Then the swab is inserted into the protective tube (with transport medium), which is labelled and immediately brought to the laboratory, as the tears contain lysozyme, with destructive action on the cell wall of gram positive bacteria, which reduces the chance of isolating these germs. 44]

VII. Infections of the CNS

a. Collecting the CSF samples (in meningitis, menigo-encephalitis) – this collection is performed by clinicians, by lumbar/suboccipital rachiocentesis, under strict aseptic conditions. 5-10 ml are collected in 3 sterile containers and immediately transported to the laboratory, at a temperature as close to 37ºC as possible. Do not refrigerate so as not to destroy certain germs!

VIII. Gastrointestinal infections

a. The faecal matter eliminated spontaneously, can be collected for the purpose of performing the coproparasitological examination or stool culture (for intestinal parasite infections, food poisoning, gastro-enteritis, colitis, typhoid fever, bacillary dysentery, cholera, etc.). The patient will defecate spontaneously in a container sterilized by boiling, scalding or autoclaving (in the case of stool culture), without contaminating the urine content. No disinfectant solutions that can prevent germ development will be used. Then, fragments will be collected using the sterile sample spoon of the stool sample container, from different portions of the stool or from potentially pathological areas - with a mucous, sanguinolent, purulent, rhiziform appearance, in a minimum volume of 3 cm^3. The collected fragments will be turned into a suspension in the transport medium from the stool sample container and immediately sent to the laboratory.

In order to detect the pathogenic enterobacteria (*Salmonella* spp., cholera vibrio, etc.), samples will be collected from the second and third stool, after having administered a purgative (15 g magnesium sulfate in 250 ml water, for adults), with the collection of the liquid part containing the flora of the small intestine.

In the patients with dysenteriform syndrome, the sample is collected using a sterile swab inserted through the anal orifice under rectoscopic control and the mucosa is wiped. After collecting the sample, the swab is introduced in the transport medium of stool sample container and sent to the laboratory.

The faecal matter in the sigmoid may also be collected using a sterile Nelaton probe, introduced at 15-20 cm in the case of adults and at approximately 10 cm in the case of children. Using a 10 ml sterile syringe, the content is aspirated and then decanted into a stool sample container with transport medium.

The technique for the coproparasitological examination is similar to the one use for stool culture, the only difference being that it does not require sterilized containers.

Samples that are not seeded on isolation media, within 2 h, must undergo a conservation process:

  ➢ By refrigeration an + 4⁰C, for maximum 24 h;
  ➢ Using special transport media, such as: the Stuart medium (useful for preserving enterobacteria, but also for enteropathogens of the *Vibrio* or *Campylobacter* type) or the Cary-Blair medium which ensures a good preservation at ambient temperature, for up to 7 days (recommended for *Enterobacteriaceae* and *Vibrio* spp.). Liquid media are now less commonly used due to the difficult transportation and inconsistent preservation of certain enteric pathogens.

b. Samples of **gastric aspirate on an empty stomach** (useful for detecting the *M. Tuberculosis* bacilli, especially in infants and small children) or of **vomit,** must be neutralized with 10% sodium bicarbonate solution, in the presence of a pH indicator (bromothymol blue solution).[45]

The technique of collecting vomit samples for bacterial culture is similar to the stool culture from spontaneous emission. Sterile Petri dishes are used as containers.

c. The selection of **foods** for examination, within an epidemiological investigation for food poisoning, is made depending on the incubation period (only the foods consumed during the last 72 h).

Table no. X Foods involved in food poisoning – adapted after [45]

NO.	FOOD	POSSIBLE ETIOLOGY
1.	Smoked foods (meat, poultry, fish)	*Salmonella,* *Staphylococcus aureus* (and its enterotoxins), *Clostridium botulinum* (and its neurotoxins)
2.	Vacuum packed foods	*Clostridium botulinum* (and its neurotoxins), *Listeria monocytogenes*
3.	Cheese	*Salmonella,* *Staphylococcus aureus* (and its enterotoxins), *E.coli*
4.	Meat and derivatives	*Salmonella,* *Staphylococcus aureus* (and its enterotoxins), *Clostridium perfringens* (and its enterotoxin), *Campylobacter jejuni,* *Yersinia enterocolitica,* *E.coli* 0157:H7
5.	Potatoes	*Bacillus cereus* (and its toxins), *Clostridium botulinum* (and its neurotoxins)
6.	Cereals and corn foods	*Bacillus cereus* (and its toxins), mycotoxins
7.	Broths, soups, stews	*Bacillus cereus,* *Clostridium perfringens* (and its enterotoxin)
8.	Home canned foods	*Clostridium botulinum* (and its neurotoxins)

9.	Crustaceans	*Vibrio parahaemolyticus,* *V.cholerae* 01
10.	Hamburger	*E.coli* 0157:H7, *Salmonella*
11.	Ice cream	*Salmonella,* *Staphylococcus aureus* (and its enterotoxins)
12.	Raw milk and derivatives	*Salmonella,* *Staphylococcus aureus* (and its enterotoxins), *Campylobacter jejuni,* *Streptococcus pyogenes,* *Yersinia enterocolitica*
13.	Powdered milk	*Salmonella,* *Bacillus cereus,* *Staphylococcus aureus* (and its enterotoxins)
14.	Mayonnaise	*E.coli* 0157:H7
15.	Legumes, beans	*Clostridium perfringens* (and its enterotoxin), *Bacillus cereus* (and its toxins)
16.	Rice	*Bacillus cereus* (and its toxins)
17.	Eggs, egg products	*Salmonella*
18.	Pastry products with milk and eggs	*Staphylococcus aureus* (and its enterotoxins), *Salmonella,* *Bacillus cereus* (and its toxins)
19.	Fish and derivatives	*V.cholerae* 01, non-01, *V. parahaemolyticus,* *Proteus* spp., *Morganella* spp.
20.	Vegetable salads with eggs or meat/fish	*Staphylococcus aureus* (and its enterotoxins), *Salmonella,* *E.coli,* *Shigella* spp.

200 ml of the liquid food and 150-200 g of the solid food (several cubic fragments, from different areas and all layers) will be aseptically collected, in sterile containers, which will be sealed, labelled and transported to the laboratory. The label must include:
  - The food name;
  - Unit name;
  - Collection date;
  - Number of the production lot;
  - Number of the collection protocol.
Thus:
  - Milk – will be sampled in its actual package or, if it exceeds 1 kg, a 200-500 ml sample will be taken;
  - Meat products - 150-200 g will be aseptically cut from different parts or a full package will be sampled (in the case of concentrates);
  - Home canned foods – an unopened package will be sampled from the same lot;
  - The consumed leftover food – will be sampled with a sterile spatula, as much as possible;

- All these will be kept in the refrigerator, at +4ºC and sent in an isothermal bag to the laboratory;
- Frozen food - a whole package will be sampled or large carcasses will be drilled/chipped with sterile instruments, so that there is evidence from both the surface and the depth. They will be kept frozen until examined;
- From rice, vegetables – samples will be taken from the surface, but also from the depth of the package and will be introduced into sterile and closed containers, safe from moisture.[45]

All samples must get to the laboratory within maximum 6 h.

IX. Environmental samples
a. Water sampling
- From central water systems - flame the tap, open it completely and allow the water to flow for 5-10 minutes; then adjust the flow so that there is a continuous water column, with a maximum diameter of 1 cm, remove the plug of the sterile vial and fill it up to 2 cm under the plug. Then close the container and label it. One sample contains 1-5 l of water;
- From tanks and basins - after removing the plug, insert the sterile vial into the tank/basin, fill it up to 2 cm under the plug and close it;
- From wells, springs - the sample must be collected directly from the well or by pouring from a bucket;
- If the collected water is chlorinated, before sterilizing the vial, add in 10 mg of sodium thiosulphate for each 500 ml of water collected for testing;[45]
- The label must include the name of the sampling point, the date/time of the collection and sample number;
- Water will be transported to the laboratory in isothermal cases, within maximum 2 h (6 h if a temperature of + 4ºC is ensured).

b. Microaeroflora sampling - is used in the epidemiological practice in the nosocomial environment, in rooms with high risk of infection for the assisted patients - operating rooms, delivery rooms, Anesthesia and Intensive Care Units, neonatology rooms. It can be performed through:
- The Koch sedimentation method - 2 sets of Petri dishes will be exposed in each room, each set comprising a blood agar plate and a nutritious agar plate. The first set will be placed in the middle of the room, on a table, and the other one in a corner, on a bedside table/shelf. The lids of the Petri dishes will be lifted, put down with the opening up and left for 10 minutes. After the interval has expired, they will be immediately closed and transported to the laboratory.

> ➤ The vacuum method – the sampling is performed using devices such as the M.A.Q.S (Microbiological air quality sampler - Oxoid) analyzer. This device involves attaching the Petri dishes with culture media in a special adapter; the air is vacuumed with a speed between 0.5-2 l/s, and a volume between 1-999 liters is analyzed. Then, after incubation, the colonies are counted and the number of germs is calculated using a mathematical formula:

$$Pr. = N\ [(1/N+1/(N-1)+1/(N-2)+….+1/(N-r+1)]$$

> where Pr. is the probable number of microorganisms in the measured air volume; N – number of orifices on the analyzer;
> r - number of CFUs (colony-forming units) on the culture dish.

The total number of germs/ m^3 of air must not exceed 500-1500, depending on the activity carried out in the room and period of the sampling day. In the operating rooms (during work periods), in the neonatology or infant wards the maximum number of germs allowed is 300 germs/air m^3, with no haemolytic flora (coagulase-positive staphylococcus or β-hemolytic streptococcus). [40]

c. The microbiological control of soft surfaces and material – is practiced for tables, bedside tables, headboards, tile walls, linen, etc. If the surfaces have been previously decontaminated, they will be sprayed before performing the sampling with an N/10 sodium thiosulphate solution in order to neutralize the chlorinated derivatives. A surface of 25 cm^2 will be wiped using a sterile pad moistened with sterile saline. The ad will be passed over the surface both horizontally and vertically, while making a rotation movement, then it will be inserted into the protective tube, homogenized, labelled and sent to the laboratory, within maximum 2 h. The surface is considered clean if less than 5 colonies/cm^2 have developed, with the absence of pathogenic germs (coagulase-positive staphylococcus, enteropathogenic *E. coli*, *Proteus* spp., etc.). [40]

d. The skin control of healthcare personnel – the most commonly targeted are hands, known to be the most common way of germ transmission in the nosocomial environment. The palmar surface of the right hand shall be wiped with a sterile pad moistened with sterile saline, including the fingers, insisting on the interdigital or periorgional spaces. The acceptable limit is of 40 colonies/ml (for the healthcare personnel), provided that no pathogenic germs are identified *(Escherichia coli; Proteus; Staphylococcus aureus; Pseudomonas* spp.; *Klebsiella* spp.; *Acintotobacter* spp.; vancomycin resistant *Enterococcus)*. If found while performing an aseptic procedure, the acceptable limit is of 10 CFU/ml. [40]

FUNDAMENTALS OF DESCRIPTIVE AND ANALYTICAL EPIDEMIOLOGY (INDICATORS USED IN MEASURING MORBIDITY/SOCIO-DEMOGRAPHIC INDICATORS, METHODOLOGY OF EPIDEMIOLOGICAL STUDIES

Epidemiological statistics is used in the study of disease distribution and of the factors contributing to their outbreak in human population. In order to quantify such phenomena, different indicators are used and they may be classified in:

A. Indicators measuring morbidity

The incidence rate – measures the frequency with which new cases of a particular disease occur in a certain population and in a certain period of time (days, months, years). It is frequently used for acute diseases, in different forms:

➢ **Annual incidence** $= \dfrac{\text{no. of new cases of disease onset in one year x } 10^n}{\text{total no. of persons from among the population at risk}}$

➢ **Cumulative incidence** $= \dfrac{\text{no. of new cases of disease from a studied period x } 10^n}{\text{no. of persons not suffering from the disease from among the population at risk at the beginning of the study}}$

➢ **Attack rate** $= \dfrac{\text{no. of new cases from among the contacts of the first cases x } 10^n}{\text{total no. of persons at risk}}$

This rate is used if population is exposed to a risk, for a limited period of time (in epidemics) or for diseases with short incubation periods (food poisoning).[46]

The prevalence of the disease – represents the total number of cases (new and old) existing in a certain population, at a specific point in time (point prevalence) or during a given period (period prevalence). It is frequently used for chronic diseases and it is calculated as following:

➢ **Point prevalence** $= \dfrac{\text{total no. of cases (new+old) at that point x } 10^n}{\text{total no. of persons examined at that time}}$

➤ **Period prevalence** = $\dfrac{\text{total no. of cases (new+old) in that period x } 10^n}{\text{total no. of persons examined in that period}}$

The hospitalized morbidity studies the frequency of the disease among the hospitalized patients.

