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

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For a correct sampling

things to keep in mind

- which are the pathological products that can contain the suspected germs, depending on the different evolutionary stages of the disease;
- what is the optimal time to take a sample;
- whether the biological product is normally sterile or contaminated with saprophytic flora;
- what is the correct sampling technique, respecting the asepsis;
- what is the required amount of that pathological product;
- how this product should be packaged and transported;
- what is the optimal time to get to the lab;
- and how it can be preserved when it can not be transported immediately.

Sampling technique

- The recipient will be enrolled with the patient's name / surname, the pathological product and the required exam and in the referral note, the number of the observation sheet, the presumed diagnosis, the date / time of the sampling, and other significant data that can guide the microbiological analysis.

- The optimal time for the sample to reach the laboratory is 1-2 h, depending on the pathological product and also on suspected germs.

- Products that are harvested in epidemiological practice can be secretions, excretions, tissue fragments obtained by biopsy or autopsy, food, water, air and so on. Most of the evidence comes from the sick, but also convalescents, healthy bearers, contacts or deceased.

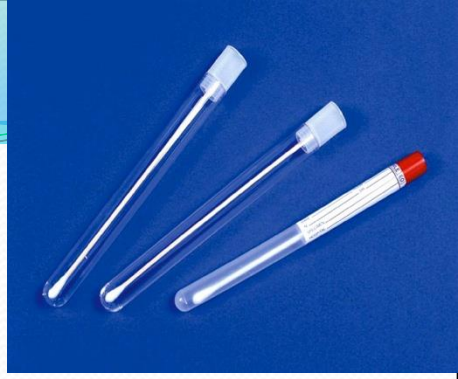
Respiratory System Infections

Taking samples of nasal exudate (in sinusitis, angina, germs colonisation)

see the movie 1 !

- is performed by wiping the vestibule of the nasal nostrils one by one with a disposable sterile buffer (one for each cavity).
- The patient is positioned with his/her head in extension, followed by gently inserting the pad until it touches the posterior wall, then rotates gently to load with nasal discharge.
- The maneuver can be repeated to increase the amount of harvested mucus.
- Subsequently, the swab is withdrawn slightly, reintroduced into the protective tube (with or without Amies / Stuart transport medium) and sent to the laboratory within 2 hours.

Respiratory System Infections

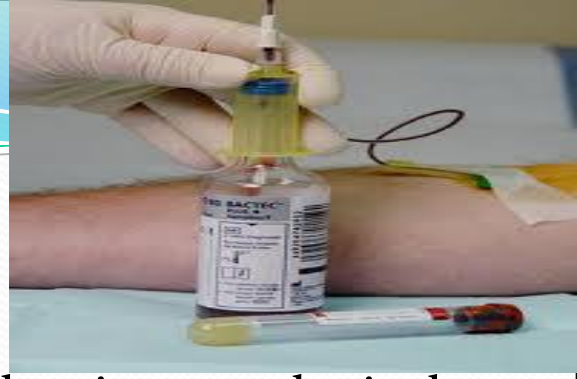


Taking samples of pharynx exudate (in angina, scarlet fever, germs colonisation)

see the movie 2 !

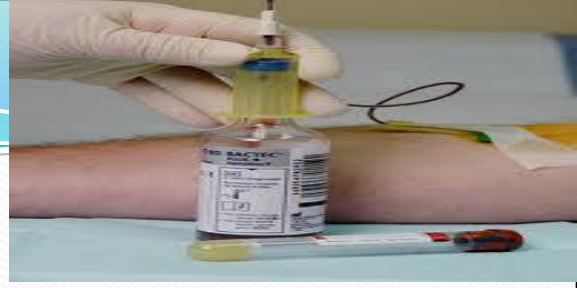
- can be done in the morning before the oral hygiene and food intake, or 3-4 hours after ingestion of food, dental brushing or the use of oral antiseptics.
- The patient having his/her head in extension will open the mouth to the maximum and pronounce the vocal A.
- Using a sterile (or disposable) spatula, the tongue's dorsal face is depressed and the buffer is inserted carefully without touching the palate, uvula or tongue.
- Wipe the back wall of the pharynx, the palatal tonsils, with a circular motion, insisting on inflamed, ulcerated or purulent areas.
- Remove the buffer with caution (not to trigger the vomit reflex), reintroduce it into the protective tube (with or without transport medium) and send it to the lab within 2 hours.

Blood Infections



- **Blood** may be harvested for biochemical, immunological, haematological or bacteriological examinations (to identify bacteria in various bacteraemia / septicemia, typhoid fever, endocarditis, etc.).
- Hemoculture is performed in case of new appearance of a chills or in the case of body temperature increase above the value of 38.5ºC through a new venous puncture, avoiding the collection of blood from preexisting venous catheters.
- It is preferable to perform hemocultures prior to the introduction of antimicrobial therapy. The optimum volume is 10 ml of blood per sample / 3-5 ml for children.
- As most bacteraemia are intermittent, a single sampling provides a sensitivity of 80%, while three samplings in 24 hours provide a sensitivity of 100%.