It is calculated as following: = $\dfrac{\text{no. of patients suffering from the "X" disease } \text{x } 100}{\text{total no. of hospitalized persons}}$

or = $\dfrac{\text{no. of hospitalization days for the "X" disease } \text{x } 100}{\text{total no. of hospitalization days}}$

B. Socio-demographic indicators

➤ **Crude birth rate** = $\dfrac{\text{no. of live births registered during the year x 1.000}}{\text{no. of inhabitants on the 1}^{\text{st}}\text{ of July}}$

➤ **Life expectancy at birth** – the average number of years a person hopes to live, according to the pattern of mortality by age groups in the population from which the person comes.

➤ **Population growth rate** = (no. of live births – no of deaths) + no. of immigrants

➤ **Natural population growth** = $\dfrac{\text{no. of live births – no of deaths recorded in one year x 1.000}}{\text{no. of inhabitants on the 1}^{\text{st}}\text{ of July}}$

➤ **Crude mortality rate** = $\dfrac{\text{no of deaths recorded in one year an } \text{x 1.000}}{\text{no. of inhabitants on the 1}^{\text{st}}\text{ of July}}$

➤ **Mortality rate from cause X** = $\dfrac{\text{no. of deaths from cause X in one year x 100.000}}{\text{no. of inhabitants on the 1}^{\text{st}}\text{ of July}}$

➤ **Infant mortality rate** = $\dfrac{\text{no. of deaths of children under 1 year of age recorded in one year x 1.000}}{\text{no. of live births in the same year}}$

➢ the same calculation methods can be used for other mortality indicators – the newborn mortality index, neonatal mortality (of infants in the first 28 days of life) or postneonatal mortality (of those over 1 month but under 12 months of age); the maternal mortality ratio;[46]

➢ **Lethality** = $\dfrac{\text{no. of deaths from the ''X'' disease over a certain period } \times 100}{\text{no. of cases diagnosed with the ''X'' disease over that period}}$

➢ **Proportional mortality** = the share of deaths grouped by a criterion (sex, age, cause of death) from the total number of deaths;

➢ **Years of potential life lost as a result of premature deaths** = the number of years that an individual who died before the age of X years (60-65 years) did not live.[46]

Indicators related to medical activity may also exist. Some of the most used ones are:

➢ **Average length of hospital stay** = $\dfrac{\text{no. of hospitalization days}}{\text{no. of discharged patients + in-patients on 31.12}}$

➢ **Patient turnover per hospital bed, in one year** = $\dfrac{\text{no. of in-patients + incoming patients}}{\text{average no. of hospital beds}}$

➢ **Hospital mortality** = $\dfrac{\text{no. of deaths among the hospitalized patients } \times 100}{\text{no. of discharged patients}}$

Indicators on the efficiency of the healthcare personnel involved in the surveillance of infectious diseases - the indicator for the detection of communicable diseases, the isolation index (see pages 61-62), as well as cost-effectiveness, cost-benefit, etc. indicators are also used.

The classification of epidemiological studies is summarized in the following table:

Table no. XI Classification of epidemiological studies

NO.	TYPES OF STUDY	EXAMPLES
1.	**Descriptive studies** - describe the characteristics of the disease in relation to people, places and time.	Individual studies: - case report, - case series, Population studies: - correlation studies, - cross-sectional studies.
2.	**Analytical studies** - checking a hypothesis; - trying to identify the causes of the disease, risk factors and cause-effect relationship.	Case-control studies, Cohort studies.
3.	**Observational studies** - there is no intervention on the subjects.	Prospective studies, Case-control studies, Cross-sectional studies, Correlation studies.
4.	**Experimental studies** - subjects are intervened on, which may involve ethical aspects.	Clinical study, Field study, Population intervention studies.

I. Descriptive epidemiological studies

They describe the distribution of a disease, providing information on the people affected by the studied pathology (characterized by age, sex, race, educational level, occupation, lifestyle - consumption of certain foods, etc.), about the outbreak place (geographical distribution, by social environment urban/rural, variations between countries, etc.), as well as some temporal characteristics (seasonality, rhythmicity of the disease, comparison of the current incidence with that of other time periods, etc.). All these data contribute to the elaboration of an epidemiological hypothesis, which will be validated or rejected with the help of other types of studies. This category includes:

➤ **Individual studies:**
- **case reports** (describing a more particular clinical case);
- **case series** (describing the characteristics of the disease in a greater number of similar cases, onset in a short period of time). The case series is useful for identifying an emerging disease or the onset of an epidemic.

➤ **Population studies:**
- **correlation (ecological) studies** – they compare the frequency of a disease in different groups of population, over the same time period or in the same population, identified at

different times. These studies are fast, easy to make, with low costs, but they cannot correlate the risk factors with the onset of the disease at the individual level, they cannot establish the temporal sequence between the disease and the exposure, and the accuracy of the data is variable, depending on the source.

- **cross-sectional studies** (synonyms: transverse, prevalence studies) – they may be either descriptive or observational. They capture the situation at a given moment (the momentary prevalence) or within a period of time (the period prevalence), in a well-established population. These studies concomitantly measure the exposure and disease, and may be the first step in investigating an epidemic outbreak with unknown causes, outlining a possible association with a risk factor. Thus, they underlie a future prospective study or are useful for defining the samples of a case-control study, they can be carried out quickly and with minimal costs, but they do not determine the temporal relationships between the risk factor and the disease, they can examine the connection with a single disease and the prevalence may be vitiated by excluding cases that have died or healed quickly.[47]

II. Analytical epidemiological studies

These studies invalidate or confirm a previously formulated hypothesis, and their results will be checked by experimental studies. Depending on the temporal criterion, they are divided into prospective or retrospective studies, i.e. they look into the future for identifying data on the onset of the disease, after an initial exposure, or they investigate the past in order to find a cause.

These studies include:

➢ **Case-control studies** (synonym: case-referent studies) – the subjects classified as sick and healthy (at the time when the study is initiated) are retrospectively followed in order to determine the exposure to a hypothetical risk factor.
  - They can also be prospective, when newly onset cases are included in the study, after the initiation of the research;
  - Errors are minimal if both the cases and the control are similar randomized samples, coming from the same population, and the selection is made according to the same criteria (all the more so when the case group is not representative for the general population);
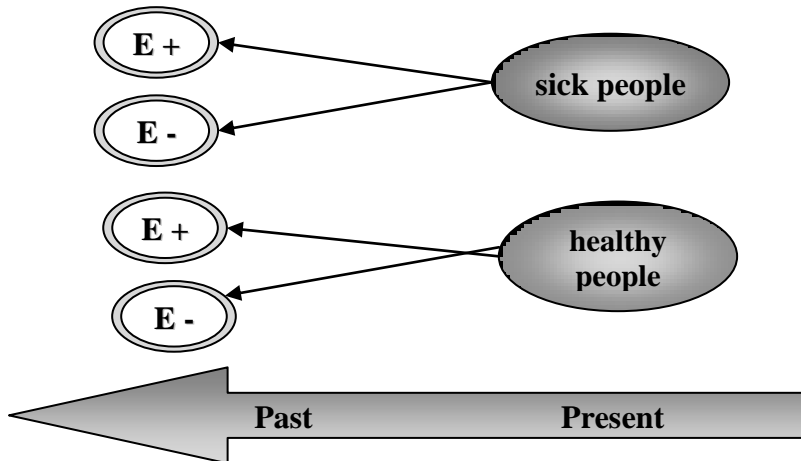
Fig.no. 2 Design of the case-control study (E = exposed people)

- Usually the ratio between the cases and controls is of 1:1, but multiple control groups may also be used (1:2, 1:3, etc.), and, if similar results are obtained compared to all these control groups, it is very likely for the observation to be real;
- In order to determine the exposure to the presumptive risk factor and the disease state, researchers use data from existing medical records, interviews, questionnaires;
- The exposure to the risk factor can be measured in a dichotomous manner (e.g.: smoker/non-smoker), in a polyhotomic manner (e.g.: non-smoker, occasional, moderate, heavy smoker) or in a continuous manner (e.g.: nicotine consumption over a certain period);
- These studies can be carried out quickly, with minimal costs, they are very practical for studying rare diseases, but also in situations where there is a long period of time between the exposure and the onset of the disease, and the need to identify the cause-effect relationships is urgent;
- However, these studies also have some disadvantages, because they are more vulnerable to systematic errors (selection, measurement errors) and encounter difficulties in determining the temporal relationships between the risk factor and the disease (e.g.: a case-control study on the relationship between depression and alcoholism reveals that patients following alcohol rehab treatments have experienced depression more frequently in the last 5 years compared to non-alcoholic subjects. The study cannot

determine whether alcoholism is secondary to depression or if depression is subsequent to alcohol consumption).[47]

➢ **Cohort studies** (synonyms: follow-up, monitoring, incidence, longitudinal studies) – the sample of *healthy* subjects, exposed to the action of the suspected risk factor, is monitored in time, as of the initiation of the study.
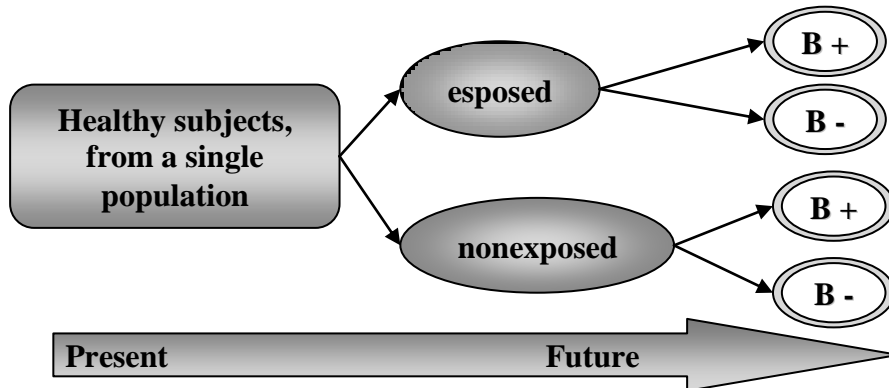
Fig.no.3 Design of the cohort, prospective study (B = disease)

- These are the only observational studies allowing a direct assessment of the risk, i.e. the probability for the healthy subject to develop a disease in a specified period of time;
- Also, the relationship between the hypothetical risk factor and several diseases may be simultaneously examined, and this is the closest observational study to the experimental ones;
- These studies minimise the errors but they are not applicable for rare diseases (as they would require the monitoring of a large sample over a long period of time) and may raise ethical issues related to the exposure to a risk factor.

➢ **Retrospective cohort studies** (historical cohort studies) – the exposed and nonexposed subjects are determined based on previous records and they are followed up over time in order to determine the current incidence of the disease.
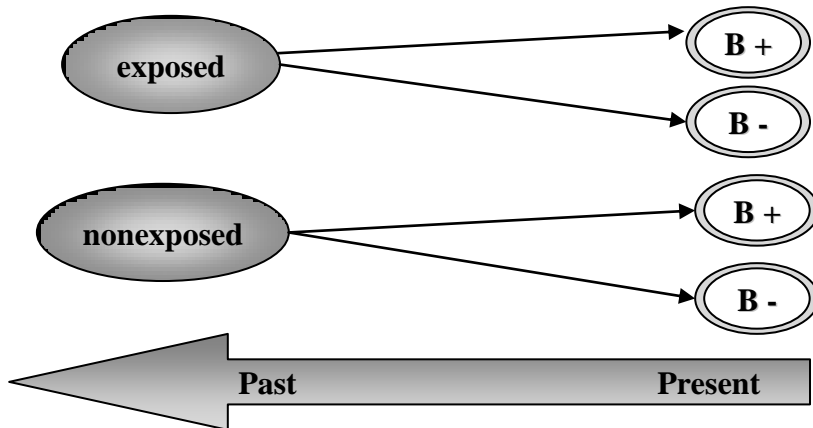
Fig.no.4 Design of the cohort, retrospective study (B = disease)

- They can be carried out quickly and with minimal costs, but they depend on the accuracy of the existing medical records. If these latter are incomplete or inaccurate, the conclusions of the study may be false.

The Hill guide for causation

In order to have a **cause – effect relationship,** the following criteria must be met:

1. The strength of association – the strong and statistically significant association between a possible cause and a possible effect, plead in favour of a cause-effect relationship more than a weak association. Establishing the causal relationship between the disease and a hypothetical risk factor is calculated using the contingency table. A significantly higher incidence of the disease in the exposed group reveals the association between the risk factor and the disease, while a significantly lower incidence of the disease in the exposed group reveals the protective effect.

	Disease +	Disease -	Total
Exposed +	a	b	a+b
Exposed -	c	d	c+d
Total	a+c	b+d	

Fig.no.5 The contingency table 2 x 2

The strength of the association between the risk factor and the disease is quantified by calculating the Relative Risk (RR) and the Odd ratio (OR), where:

$$\checkmark \quad \textbf{RR} = \frac{\text{the risk of disease in the presence of the risk factor}}{\text{the risk of disease in the absence of the risk factor}}$$

$$\textbf{RR} = \frac{\textbf{P(B+/E+)}}{\textbf{P(B+/E-)}} = \frac{\textbf{a/a+b}}{\textbf{c/c+d}}$$

$$\checkmark \quad \textbf{Odd ratio} = \frac{\text{the probability for the exposed individuals to get sick}}{\text{the probability for the nonexposed individuals to get sick}}$$

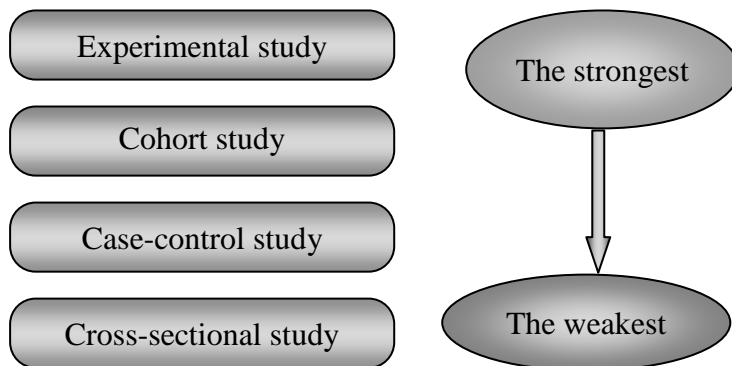$$\textbf{OR} = \frac{\textbf{P(B+/E+)}}{\textbf{P(B-/E+)}} \bigg/ \frac{\textbf{P(B+/E -)}}{\textbf{P(B-/E-)}} = \frac{\textbf{a/b}}{\textbf{c/d}}$$

- Usually, the OR is higher than the RR. If OR > 1 (RR >1) and the entire confidence interval > 1, the exposure factor meets the requirements for being considered a risk factor, regardless of the type of study.[48]

2. **Consistency** – several researchers, using different types of study, at different times, in different circumstances and locations, come to the same conclusions;
3. **A correct temporal relationship** – the exposure must precede the disease;
4. **Dose-effect relationship** – risk is directly proportional to the intensity of the exposure;
5. **Reversibility** - the causal association is strengthened if the removal of the cause leads to a decrease in the risk of disease;
6. **Plausibility** – a cause-effect association is plausible if it is consistent with the recognized scientific knowledge. The lack of

plausibility may rather reflect the lack of knowledge than the irrevocable absence of causation;

7. **Specificity** - a single possible cause is related to a single effect (e.g.: in infectious, genetic diseases);

8. **Analogy** - the existence of another cause-effect relationship similar to the one studied, gives it credibility.[1, 47]

Hill's criteria are necessary but not sufficient for establishing a causal relationship!

The power of studies in establishing the causation of a disease is illustrated in the following figure:

Experimental study

Cohort study

Case-control study

Cross-sectional study

The strongest

The weakest

Fig.no.6 The power of epidemiological studies in determining the causation relationship

---

ERRORS IN THE RISK ANALYSIS STUDIES

The systematic differences between the compared groups may affect the internal validity and invalidate the conclusions of the study.

 1. Confusion error: Variables may be confused or there may be co-interventions that are not take into account:

➤ e.g.: smoking is usually associated with coffee consumption and this association can generate confusion in vasoconstriction studies;

➤ e.g.: A prospective cohort study has analyzed the incidence of neoplasia over a 10-year period, on 2 randomized samples, one in the state of Nevada, where gambling is legal, the other one in Utah, where gambling is illegal. The high incidence of oncological pathology in the first batch may lead to the

conclusion that gambling is a risk factor! In fact, the excessive use of alcohol and smoking, present among the subjects in Nevada, is confused with the participation in gambling. The control group in Utah, most of them Mormons, did not consist of smokers or alcohol consumers.[47]

2. Selection errors – the researcher compares groups of subjects who differ by other elements than the disease state or the risk factor.

> e.g.: a study on the effect of jogging on coronary heart diseases compares the incidence of the disease among people who practice jogging, compared to a sample of the general population.
> There is a selection error, because the people who practice fitness activities are more careful with their health, and have a hypolipid diet, which can affect any conclusion of the study.[47]

3. Migration errors - appear when subjects leave the study or move from one group to another:

> e.g.: A prospective study on the effects of nutrition on school performance was carried out on a group of students from a private school, with a good nutrition status, and a group of children from a district school, considered to have a poorer nutrition status. The loss of some subjects in the last group, as a result of giving up the education process, with a higher rate than the first sample, will invalidate the results of the study.[47]
> When the subjects who left the study systematically differ from the remaining ones, the results can only be applicable for the final sample;
> Even if the sample was initially representative for the population, the migration biases may restrict the generalization of the results;
> The more subjects are lost, the less the results can be generalized.

4. Measurement/surveillance errors - appear as a result of the systematic differences in the measurement of the variable among the 2 groups.

> e.g.: a prospective study focusing on determining the association between postmenopausal estrogen administration and the onset of uterine neoplasm. As the treated group undergoes gynecological examinations more frequently, more cases may be identified even if the incidence is similar in both samples.

5. Information errors - patients and their next-of-kin are more persistent in identifying previous exposures compared to the control group. Sometimes they can systematically overestimate or underestimate the exposure.

> e.g.: mothers of newborn with malformations tend to overestimate medicine use during pregnancy.

6. Sampling errors - systematic differences appear between the studied sample and the general population, the external validity being affected and therefore the possibility of generalizing the results.

➢ Case-control studies based on prevalence are more susceptible to these biases, compared to those based on incidence, because they exclude the patients who died as a result of the disease or those who have healed quickly;

➢ The control groups selected from hospitalized patients generally have an increased morbidity compared to the general population;

➢ e.g.: the Berkson bias: in a case-control study on the association between asthma and emotional disorders in children, because those suffering from asthma and psychological disorders are more often hospitalized, the association between asthma and emotional disorders will increase artificially (a result that is not representative for the general paediatric population).[47]

➢ Sampling errors may also occur due to the motivation of the subjects, generating differences between the studied sample and the general population.

Random errors occur when the analysis is performed on a non-representative sample of population, and the results obtained cannot be extrapolated. Such an error may only be reduced, by increasing the samples.

Error control may be obtained by multiple methods:

1. **Restricting** the subjects' access in the study, in order to minimise confusions:

➢ e.g.: Black people consume more salt than other racial groups, so the association between hypertension and salt may be confused with the connection between the pathology in question and race. In order to avoid confusion, only subjects belonging to a single race will be accepted; [47]

➢ By this method, the connection between the restricted factor and the disease cannot be studied.

2. **Using pairs of subjects** who are similar from multiple points of view:

➢ e.g.: in a study on the role of smoking in baldness, the cases were selected from among the men who requested specialized medical care for this problem, and the control group from among the patients of the GP surgery. Both baldness and smoking are more common in the elderly. In order to eliminate any confusion, the pairs bald /not bald person had the same age.[47]

3. **Stratification:**
   - ➢ Subgrouping the study subjects based on similar characteristics and analyzing the data for each sub-sample, separately;
   - ➢ e.g.: a case-control study marked out an association between caffeine and coronary heart diseases. A detailed analysis shows that smokers consume more coffee than non-smokers and the sample of patients suffering from coronary heart disease included more smokers. In order to avoid any confusion between caffeine and smoking, both the patient group and the control will be stratified according to the smoker status.

4. **Standardization of rates –** the following can be calculated based on the observational studies:
   - ➢ **Crude rates** of morbidity/mortality;
   - ➢ **Specific rates** – specific mortality by age groups;
   - ➢ Standardized **adjusted rates**, so as to compare populations with different fundamental characteristics.

5. **Assuming the "worst case scenario" –** when confusion cannot be avoided or has a minor impact:
   - ➢ The effect of confusion is estimated by assuming the "most unfortunate" distribution of the factor among the compared groups;
   - ➢ It is especially useful for the control of sampling errors due to non-compliance;
   - ➢ e.g.: in a study conducted for determining the incidence of coronary heart diseases, all subjects included but non-compliant will be considered as cases suffering from the pathology in question.

6. **Statistical methods –** in order to adjust the values of the variable that depends on the influence of one or more independent variables - sources of confusion, researchers use the multivariate regression (statistical procedures such as logistic regression, the model of proportional hazard regression - Cox, covariance analysis).

III. Experimental epidemiological studies

Unlike the observational studies, experimental studies provide a high level of control over the conditions and participating subjects, resulting into more relevant results.
   - ➢ The **randomized clinical trials** (with the random distribution of subjects in the case group or in the control group) are the most commonly used, for assessing the efficiency of certain therapeutic

procedures/preventive measures, based on the voluntary participation of informed subjects who consent to take part in the study, after a prior approval by an institutional committee and a medical ethics committee. There may be:

- **controlled clinical trials** - efficiency is assessed by comparing the evolution of the treated group with a similar control group (who received a standardized conventional treatment or placebo). A standardized treatment means an effective treatment, already existing and used in medical practice. When such treatment exists, not administering the therapy or administering a placebo treatment may raise ethical concerns. The most common situation is the administration of a placebo treatment, with an identical form of administration but without the active principle;

- **uncontrolled clinical trials** - efficiency is determined by comparing the same subjects, before and after the treatment. They are less commonly used, because the results obtained may have alternative explanations. Thus, the natural evolution of many diseases leads to recovery, even in the absence of a treatment, if the study period is long enough. In other diseases (multiple sclerosis; ulcerative colitis) the periods of symptomatic exacerbation alternate with those of remission. If the experimental treatment was initiated in a florid state, its "efficiency" may be mistaken for a spontaneous remission. Moreover, some volunteer patients feel the desire to please the investigating physician in exchange for the attention they receive (the Hatwhorne effect). This may consciously or unconsciously lead to the introduction of biases in the study.[49]

The stages of a controlled clinical trial are:

1. Clinical formulation;
2. Establishing the working method (type of trial, sample size, selection of the control group);
3. Dividing the subjects into groups;
4. Treatment administration and variable monitoring;
5. Data analysis;
6. Critical interpretation of the results with an indication of the clinical applicability, external validity (the possibility to generalize the outcomes at the level of the entire population), possible biases, and determination of their sources;
7. Final assessment of the study.[49]

In order to prevent the biases that may occur during the intervention and during the measurement of the variables, which may compromise the internal validity of the trial, blind studies are preferred. Thus, there may be:

> ➢ Single-blind studies – the subjects don't know whether they receive the active principle or the placebo, for the purpose of estimating the adverse reactions or efficiency;
> ➢ Double-blind studies – neither the subjects nor the physician know whether they belong to the study/control group, in order to reduce the errors due to surveillance;
> ➢ Triple- blind studies - neither the subjects, nor the physician or the assessor know whether they belong to the study/control group.

Possible errors in the case of clinical trials may depend on the studied population (selection errors), on the assessment technique (due to the subject or assessor), on different errors (generated by the co-intervention of another contaminating factor, non-compliance of the subjects), on the external validation (when there are systematic differences compared to the general population) or on statistical errors (as a result of incorrectly applying the statistical methods).

The randomized clinical trial is considered a "gold standard", as it provides the possibility to minimize the errors, but its high cost, ethical issues and, the sometimes reduced compliance of the subjects, limits the performance of such studies.[50]

Besides the clinical trials, we also have:

> ➢ **Field studies –** which include persons who do not suffer from the investigated pathology, but who are supposed to be exposed to this risk. The compared groups are a "protected" group versus an "unprotected" one, because these studies are used to assess certain control or preventive measures.
> ➢ **Population intervention studies** – which use a community in the "protected" group. They are used for assessing the prophylactic measures, in some diseases with a social component, which could be influenced by the behaviour of that population. They can be applied in a small number of communities, each with certain characteristics, selection cannot be randomized, so their use is more limited.[50]

MONITORING, PREVENTION AND DIRECTLY OBSERVED THERAPY

Epidemiological surveillance is the system of systematic collection, analysis, interpretation and dissemination of health data in a population, for the purpose of carrying out prevention and control activities. [51]

The term of "epidemiological monitoring" is not synonym to "medical surveillance" during the maximum incubation period of the contacts, for the purpose of early detecting the first signs of an infectious disease or of persons at risk for a non-communicable disease.

The WHO asks all states to currently report certain conditions: cholera, plague, yellow fever, HIV. In addition to these, the Public Health authorities in each country establish a list of other reportable pathologies, depending on the needs. Subsequently, this system was extended to chronic non-communicable pathologies or to the immunization of the population.

The analysis of the data collected by epidemiological surveillance serves for:

- ➢ Knowing how diseases develop, while detecting the changes that have occurred;
- ➢ Recognizing the epidemiological connection between cases;
- ➢ Making the control and prevention measures more efficient;
- ➢ Establishing the public health policy;
- ➢ Obtaining additional data necessary for characterizing and understanding the morbidity phenomena, and also checking certain hypotheses.

The stages of the surveillance system are the following:

1. Selecting the population and event by defining the case;
2. Choosing the surveillance method that is adequate for the proposed objectives;
3. Systematic data collection;
4. Centralizing the collected data;
5. Data analysis and interpretation;
6. Disseminating the results by reporting them to the higher levels and communicating them to the in-house personnel;
7. Assessing the surveillance system.

1. Selecting the population and event by defining the case – The first step is to establish the case definition, as clearly, completely, and easy to understand, and to distribute it to the entire medical personnel with a role in case identification and reporting. There are definitions for the

confirmed cases, but also for suspected cases. The monitored population is also determined, in a certain geographical area or in a representative sector.

In Romania, the surveillance of the community-acquired infectious pathology is regulated by Decision no. 589/13.06.2007 on establishing the data reporting and collection methodology for monitoring communicable diseases, with the implementation of the Unique case report form. [35] Regarding the nosocomial infections, the methodology is found in the Order of the Ministry of Health no.1101/30.09.2016 on the approval of the Rules for the monitoring, prevention and control of the nosocomial infections in the healthcare units.

2. Choosing the surveillance method that is adequate for the proposed objectives – Depending on the method of data collection:

> ➢ A passive surveillance – by the periodic reports of the medical network on the diseases for which the nominal or numerical reporting is compulsory;
> ➢ An active surveillance – by direct contact, without waiting for the periodic reporting;
> ➢ A surveillance based on the "sentinel" system, by taking over the date from specially appointed healthcare personnel, from a well-defined area and population. Such an example is the surveillance of influenza.

Traditional surveillance is based on reporting the confirmed cases. Lately, the syndromic surveillance has developed, based on clinical signs and symptoms that are insufficient for a firm diagnosis, but signal with sufficient probability a case or an epidemic onset, allowing faster detection, a more prompt response from the Public Health System and possible decrease of the morbidity/mortality.

The design of the surveillance system must take into account the proposed objectives, targeted duration, personnel available, value of the active methods compared to the passive ones. The advantages and disadvantages of each method are presented in table no. XII:

Table no. XII Comparative analysis of the surveillance methods – adapted after [52,53,54,55,56,57,58]

MONITORING	DESCRIPTION	ADVANTAGES	DISADVANTAGES	EXAMPLES
Passive	- it is made by the reports of the healthcare personnel in the system.	- less expensive.	- the personnel is not specially trained in the field of surveillance and epidemiology; - it may deviated from the standard definitions; - it is an additional task to many others, leading to underreporting/non-reporting in a timely manner.	- surveillance of rubella, varicella, pertussis, epidemic parotiditis, viral hepatitis.
Active	- it is made by the direct involvement of the professional healthcare personnel (by telephone, regular visits, etc.).	- it observes more strictly case definitions; - it provides a higher quality monitoring, from multiple sources.	- it requires specialized personnel in this field; - it is more expensive; - it has less chances of continuity, especially in systems with limited resources.	- surveillance of nosocomial infections by the personnel of the SPIAAM (*The Department for the Prevention of health-care associated infections*) department.
Continuous	- the continuous surveillance over several years.	- it determines an overview of the issue; - it allows the storage and comparison of the data in order to capture temporal trends.	- it requires considerable time and efforts.	- surveillance of the Lyme disease in a CD ward for a long period of time.
On a limited period (by turns)	- surveillance by turns: - it may be a total surveillance for a certain period, carried out successively in each hospital ward; - or it may alternate a global surveillance on a certain period with a targeted surveillance for certain types of infection, during other periods.	- it allows the surveillance with a small number of personnel; - it allows the assessment of control measures.	- the short surveillance period may lead to wrong conclusions and the nosocomial problem can be left unsolved.	- surveillance of the nosocomial urinary tract infections in an area with high rates, until prevalence decreases.

Global	- collecting, analyzing and disseminating all the data on a certain pathology in a certain area.	- it determines an overview of the issue.	- it overloads the personnel, and it is less feasible in practice.	- surveillance the nosocomial problem in a tertiary healthcare unit.
Targeted	- on a certain ward; - on a certain type of infection; - on a certain group of patients.	- it increases the accuracy of the collected data which is reflected in the accuracy of the monitoring; - it allows the selection of the criteria: frequency, mortality, costs, prevention possibilities; - it is more efficient by restricting the collection area and saving time; - it can be easily associated with other methods.	- it cannot detect infections in other unmonitored areas; - in some high-risk wards, the severity of the patients' pathology increases the time allocated for reading the medical records; - the specific results obtained cannot be compared with the rest of the hospital.	- surveillance the nosocomial problems in an Intensive Care unit; - surveillance the post-operative infections in a surgical ward.
Post-hospital discharge	- contacting the patient after hospital discharge by telephone, email or post; - contacting the attending physician/surgeon; - detecting hospital readmissions; - surveillance the antibiotic therapy in outpatients during the postoperative period.	- it allows the identification of an important segment of post-operative nosocomial wound infections, under the conditions of the permanent reduction of the hospitalization period (it is considered that about 70% of the cases occur after discharge).	- there is no standard method; - errors occur in identifying an infection; - reduced adherence to reporting by the healthcare personnel; - the impossibility to determine the clinical status of patients lost to follow-up; - difficulties in monitoring the ambulatory antibiotic therapy.	- surveillance the post-cardiac surgery endocarditis or post-operative wound infections.
Retrospective	- carried out by medical recordings, after the discharge of the patient; - all new cases in an anterior period are registered.	- each patient is investigated only one time; - it is very useful in epidemic situations; - it needs limited resources.	- the quality of the surveillance depends on the quality of the clinical documentation; - going too much in the past decreases the usefulness of the method due to the lack of data.	- investigating an epidemic with post-operative wound infections.

Prospective	- patient surveillance by repeated check-ups, for the entire hospitalization period.	- it uses all available data sources; - further investigations or further interventions may be initiated; - it increases the visibility of the specialized personnel by wards; - it facilitates the feedback on results.	- it requires more time and extensive efforts.	- surveillance cases of septicemia in an Intensive Care unit.
By longitudinal (incidence) study	- recording all newly onset infection cases in an area and calculating the incidence.	- it determines an overview of the issue; - it allows the analysis of the risk factors.	- it overloads the specialized personnel; - the adjusted rates cannot be calculated; - precise prevention objectives cannot be monitored; - it does not allow comparing the rates with those in other hospitals/areas.	- determining the incidence of food poisoning.
By cross-sectional (prevalence) study	- recording all infection cases (new + old) in a population of patients, in a single day or a certain period, with the calculation of the momentary (punctiform) or period prevalence.	- it requires limited time and efforts; it is carried out quickly by a trained team; - it is useful when wanting a quick and inaccurate assessment of a problem; - it makes it possible to determine the sensitivity of the surveillance system; and to analyze the efficiency of the intervention strategies; - it can be used for assessing the problem in an institution where there is no other surveillance system.	- less efficient in acute or short-term infections; - the prevalence rate is influenced by the duration of the infection and ends up overestimating the risk of patients for the nosocomial pathology; - result interpretation, with the detection of statistically significant differences, is difficult due to the small number of studied patients; - it does not allow comparing the rates with those in other hospitals/areas.	- prevalence of HCV infections in the patients of a healthcare unit, on a given day.

Sentinel	- collecting the data on certain infections from a geographically representative sample of interested medical units.	- it informs about the changes in global incidence; - it has a good sensitivity; - it requires limited time and effort; - case reporting is optimal.	- it only collects the number of cases with that pathology, without or with little information about the cases; - with low specificity.	- sentinel surveillance of the HCAIs (for bloodstream infections, post-operative wound infections) or of influenza.
Syndromic	- based on clinical signs and symptoms that precede the diagnosis but signal with sufficient probability a clinical case or epidemic onset.	- it ensures faster detection and a possible reduction of morbidity/mortality through the implementation of control measures.	- lack of experience of the healthcare personnel; - difficulty in choosing the value of the alert threshold; - too much geographical focus or focus on the health system levels; - it requires the creation of a software for the automatic collection and analysis of the clinical, and, possibly, laboratory data.	- the early identification of infectious diarrheal disease community epidemics, - early warning in case of bioterrorist attacks.
Of the alert microorganisms	- surveillance the incidence of strains such as MRSA, VISA, VRE, *Ps.aeruginosa/ Acinetobacter baumannii/* multidrug-resistant *Mycobacterium tuberculosis, C.difficile,* etc.	- it is easy, cost-effective, it can be automated in computerized laboratories; - it can show the evolutionary trends in time/by wards of the prevalence these specific strains.	- the isolation of these agents does not automatically mean infection, as their non-isolation does not mean the absence of infection/colonization.	- urgent reporting of the VISA/VRSA strains (in compliance with the CLSI standards).

3. The systematic data collection – There are multiple useful sources:

a. The morbidity data provided by the healthcare personnel (clinicians or representatives appointed by the healthcare units for this purpose) or from the official reports on the health status;

b. The mortality data, from vital records statistics, forensic services, etc. This information is important for diseases with high mortality rates;

c. The laboratory data identifying the etiological agent, its sensitivity characteristics, phenotype, genotype and helping to certify cases;

d. Reports on the epidemic outbreaks of typically manifest clinical cases (the atypical, subclinical or sporadic cases that may escape the surveillance system);

e. The epidemiological investigations of communicable disease cases and outbreak investigation reports, which can actively identify other undeclared cases;

f. The data relating to the animal sources of pathogens, vectors - especially important in zoonoses;

g. The demographic data for the characterization of the population - sex, age, profession, economic-social status, domicile. Even data on school and work absenteeism may be useful for the surveillance of infectious diseases;

h. The data provided by written or audiovisual media, data on medicine use, etc.

The clinician, official representatives of the health-care units report the communicable disease cases to the local Public Health authorities, and, from here, the information is disseminated to the regional centres, and then to the Ministry of Health. Depending on the report, there are several categories of communicable diseases:

➢ With mandatory individual reporting: cholera, plague, yellow fever, exanthematic typhus, malaria, polio, tetanus, anthrax, etc.;

➢ With periodic numerical reporting: rubella, varicella, pertussis, epidemic parotiditis, etc.

➢ With mandatory reporting of the epidemics, individual case reporting not being mandatory: staphylococcal food poisoning;

➢ Without reporting, in typical sporadic diseases or which do not impose control measures: common viral respiratory infections.[51]

The precise determination of the incidence of the monitored disease depends on the reporting quality, but also on the quality of the surveillance system.

4. Centralizing the collected data – It fully benefits from software applications, facilitating the statistical interpretation and table or graphic illustration. Additionally, the use of a computer network system allows to provide information feedback to the personnel involved in the preventive and control practice, favours a prompt response, including from the management departments, and a Web network allows the provision of specialized support from regional or national institutions.

The implementation of a national online system leads to the reduction of reporting delays, to a more efficient use of the working time, and to the increase of the potential for carrying out evolutionary analyses of the diseases. The inclusion of all microbiology laboratories in the network (as in the case of the OSIRIS infectious disease surveillance system in the Netherlands) allows access to the positive and negative results (facilitating the syndrome-based case definition), as well as the automatic interconnection with other surveillance systems (e.g. EARSS).[59]

5. Data analysis and interpretation - It consists of analyzing the data obtained depending on the temporal criterion, and location of the cases and affected persons. Finally, the data are summarized in tables, charts, maps. Data interpretation includes comparisons with similar periods in the past, with other areas, or case analyses by sex, age, occupation, environment of origin, etc.

6. Result dissemination - It is made both to the healthcare personnel who provided the data and to the decision makers. Additionally, the data may be communicated to the neighbouring countries, the WHO, especially in the case of diseases with international surveillance or epidemics.

Data presentation and interpretation in a suggestive form, followed by an interactive discussion, can have a positive impact on the implementation of the control measures. It is imperative to comply with data confidentiality and to use them strictly for the purpose of making the preventive and control activity more efficient (not for punitive purposes).

7. Assessing the surveillance system - A surveillance system should be simple, sensitive, flexible, acceptable to the personnel involved in this activity, prompt, representative and with a positive predictive value, i.e. the reported cases are real.

Modern surveillance/control systems have started to even use methods specific for molecular epidemiology, which come out of the exclusive sphere of medical research and find their place in the reference laboratories or Public Health institutions. Using molecular biology in the field of surveillance requires the identification of a sufficiently stable and discriminatory set of markers. These molecular markers are used for the fragmentation of the genome or proteome according to the monitored characteristics and can be represented by restriction fragments produced by pulsed-field gel electrophoresis (PFGE) or analysis of the restriction fragment length polymorphism (RFLP), variable number of tandem repeats (VNTR) from the multilocus analysis (MLVA), polymorphic sequences used in the multilocus sequence typing (MLST), whole genome sequencing (WGS), mononucleotide polymorphism (SNPs) or sequence of the influenza neuraminidase used for measuring the antiviral agent sensitivity. [60]

In 1996, the CDC initiated a molecular subtype surveillance network - PulseNet, which, starting with 2000, was also expanded to Canada, Europe, Latin America and Asia (including China), focusing on foodborne germs (including *Enterobacteriaceae* such as: *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp.). [61] In the field of nosocomiality, such programs operate for monitoring the MRSA and VRE, and there is a European project for storing molecular subtyping data on the priority germs for the control of hospital-acquired infections - MRSA, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* and certain viruses. [62]

Prevention refers to all medical, economical, social and political actions taken for maintaining and promoting health, i.e. for reducing the risks for a disease to outbreak and expand at the population level.[63]

Prophylactic (preventive) medicine is the branch of Medicine based on the application by physicians of prophylaxis measures.
The ancient saying "*It is easier to prevent than to treat a disease*" really finds its applicability in the context of modern medicine, when the costs are higher and higher, the number of elderly people is more and more significant in the population, and we are facing the emergence and re-emergence of certain communicable diseases. The medical specialties with an important role in Preventive Medicine are: Family Medicine, Epidemiology, Public Health, Hygiene, but every clinician's activity also includes a preventive component.
Prevention includes the following levels:

1. **Primordial prevention** - with the role of fighting the emergence and consolidation of certain social, cultural, economic habits that increase the risks of disease in the population. It includes the improvement of living conditions, hygiene, providing drinking water, food, fighting smoking, alcohol consumption, speed reduction campaigns; [1]
2. **Primary prevention** identifies and controls risk factors, for the purpose of preventing the onset of the disease at the individual and population level. It includes measures for maintaining an optimal body weight, for fighting the sedentary lifestyle, for immunizing the population against some communicable diseases, etc. It is achieved by:
   ➢ protection programs for people at risk;
   ➢ and population programs.
3. **Secondary prevention** includes measures for the early detection and correction of reversible imbalances that occur before the clinical manifestation of the disease (during the incubation or latency period). It is based on screening tests using the most accurate methods for early detection, which are less invasive, less expensive, and applicable at the population level (the detection of cervical cancer in the early stages using the Papanicoulaou test).
4. **Tertiary prevention** includes the medical activities by which the complications of the disease are prevented or reduced. It is part of the therapy of chronic pathologies and it includes measures for reducing the suffering, lesions, disabilities, as well as for helping the patient adapt to his/her new condition.[1,63]

Depending on the type of pathology on which prevention acts, we distinguish:
   ➢ **Prevention of communicable diseases:** of influenza, viral exanthems from early childhood, pertussis, polio, tetanus, rabies, A/B viral hepatitis, etc.;
   ➢ **Prevention of non-communicable diseases:** of cardiovascular, respiratory, digestive, oncological diseases, etc.

Depending on the specificity level, we distinguish:
   ➢ **General prevention**, by complying with personal and collective hygiene standards, health education, decontamination/disinfection/deratization measures, etc.
   ➢ **Prevention specific** for the pathology in question, by a specific active and/or passive immunization.

The directly observed therapy (**medical surveillance)** is the active detection activity of people or of a population segment at risk. There are 3 types of directly observed therapy:

> - **The directly observed therapy for protection purposes** is strictly related to primary prevention, by which the possibility for people at risk to develop the disease is avoided;
> - **The directly observed therapy for rebalancing**, associated with secondary prevention, consisting of measures to improve the living and working conditions, and, possibly, the pharmacological conditions, for those early detected with reversible homeostatic conditions;
> - And **the directly observed therapy for reconstruction-recovery**, addressed to the patients, for preventing complications and severe evolution.

Directly observed therapy is adapted to the types of risk factors, community, and pathology, in the form of programs with broad addressability. [63]

PREVENTION OF THE HEALTHCARE-ASSOCIATED INFECTIONS

Modern medicine, especially tertiary medicine, is tributary to a continuously increasing degree of (diagnostic and therapeutic) invasiveness, it addresses more and more patients with pronounced comorbidities, severe acute pathologies or immunosuppression, and, in addition, it is forced to withstand the assault of the microbial world, whose pathogenicity arsenal is constantly evolving. Under these conditions, healthcare-associated infections (HAI) have become an increasingly important problem.

According to the last European survey on the prevalence of healthcare-associated infections, carried out in 2016/2017, 6.5% of the acute patients and 3.9% of the residents of long-term care facilities develop at least one HAI, i.e. 1 patient out of 15.[64] Under these conditions, each hospital or healthcare unit is required to implement patient safety/risk reduction programmes for nosocomial infections. These guides/programmes are conceived and periodically updated, by the infection control committee, after having reviewed their own data and the data provided by the literature. They include complying with the standard precautions taken for each patient, regardless of the known infectious status, in order to prevent cross-transmission. Such precautions refer to hand hygiene, wearing protective equipment as needed (gloves, surgical gown, apron, mask, safety glasses/face shield, etc.), safe injection practices, safe handling of the medical equipment or specific procedures after contact with potentially contaminated surfaces, and the cough etiquette. [41]

Standard precautions together with current decontamination are applied to all patients. Blood, biological fluids (pericardial, pleural, peritoneal, amniotic, synovial, cerebrospinal fluid, sperm, vaginal discharge, tissues), excretions and secretions from all patients should be regarded as potentially infectious and appropriate precautions should be taken so as to minimize the risk of germ transmission. The concept of standard precautions includes:

1. hand antisepsis with alcoholic solutions or hand sanitation with water and soap, extremely important given that 70-80% of the exogenous HAIs are transmitted through the hands of the healthcare personnel. Hands can be contaminated with *Staphylococcus aureus*, enterococci, Gram-negative bacilli, *Clostridium difficile*, viruses, etc., by touching the skin, infected areas, contaminated surfaces from the nosocomial environment, especially those in the immediate vicinity of the patient. The direct contact with the patient, with his/her

fluids/excreta, catheter and wound care, determine an important microbial load, all the more persistent as long nails and/or rings are worn.[65]

The WHO guideline on hand hygiene in health care includes the following indications:

➢ wash hands with soap and water when the skin is visibly soiled with blood or other biological fluids, when potential spore-forming pathogens are suspected (*C. difficile,* noroviruses) or after using the toilet;

➢ the use of alcohol-based handrub is preferable if hands are not visibly soiled, in the following situations:
- Moment 1: before and after touching a patient;
- Moment 2: before performing a clean/aseptic procedure, regardless of whether gloves are used or not;
- Moment 3: after contact with biological fluids, excretions, mucous membranes, injured skin of the patient;
- Moment 4: after contact with the patient;
- Moment 5: after contact with inanimate surfaces or objects in the immediate vicinity of the patient;
- after removing sterile or non-sterile gloves.[66]

➢ soap and handrub should not be used concomitantly so as to avoid skin irritation.

Healthcare personnel should not wear rings, bracelets, watches (which interfere with hand hygiene), nails should be cut short and kept neat. Both in the washing and in the antisepsis of the hands, the 6 stages technique imposed by the WHO will be complied with: palm to palm, palm over dorsum of hand, palm to palm with fingers interlaced, fingers, nails, thumbs. It can be completed with the wrist, the duration being of 20-30 seconds. For washing, use water and liquid soap, with 2 consecutive soap applications, followed by the mandatory wiping with disposable paper towel.

2. use of personal protective equipment: Gloves reduce hand contamination, and pathogen transmission if used properly, but can also facilitate cross-transmission (if the same pair is worn for a long time, touching several patients and/or surfaces).

They must be worn when anticipating contact with blood, other biological fluids, secretions/excretions, mucous membranes; in case of venous or arterial access; CSF sampling; contact with patients who have open wounds, bedsores; handling contaminated instruments, for decontamination and disinfection. Gloves shall be changed when going from one patient to another and even for the same patient, if moving from a contaminated area to an intact one. Personnel will be trained on the

technique of putting on/removing the gloves. After use, gloves shall be removed by holding the first glove on the outside edge; then, the first glove will be thrown into the medical waste container and the other one will be grasped with the free hand from the inside, then carefully removed and thrown into the same container placed at hand. Hand washing or antisepsis should be performed before putting on the gloves and after having removed them as well. Do not reuse disposable gloves! [67]

The white coat must be worn (and sometimes, a protective plastic apron), in case of contact with the patient or his/her bed/care equipment/ dirty linen or with projections of biological fluids. The mask, safety glasses, face shield are recommended during medical procedures that can generate projections of blood, biological fluids and secretions, especially during wound irrigation, oral suction and endotracheal intubation.

3. safe injection practices – for avoiding the transmission of parenteral germs (HBV, HCV, HIV, etc.), from one patient to another or from one patient to the medical personnel. Asepsis will be complied with, starting from the sterility of the equipment and up to the hygiene rules in the preparation and administration of parenteral treatments. Prior to the parenteral administration of the treatment healthcare personnel must:

- Check the validity period of the syringe and needle sterilization;
- Check the validity period of the solutions, their appearance (they should be clear, transparent, without precipitate) as well as the integrity of the vial/ampoule;
- Suspensions should be stirred until the solubilisation of deposits;
- Wash the hands with water and soap/carry out the antisepsis procedure with alcoholic solution; for hemoculture, sterile gloves must be worn;
- Disinfect the vial plug;
- Insert the needle into the vials without touching the edges or tip of the vial;
- Must not keep the opened glass vials;
- If broken glass gets into the solution when opening a vial, its contents will no longer be used!
- Throw away the unlabelled vials/ampoules or vials/ampoules with illegible inscriptions!
- Avoid multidose vials as much as possible - when this is not possible, use a sterile needle every time the solution is extracted from the vial. No needle shall be left in the

rubber plug between manoeuvres! (this facilitates content contamination);

  – Must not perform inoculations in infected skin areas or areas with dermatological changes;
  – Antiseptize the site of inoculation with 70° alcohol ± another antiseptic;
  – Remove the air will from the syringe before administration;
  – Check the needle position by suction.

- The indications and contraindications for each administration method shall be strictly complied with:
  – Observing the chosen site;
  – Iso- and hypertonic solutions shall not be administered subcutaneously and intramuscularly (caustic effect!)
  – Oily solutions shall not administered intravenously (due to the risk of embolism).

4. handling in safe conditions the medical equipment/specific procedures after contact with potentially contaminated surfaces: All care equipments, any surface in the environment and particularly those which are frequently touched by patients shall be rigorously decontaminated. All reusable equipments/instruments shall be reprocessed in an appropriate manner before being used for another patient and linen shall be handled with gloves, in order to prevent the transfer of microorganisms.[41]

5. respiratory hygiene and cough etiquette includes covering the oral cavity in case of coughing, sneezing, using disposable tissues, followed by hand hygiene, and positioning at minimum 1 meter from another person. This behaviour should be applied by both healthcare personnel and patients, visitors or other categories of people. [41]

Complying with the concept also imposes certain requirements related to the nosocomial environment. Thus, the distance between beds should be sufficient, so that there is no possibility to touch both beds at the same time. Increasing this distance decreases the risk of pathogen transfer. A sufficient number of sinks is required in order to ensure hand hygiene. Alcohol-based handrub dispensers must be available and placed in accessible places.

In case of known infection, additional precautions should be taken, based on the transmission method. [41] These are:

Contact precautions – include the use of medical protective equipment by the healthcare personnel, when there is a risk of coming into contact with germs transmitted in this way, and placing the patient alone in one room. If not available, the risk of placing the patient in

question with other patients (cohorting with other patients infected with the same pathogen) should be assessed. If a substantial contact with the patient, environmental surfaces or objects in the hospital room is anticipated, it is recommended to wear gloves, the white coat/sometimes even an impermeable apron. The white coat and gloves should be put on before entering the hospital room and removed before getting out, first the gloves and then the coat. Hand antisepsis or current and terminal cleaning and decontamination are required. Contact isolation is required in case of: hepatitis A virus; *Herpes simplex*, enteral pathogens: *Cl. difficile*, enterobacteria; multi-resistant bacteria: MRSA, VRE. These are also extended to patients with possibly contagious secretions:

- Wound infections, drained abscesses, bedsores;
- Impetigo;
- Scabies;
- Patients with incontinence (including infants, children, patients with altered mental status), etc.[41, 68]

Droplet precautions – it is recommended to place the patient alone in a hospital room (or with another patient infected with the same pathogen) and to wear face protection, when working at a short distance (under 2-3 m) from the patient. Wearing a mask, face shield or safety glasses can prevent secretions from coming into contact with mucous membranes and can provide protection against airborne germ transmission. If the patient must be transported outside the isolation room, he/she will be recommended to wear the respiratory mask. Droplet isolation is required for germs transmitted via Flűgge droplets: *Haemophylus influenzae; Neisseria meningitidis; Streptococcus pyogenes; Corynebacterium diphteriae; Bordetella pertussis;* influenza, rubella, urlian, syncytial virus, etc.

Respiratory precautions – are taken for the airborne germs, transmitted by very small particles, which makes them very contagious (*Mycobacterium tuberculosis*, varicellozosterian virus, measles virus). They require placing the patient alone in a hospital room with a bathroom and special ventilation, and wearing a mask. An isolation room with negative air pressure compared to the hallway, direct air elimination in the exterior environment or air recirculation through a highly efficient HEPA filter (6-12 air changes per hour) is recommended. Respiratory isolation requires visitor triage and current and terminal decontamination. [41]

For patients with allergenic hematopoietic stem cell transplantation, **protective isolation** is required, in a room with positive air pressure compared to the hallway, with inlet air filtration including over 12 changes per hour and strict control, in order to prevent exposure to

environmental fungal spores. In general, isolation is associated with negative psychological effects, decreased contact with the physician and other adverse effects, therefore it should be interrupted as soon as possible.

As healthcare-associated infections may also affect medical personnel, not only the patients, an important chapter refers to the prevention of occupational accidents. This exposure may occur by percutaneous inoculations (pricking; cutting), pre-existing continuity solutions or contamination of mucous membranes during invasive procedures, handling of biological products, potentially infected instruments/materials and medical waste.

Occupational accidents may be prevented through:

1. the management of sharp objects with the reduction of parenteral manoeuvres to the minimum level required; collection in medical waste containers for sharp objects for destruction; avoiding needle recapping; eliminating the syringes with their needles and carefully handling the sharp instruments.

2. the management of linen with minimum handling, in places specifically intended for that purpose; wearing adequate protective equipment, collecting it in waterproof bags and ensuring a proper decontamination process.

3. the management of infectious waste and collection in labelled medical waste containers and neutralization by burning/autoclaving;

4. environment decontamination by cleaning, disinfection and sterilization;

5. a rigorous personal hygiene, avoiding skin continuity solutions and immune deficiencies.[69]

PREVENTION OF THE MAIN HEALTHCARE-ASSOCIATED INFECTIONS

Prevention of the nosocomial urinary tract infections

Although frequent, this type of healthcare-associated infections is encumbered by a lower fatality rate. However, they lead to an increased length of hospital stay, to the increase of the cost of treatment and may lead to important complications, especially since multidrug-resistant or extended-spectrum strains are frequently involved. Their prevention includes the following measures:

- limiting the indications and duration of bladder catheterization;
- continuously training the healthcare personnel and establishing teams that are periodically trained on the technique of bladder catheterization and possible complications;

- using the closed urinary catheterization system, where the catheter and the drainage bag are assembled together, further disconnections are avoided, and the aseptic emptying of the bag is done through a lower valve;
- antisepsis of the hands before or after any handling of the catheter;
- wearing sterile gloves for the personnel carrying out the catheterization;
- preparing the genito-urinary area by washing with sterile water or antiseptic solution;
- lubricating the catheter with a single-dose lubricant (not usually recommended);
- the catheter lumen should be adequate, as small as possible in order to prevent harming the urethra;
- the catheter and the discharge bag should be installed by an aseptic technique;
- the catheter should be fixed so as to prevent the movement and traction of the urethra;
- the discharge bag should be fixed so as to allow the regular elimination of the urine, while preventing the contact of the bag with the floor;
- urinary samples shall be taken in strict compliance with the asepsis procedure;
- the periodic emptying of the discharge bag into an individual container (when ¾ full), for each patient, without a direct contact between the bag and the non-sterile container;
- the catheterized patient requires a rigorous daily cleaning, proper hydration, clinical supervision;
- bladder washes and irrigation procedures should be avoided (except in the case of possible obstructions, when they will be performed in strict compliance with the asepsis procedure);
- if antibiotic therapy is required, the duration should be as short as possible;
- if the catheter is not removed, the therapeutic decision and efficiency assessment shall be based on the clinical evolution and less on the microbiological results;
- removing the catheters after arbitrarily established intervals should be avoided;
- in order to prevent the cross-transmission of germs, healthcare workers must avoid placing the infected patients in beds located next to the uninfected ones.[70]

Prevention of exogenous pneumonia

Along with bloodstream infections, nosocomial pneumonias are among the most severe nosocomial infections. Prophylaxis measures focus on:

- hand decontamination by washing with antimicrobial soap or alcohol-based antiseptic agents, before and after contact with the patient;
- the use of gloves when handling the endotracheal tube or when suctioning the bronchial/oropharyngeal secretions;
- sterilizing/disinfecting all the instruments – intubation catheters, nasal catheters, oxygen therapy masks, equipment for assisted ventilation or general anaesthesia (or using disposable materials);
- changing nasal catheters or masks for oxygen administration, as patients change, and using sterile water for oxygen humidification;
- humidifiers must be cleaned, disinfected on a daily basis;
- checking the air conditioning system in the hospital in order to avoid the pathology determined by *Pseudomonas aeruginosa* or *Legionella pneumophila*;
- the "droplet" isolation for patients infected with respiratory viruses or colonized/infected by multidrug-resistant airborne bacteria;
- establishing quarantine/restricting the visitors' access during the periods of community respiratory epidemics.
- the reusable components of the anaesthesia equipment, which come into direct contact with the oral and tracheal mucosa (face mask, tracheal catheter) or can be contaminated with respiratory secretions (the Y-piece, inspiratory and expiratory tubes, humidifier):
  - must be cleaned and subjected to sterilization by either autoclaving or by using ethylene oxide (when the material allows it), or to high level disinfection by using chemical disinfectant liquids.
  - and then rinsed with sterile water in order to prevent any microbial contamination.
    - intubation catheters, tracheotomy tubes are disposable
- masks shall be subjected to high level chemical disinfection between 2 uses;
- anti-bacterial and antiviral filters can be used for preventing cross-transmission.[71]

Prevention of endogenous pneumonia

It is essential for Intensive Care services, intubated patients, with high risk of reflux. Therefore, the following measures are required:

- using gloves when handling the endotracheal tube or when suctioning the bronchial/oropharyngeal secretions;
- preventing the suctioning of the gastric fluid by raising the cephalic extremity at an angle of 30-45°C, avoiding deep, prolonged sedation and not recommending the routine administration of antacids to mechanically ventilated patients;
- preventing the suctioning of bronchial/oropharyngeal secretions by oropharyngeal antisepsis and periodical suctions at regular intervals (3-4 h), after a prior washing with antiseptic solutions or sterile saline;
- using non-invasive ventilation, if the clinical condition allows it/preferring the orotracheal intubation, if assisted ventilation is indispensable;
- avoiding the colonization of the lower respiratory tract by maintaining the cough reflex/superficializing the sedation;
- the colonization of the respiratory tract is also tackled with aerosols containing antimicrobial solutions, in the perioperative period;
- avoiding the excessive use of antibiotic therapy in order to minimize the risks of multidrug resistance;
- in the ICU services, where there are high risks of contacting endogenous nosocomial pneumonia:
  - different schemes of selective decontamination of the digestive tract can be applied, by administering non-absorbable antibiotics, or systemic anti-infectious chemotherapy, but the benefits are counterbalanced by the increase of the potential for bacteria to develop multidrug resistance;
  - therefore selective decontamination schemes are not routinely recommended for intubated/mechanically ventilated patients in the ICUs!

Prevention of postoperative pneumonia

After surgery, due to prolonged clinostatism, pain at the incision level, causing cough avoidance, bronchial secretions persist in the bronchial tree and may lead to lower respiratory tract infections. In order to avoid them, the following measures are recommended:

- no smoking, at least 15 days before the intervention;

- treating the preoperative respiratory infections;
- facilitating the drainage of respiratory secretions through pre- and post-operative kinesitherapy - deep inspiration and favouring cough reflux;
- early mobilization and
- permissive postoperative analgesia for cough.

Prevention of the nosocomial surgical site infections

Surgical interventions, through the wide openings they make, create multiple contamination/infection possibilities. Therefore, the prevention of postoperative infections starts from the preoperative phase, it is essential during the intervention and continues after surgery. Prevention guidelines include the following measures:

Preoperative measures
- maximum limitation of the preoperative hospitalization period, with the performance of some examinations outside the hospital;
- treating the preoperative infections before the intervention (e.g. urinary tract, skin infections);
- **laughing is forbidden!** If depilation is necessary, it is preferable to cut the hair using a special machine, right before the operative act (possibly chemical hair removal, after having performed a skin sensitivity test);
- the patient's personal cleaning includes a general shower (including the hair) with antiseptic soap, performed in the evening before the intervention and repeated, if possible, in the morning;
- the patient is asked about his/her allergic history before choosing the antiseptic solution;
- the antisepsis of the incision area is performed with antiseptic solutions in concentric circles/parallel lines, from the centre to the periphery;
- access to the operating theatre is restricted, with strict limitation to the authorized medical and care personnel;
- the surgical lavage of the hands and dressing of the operating team according to the protocols in force;
- decontamination of the **operating theatre** by cleaning followed by high level disinfection or sterilization of all materials allowing such a process, according to the existing protocol;
- permanently checking the proper functioning of the air conditioning system in the operating theatre;

- obliging the healthcare personnel to declare the infectious pathology, with the temporary relief from professional duties, without being afraid of possible repercussions;

Intraoperative measures
- wearing the adequate, sterile protective equipment, by all members of the operating team – surgical gown, mask, cap, safety glasses, gloves. It is recommended to use 2 pairs of sterile surgical gloves, changing the external pair after each operating time or in case of contamination/perforation/penetration of blood or other biological products;
- using correctly sterilized surgical instruments;
- strictly complying with the asepsis procedure and minimizing surgical traumas (efficient hemostasis, minimal tissue devitalisation);
- correct suturing, avoiding the devitalized spaces (especially in the case of obese patients);
- performing the drainage by a separate incision;
- a good intra- and post-operative tissue oxygenation, as well as the control of body temperature and blood glucose below 200 mg/dl (for both diabetic and non-diabetic patients) are ensured;
- the parenteral antibioprophylaxis is indispensable in the case of class I surgery interventions (clean - vascular, cardio-vascular surgery) and in clean-contaminated/contaminated interventions or interventions with a NNIS risk ≥1 using anti-infectious chemotherapy with proven efficacy according to specialized studies;
- in septic interventions, the antibiotic therapy is chosen and applied depending on the location, severity of the infection and microorganisms involved;
- antibioprophylaxis starts when anaesthesia is induced and is limited to the duration of the intervention (with every 3 hours re-administrations in case of prolonged interventions), and for maximum 24-48 hours after surgery (for class I);
- the extension of postoperative antibioprophylaxis does not reduce the HAI rate but it increases the risk for enterocolitis caused by *Clostridium difficile* and of multidrug resistance of bacteria;
- oral antibioprophylaxis may be used in cases where there is a risk of endocarditis.[72]

Postoperative measures
- a sterile dressing shall be applied (after the formation of the fibrin network, a biological environment that is less influenced from the exterior is created);
- the healthcare personnel must insist on a rigorous hand hygiene before and after changing the dressing or in case of any contact with the surgical suture area;
- the dressing shall be changed in strict compliance with the asepsis procedure;
- the patient shall be educated on reporting any symptoms that may appear during the postoperative period;
- early mobilization for the prevention of bedsores.[72]

Prevention of bloodstream infections after catheterization

Nosocomial septicaemias are very fatal (about 50% of the deaths occurring in a hospital are due to primary or secondary septicaemias). Multiple measures should be applied, including:
- limiting the indications;
- compliance with the protocol for the implantation, maintenance of the intravascular device and for diagnosing of catheter infections;
- the introduction shall be performed under rigorous asepsis conditions by experienced personnel;
- preferential use of the subclavian access, in case of central catheterization;
- antisepsis of the venipuncture site;
- catheter anchorage in order to reduce the risk of colonization;
- using a sterile dressing, preferably semipermeable, and transparent, which allows the permanent examination of the insertion site;
- no topical antibiotics or ointments shall be used on the insertion site, due to the risk of developing microbial resistance;
- no intranasal or systemic antimicrobial prophylaxis shall be administered;
- using only sterile infusion solutions, after having checked their validity period and appearance!
- quick detection of the local inflammatory signs by regular visualization and palpation, while also asking the patient to report any discomfort related to the intravascular device;
- using the Visual Infusion Phlebitis score (VIP Score by Andrew Jackson), and removing the venous catheter at a score equal to and greater than 2;

- the catheter insertion date and time, the name of the person who implanted it, the date when the dressing was change and the date when the intravascular device was removed will be recorded in the medical record;
- the personnel shall comply with the hygiene rules, by washing their hands with water and antibacterial soap or using an alcohol-based product;
- limiting the system handling, by administration of additional medicines, in the solution container, at the end of the infusion line;

For peripheral catheterization
- avoid implementing the peripheral catheter in the veins of the lower limbs;[73]
- change the infusion tubing and attachments in case of i.v. administration of blood preparations or lipid solutions, after each product has passed;
- connections must be disinfected before each administration.

For central catheterization
- training the healthcare personnel to perform the surgical lavage of the hands, and putting on the sterile equipment (mask, cap, surgical gown, gloves);
- preparing the catheter insertion area:
    - hair removal is not recommended (if depilation is indispensable, use a special trimming device or hair removal cream);
    - the antisepsis of the place shall be carried out on an area larger than the insertion area;
    - a sterile field shall be installed over the insertion area;
- the catheter shall be secured to the skin using a non-absorbable, solid suture;
- the insertion area shall be covered with a sterile dressing, so as the catheter may be inspected on a daily basis.

Annex I. The technique of antisepsis for hands of the medical personnel - total duration of the procedure 20-30s (World Health Organization 2009) [43]
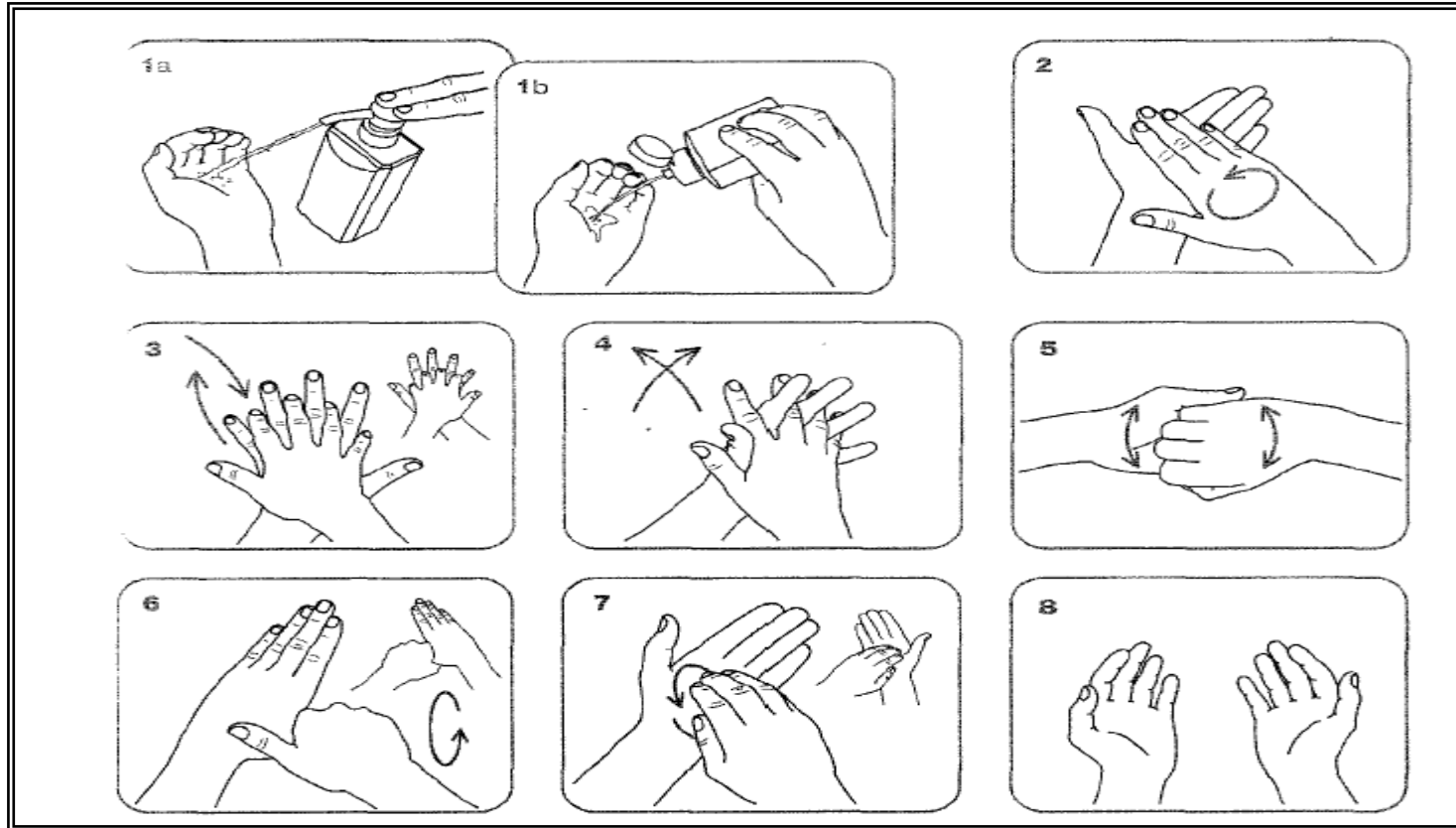
Annex II. The technique of hand washing for medical personnel - total duration of the procedure 40-60 s. (World Health Organization 2009) [43]
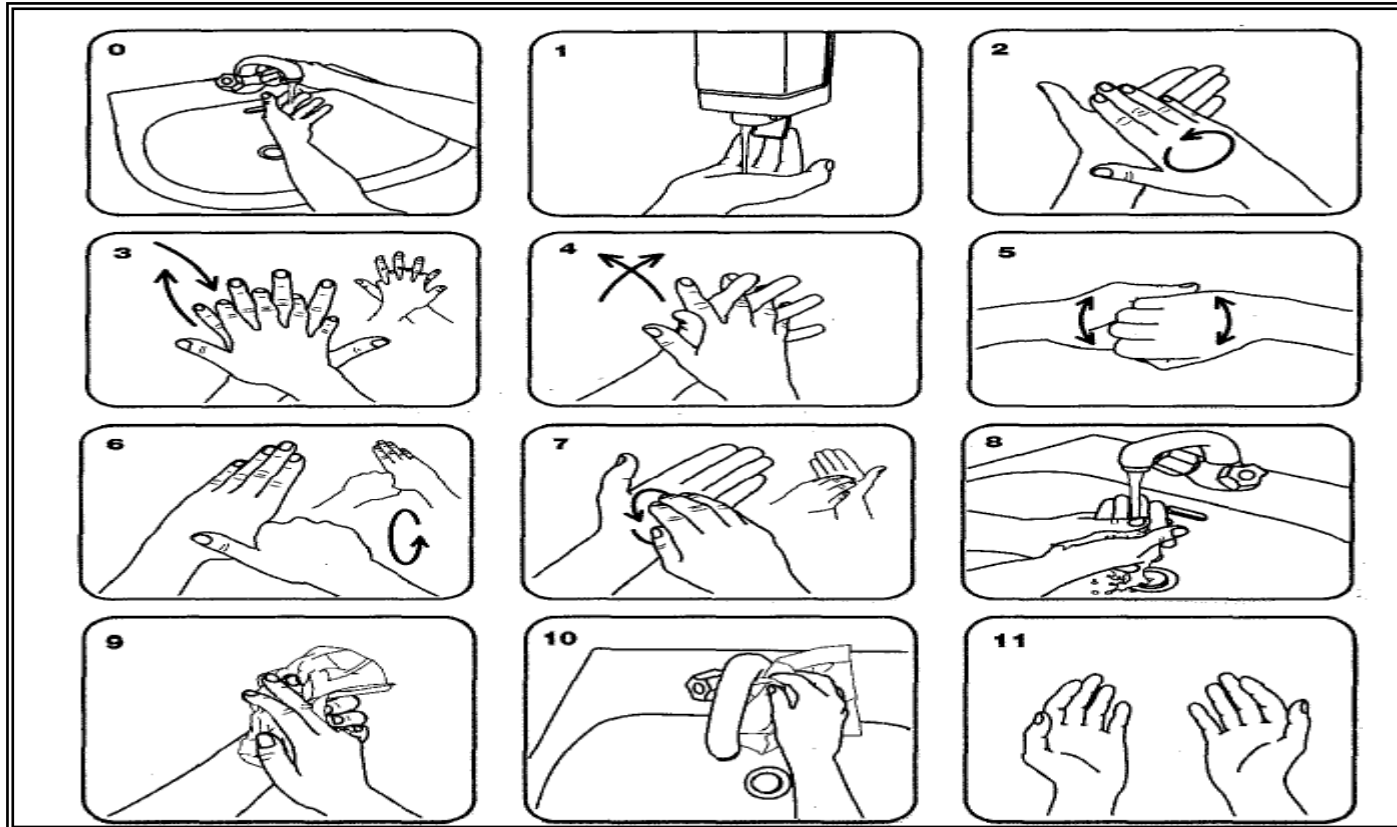
BIBLIOGRAPHY

1. Ioan Stelian Bocşan, Amanda Rădulescu, Irina Brumboiu, Ofelia Şuteu, A.Achimaş. Epidemiologie practică pentru medicii de familie, Editura medicală universitară "Iuliu Haţieganu", Cluj-Napoca, 1999: 81-85; 609-629

2. Irina Maria Brumboiu, Ioan Stelian Bocşan. Vaccinuri şi vaccinări în practica medicală, Editura medicală universitară "Iuliu Haţieganu", Cluj-Napoca, 2005: 38; 75-124;145-161

3. *** Ordinul nr. 978/2019 pentru prelungirea termenului de aplicare a prevederilor Normelor tehnice de realizare a programelor naţionale de sănătate publică pentru anii 2017 şi 2018, aprobate prin Ordinul ministrului sănătăţii nr. 377/2017, precum şi modificarea şi completarea acestora. *https://lege5.ro/Gratuit/gmztqmjzgiyq/ordinul-nr-978-2019-pentru-prelungirea-termenului-de-aplicare-a-prevederilor-normelor-tehnice-de-realizare-a-programelor-nationale-de-sanatate-publica-pentru-anii-2017-si-2018-aprobate-prin-ordinul-mi*

4. ASEAN Disaster News Watch *http://tsunami.aseansec.org/index.php?OP=NEWS&NEWS=1108729929*

5. *** General recommendation on immunization - Recommendation of the Advisory Committee on Immunization Practice and the American Academy of Family Physicians, MMWR, 2002, 51, RR-2: 1-35

6. Susan Plotkin, Stanley Plotkin. A short history of vaccination in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 11

7. Kim Connelly Smith, Ian M. Orme, Jeffrey R. Starke. Tuberculosis Vaccines, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 857-878

8. *** *http://www.who.int/mediacentre/factsheets/fs114/en/*

9. *** *http://www.mediafax.ro/social/alerta-dupa-anuntul-oms-privitor-la-o-epidemie-de-poliomielita-6139348*

10. *** *http://www.polioeradication.org/Dataandmonitoring/Poliothisweek.aspx*

11. E Anis, E Kopel, S R Singer, E Kaliner, L Moerman, J Moran-Gilad, D Sofer, Y Manor, L M Shulman, E Mendelson, M Gdalevich, B Lev, R Gamzu, I Grotto. INSIDIOUS REINTRODUCTION OF WILD POLIOVIRUS INTO ISRAEL, 2013, *http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20586*

12. *http://www.scientificamerican.com/article/polio-re-emerges-in-syria-and-israel-threatening-europe/*

13. *http://adevarul.ro/international/in-lume/focarul-poliomielita-siria-amenintare-reala-europa-1_ 527f7192c7b855ff56db8c83/index.html*

14. Rolland W. Sutter, Olen M. Kew, Stephen L. Cochi. Poliovirus vaccine-live, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 631-677

15. Stanley A. Plotkin, Emmanuel Vidor. Poliovirus vaccine-inactivated, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 605-625

16. Charles R. Vitek, Melinda Wharton. Diphtheria toxoid, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 139-152

17. Kathryn M. Edwards, Michael D. Decker. Pertussis vaccines, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 467-510

18. Steven G.F.Wassilak, Martha H.Roper, Katrina Kretsinger, Walter A. Orenstein. Tetanus toxoid, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 805-830

19. Aruna Chandran, James P. Watt, Mathuram Santosham. *Haemophilus influenzae* vaccines, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 157-170

20. Sanofi Pasteur. Prospect vaccin Pentaxim

21. Peter M. Strebel, Mark J. Papania, Gustavo H. Dayan, Neal A. Halsey. Measles vaccine, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 353-386

22. Stanley A. Plotkin, Susan E. Reef. Rubella vaccine, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 735-761

23. Stanley A. Plotkin, Steven A. Rubin. Mumps vaccine, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 435-458

24. Eric E. Mast, John W. Ward. Hepatitis B vaccines, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 205-233

25. WHO, Hepatitis B, Fact sheet. *https://www.who.int/news-room/fact-sheets/detail/hepatitis-b*

26. John G.Bartlett. Ghid de buzunar - Tratamentul bolilor infecţioase, Editura Medicală Amaltea, Bucureşti, 2007: 96-125

27. Carolyn B. Bridges, Jacqueline M. Katz, Roland A. Levandowski, Nancy J. Cox. Inactivated influenza vaccines, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 259-284

28. *** *http://en.wikipedia.org /wiki/Influenza_A_virus_subtype_H1N1*

29. *** Surveillance Report. Weekly influenza surveillance overview, 09.04.2010,*http://www.ecdc.europa.eu/en/activities/surveillance/EISN/Pages/index.aspx*

30. Sanofi Pasteur. Prospect vaccin Vaxigrip

31. Robert B. Belshe, Robert Walker, Jeffrey J. Stoddard, George Kemble, Husein F. Maasab, Paul M. Mendelman. Influenza vaccines-live, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008: 291-305

32. Anthony E. Fiore, Stephen M. Feinstone. Hepatitis A vaccines, in Stanley Plotkin, Walter Orenstein, Paul Offit. Vaccines, Saunders Elsevier, Fifth Edition, 2008:177-196

33. Sorina Maria Denisa Laitin. Ancheta epidemiologică. Îndreptar pentru studenţi, Editura Mirton, Timişoara, 2003: 12-34; 64-76

34. Ioan Stelian Bocşan. Combaterea (controlul) în bolile transmisibile, în Ivan A. şi colaboratorii, Tratat de epidemiologie a bolilor transmisibile, Editura Polirom, Bucureşti, 2002: 119-130

35. *** Hotărârea Ministerului Sănătăţii Publice nr.589 din 13.06.2007 privind stabilirea metodologiei de raportare şi de colectare a datelor pentru supravegherea bolilor transmisibile

36. Maftei I. Maftei, I. M. Golopentia, Asepsie, antisepsie, sterilizare, Editura Cerma, Bucureşti, 1994

37. T. Marlind. Comparison of Different Sterilization Methods, Pharmaceutical and Medical Packajing, Copenhagen, Denmark, 1997

38. Gerald E. McDonnell. Antisepsis, disinfection, and sterilization: types, action, and resistance, Editura Wiley-Blackwell, 2007

39. Gurusamy Manivannan. Disinfection and decontamination: principles, applications and related issues, Editura CRC Press, 2008

40. *** Ordinul nr. 961/2016 pentru aprobarea Normelor tehnice privind curăţarea, dezinfecţia şi sterilizarea în unităţile sanitare publice şi private, tehnicii de lucru şi interpretare pentru testele de evaluare a eficienţei procedurii de curăţenie şi dezinfecţie, procedurilor recomandate pentru dezinfecţia mâinilor, în funcţie de nivelul de risc, metodelor de aplicare a dezinfectantelor chimice în funcţie de suportul care urmează să fie tratat și a metodelor de evaluare a derulării şi eficienţei procesului de sterilizare. *https://lege5.ro/Gratuit/gezdombzgmya/ordinul-nr-961-2016-pentru-aprobarea-normelor-tehnice-privind-curatarea-dezinfectia-si-sterilizarea-in-unitatile-sanitare-publice-si-private-tehnicii-de-lucru-si-interpretare-pentru-testele-de-evaluar*

41. *** Ordinul nr. 1101/2016 privind aprobarea Normelor de supraveghere, prevenire şi limitare a infecţiilor combined asistenţei medicale în unităţile sanitare. *https://lege5.ro/Gratuit/geztanjsgmzq/ordinul-nr-1101-2016-privind-aprobarea-normelor-de-supraveghere-prevenire-si-limitare-a-infectiilor-combined-asistentei-medicale-in-unitatile-sanitare*

42. William A. Rutala. Disinfection, Sterilization, and Antisepsis, APIC 2006

43. Benedetta Allegranzi, Claire Kilpatrick, Didier Pittet. Hand Hygiene in IFIC Basic Concepts of Infection Control, International Federation of Infection Control, 2 Edition, Portadown, 2011: 142-143

44. Roxana Moldovan şi colaboratorii. Îndreptar de lucrări practice, Uiversitatea de Medicină şi Farmacie „Victor Babeş" Timişoara, LITO 2002: 38-44

45. D.Buiuc, G.Coman, M.Neguţ. Microbiologie clinică, Editura Didactică şi Pedagogică R.A., Bucureşti 1998: 239-246; 392-399

46. Georgeta Yanoschi, Maria Liliana Iliescu. Metode de evaluare statistică cu largă utilizare în epidemiologie, în Ivan A. şi colaboratorii, Tratat de epidemiologie a bolilor transmisibile, Editura Polirom, Bucureşti, 2002: 144-148

47. Rebecca Knapp, M.Clinton Miller III. Risk and Causality in Clinical Epidemiology and Biostatistics, Williams & Wilkins, Baltimore, Maryland, 1992: 109-122

48. Gheorghe Ioan Mihalaş. Metodologia cercetării ştiinţifice medicale, Scoală doctorală, Universitatea de Medicină şi Farmacie „Victor Babeş" Timişoara, 2006

49. Margit Şerban. Evaluarea atitudinii terapeutice, Scoală doctorală, Universitatea de Medicină şi Farmacie „Victor Babeş" Timişoara, 2006

50. Ioan Stelian Bocşan, Irina Maria Brumboiu. Metode de lucru şi cercetare în epidemiologie, în Ivan A. şi colaboratorii, Tratat de epidemiologie a bolilor transmisibile, Editura Polirom, Bucureşti, 2002: 63-75

51. Ioan Stelian Bocşan. Supravegherea epidemiologică în sănătatea comunitară, în Ivan A. şi colaboratorii, Tratat de epidemiologie a bolilor transmisibile, Editura Polirom, Bucureşti, 2002:85-103

52. Eggimann P., Pittet D. Infection Control in the ICU. Chest 2001; 120: 2059-2093

53. Gastmeier P., Coignard B., Horan T. Surveillance for healthcare-associated infections, in M'ikanatha N.M., Lynfield R., Van Beneden C.A., de Valk H. Infectious Disease Sueveillance, 1th edition, Oxford Blackwell Publishing 2007: 159-170

54. Horan T.C., Gaynes R.P. Surveillance of nosocomial infections, in Mayhall G.C. Hospital Epidemiology and Infection Control, 3th edition, Atlanta: Lippincott Williams&Wilkins 2004: 1659-1700

55. Giesecke J. Routine surveillance of infectious diseases, in Giesecke J. Modern Infectious Disease Epidemiology, 2th Edition, London: Arnold Editure 2002: 148-159

56. Yunesian M. Nosocomial Infection Surveillance Methods, *http://www.pitt.edu/~super1/lecture/lec2041/index.htm*

57. Borchardt S.M., Ritger K.A., Dworkin M.S. Categorization, Prioritization and Surveillance of Potential Bioterrorism Agents, in Khardori N., Moellering R.C. Bioterrorism and Bioterrorism preparedness; Infectious disease clinics of North America 2006; 20(2): 216-224

58. French G. Surveillance for Healthcare-Associated Infections in IFIC Basic Concepts of Infection Control, International Federation of Infection Control, Portadown, 2011: 19-26

59. Bosman A., van Vliet. H. The Netherlands'Infectious Diseases Surveillance Information System (ISIS), in M'ikanatha N.M., Lynfield R., Van Beneden C.A., de Valk H. Infectious Disease Sueveillance, 1th edition, Oxford Blackwell Publishing 2007: 294-302

60. Besser J. Use of molecular epidemiology in infectious disease surveillance, in M'ikanatha N.M., Lynfield R., Van Beneden C.A., de Valk H. Infectious Disease Sueveillance, 1th edition, Oxford Blackwell Publishing 2007: 393-440

61. Swaminathan B., Gerner-Smidt P., Ng LK et al. Building Pulse-Net International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne disease, Foodborne Pathog Dis 2006; 3(1):36-50

62. Health Protection Agency. Microbial Identification and Typing Databeses, *www.hpa.org.uk/cfi/bioinformatics/dbases.htm*

63. Aurel Ivan. Prevenția, în Ivan A. și colaboratorii, Tratat de epidemiologie a bolilor transmisibile, Editura Polirom, București, 2002:104-117

64. Carl Suetens, Katrien Latour, Tommi Kärki, Enrico Ricchizzi, Pete Kinross, Maria Luisa Moro, Béatrice Jans, Susan Hopkins, Sonja Hansen, Outi Lyytikäinen, Jacqui Reilly, Aleksander Deptula, Walter Zingg, Diamantis Plachouras, Dominique L Monnet, the Healthcare-Associated Infections Prevalence Study Group . Prevalence of healthcare-associated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and long-term care facilities: results from two European point prevalence surveys, 2016 to 2017. *https://www.eurosurveillance.org/ content/10.2807/1560-7917.ES.2018.23.46.1800516*

65. Monica Licker, Emilia Nicoară, Luminița Bădițoiu, Elena Hogea, Delia Munteanu, Florin Horhat, Roxana Moldovan. Ghid pentru prevenția multirezistenței bacteriene, Eurobit, Timisoara 2011: 130-137

66. *https://www.who.int/infection-prevention/campaigns/clean-hands/ 5moments/en/*

67. Institutul Național de Sănătate Publică. Analiză de situație: Campania anuală mondială „SALVEAZĂ VIEȚI : IGIENA MÂINILOR!", 5 MAI 2015, *http://insp.gov.ro/sites/cnepss/wp-content/uploads/2016/01/ ANALIZA-DE-SITUATIE-2015.pdf*

68. Brenner P., Ransjo U. Isolation Precautions in IFIC Basic Concepts of Infection Control, International Federation of Infection Control, Portadown 2007:75-82

69. *** Ordinul nr. 916/2006 privind aprobarea Normelor de supraveghere, prevenire și limitare a infecțiilor nosocomiale în unitățile sanitare. *https://lege5.ro/Gratuit/geydcnbsha/ordinul-nr-916-2006-privind- aprobarea-normelor-de-supraveghere-prevenire-si-control-al-infectiilor- nosocomiale-in-unitatile-sanitare*

70. Carolyn V. Gould, Craig A. Umscheid, Rajender K. Agarwal, Gretchen Kuntz, David A. Pegues, and the Healthcare Infection Control Practices

Advisory Committee (HICPAC). GUIDELINE FOR PREVENTION OF CATHETER-ASSOCIATED URINARY TRACT INFECTIONS 2009. *https://www.cdc.gov/infectioncontrol/pdf/guidelines/cauti-guidelines-H.pdf*

71. Ofelia C. Tablan, Larry J. Anderson, Richard Besser, Carolyn Bridges, Rana Hajjeh. Guidelines for Preventing Health-Care--Associated Pneumonia, 2003. *https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5303a1.htm*

72. Institutul Național de Sănătate Publică. Analiză de situație: Campania mondială multi-anuală „SALVEAZĂ VIEȚI : IGIENA MÂINILOR!", 5 MAI 2017, „COMBATE REZISTENȚA MICROBIANĂ-ESTE IN MÂINILE TALE". *http://insp.gov.ro/sites/cnepss/wp-content/uploads/2017/10/ANALIZA_Igiena_Mainilor_2017.pdf*

73. CDC. Intravascular Catheter-related Infection (BSI). Summary of Recommendations. *https://www.cdc.gov/infectioncontrol/guidelines/bsi/index.html*