Blood Infections



- The venous puncture is done on the anterior face of the elbow or the jugular vein (for newborns or infants), after a broadest antiseptic treatment with Betadine, then with alcohol, to leave the skin dry.
- The medical staff carrying out this work will wear sterile, disposable gloves. After the application of the tourniquet, the most obvious vein is restrained with the left hand pointer, and with the right it is punched in the vena shaft at an angle of 30º, the needle opening being upwards.
- When the piston retracts, the blood must penetrate into the syringe if it is correctly positioned. Subsequently, remove the tourniquet, retract the needle from the vein and exert a constant pressure with a sterile swab until complete haemostasis.
- After harvesting, the blood is distributed in the hemoculture vials for aerobic, anaerobic, possibly fungi (with pre-disinfected stoppers), gently agitating for environmental homogenization.
- These vials are sent to the laboratory as soon as possible (maximum 1 h) and placed in automated systems (eg BACTEC), where they are monitored for 10 days.
- **see the movie 3 !**


BACTEC
PLUS +
AEROBIC/F
8-10 mL
BACTEC
PLUS + ANAEROBIC/F
8-10 mL
BACTEC
MYCO/F LYTIC
1-5 mL Inoculum / Inokulum
BACTEC
PEDS PLUS/F
1-3 mL
BACTEC
Standard 10
Aerobic/F
BACTEC
Standard
Anaerobic/F


BACTEC 9050


Artisan Technology Group

Infections of the Urinary System

Harvesting urine for uroculture (in lower / upper urinary tract infections) is taken from the middle urine jet in case of non-catheterized patients in a sterile broad-necked container identical to men and women, after a rigorous water and soap wash of the external genitalia.

- It is preferable to take the sample from the first morning urine or at least 4 hours after the previous micturition.
- Regarding patients with prolonged catheterization (for urologic or neurological reasons), harvesting is performed after decontamination of the distal end of the catheter with 70% alcohol. 5 ml urine is harvested with a sterile syringe, then passed under aseptic conditions to the urinalysis machine. It is forbidden to sample directly from the drainage bag or to cultivate the catheter tip.
- Transcutaneous suprapubic puncture is reserved for carefully selected cases, being performed under surgical asepsis conditions. It is indicated for identification of infections with anaerobic bacteria, being very effective in avoiding urethral contamination of the samples.
- Samples should be processed within approximately 2 hours after harvesting to prevent the microbial flora from growing. If this condition can not be met, the urine is kept at + 4ºC until processing.
- **see the movie 4,5 and 6 !**

Skin Infections

a. **From closed purulent collections** (abscesses, phlegmon, boils, hydrosadenite, aso.) - is harvested by the surgeon at the opening of the collection or by aspiration puncture with a fine needle syringe in depth after a prior antisepsis of the skin. The product is transferred to an anaerobic sterile transport system and sent to the laboratory immediately.

b. **From open fistulous collections** – on the intact skin around it is performed antisepsis with Betadine, and the exudate from the surface of the lesion is first wiped off with sterile physiological serum. The sterile buffer is inserted into the fistula tract, cure as deeply as possible, then insert it into the tube with the Amies transport medium and immediately dispatch it to the laboratory.

c. **From wound secretions** (surgical wounds, skin ulcers, burns) - after a previous wound toilet using physiological serum (Betadine for the circumscribed area), swirl the tip of the buffer for 5 seconds on a 1 cm2 area, firm enough to cause a slight bleeding, then insert it into the tube and transport it to the laboratory within one hour.

see the movie 7 and 8 !

Digestive Infections

- **Faeces,** spontaneously eliminated, may be harvested for stool parasites examination or stool culture (in intestinal parasitoses, food toxicities, gastro-enteritis, colitis, typhoid fever, bacillary dysentery, cholera, aso.).
- The patient will spontaneously defecate in a sterilized container by boiling, scalding or autoclaving (in case of coprocultivation) without contaminating the contents with urine.
- Do not use disinfectant solutions which may prevent germ growth. Then, using the sterile spoon of the sampling recipient, take fragments from different parts of the stool or from potentially pathological areas - mucous, sanguinous, purulent, rhizome, in a minimum volume of 3 cm3.
- The harvested fragments are suspended in the transport medium of the sampling recipient and sent immediately to the laboratory.
- **see the movie 9 !**

Digestive Infections

- For the detection of pathogenic enterobacteria (Salmonella spp., Vibrio cholerae, aso.), the second and third stools are harvested after a purgative is taken (15 g magnesium sulphate in 250 ml water for adults), focusing on the liquid part containing the small intestine flora.
- In patients with dysentery syndrome, harvesting is done with a sterile swab inserted through the anal orifice, under proctoscope control and use it to wipe the mucosa. After sampling, the buffer is introduced into the transport medium of the sampling recipient and sent to the laboratory.
- Sigmoid faeces can also be harvested using a sterile Nelaton probe, inserted 15-20 cm for adults and approximately 10 cm for children. Using a 10 ml sterile syringe, aspirate the contents and decant it into a sampling recipient with transport medium.

Digestive Infections

- The stool parasites test has a similar technique to stool culture, with the only difference that it does not require sterilized containers.
- Samples not sown on containment medium within 2 hours must be subjected to a preservation process :
- By refrigeration at + 4ºC, maximum 24 hours;
- Use of special transport mediums such as: Stuart medium (useful for the preservation of enterobacteria and also for enteropathogens of the Vibrio or Campylobacter genus) or Cary-Blair medium which provides good conservation at ambient temperature for up to 7 days (recommended for Enterobacteriaceae and Vibrio spp.).
- Liquid mediums are less commonly used today due to difficult transport and inconsistent preservation of enteric pathogens.

Digestive Infections

- **The "a jeun" gastric juice** aspirates (useful for the detection of M. tuberculosis bacillus, especially in infants and young children) or **vomiting** samples should be neutralized with 10% Na bicarbonate solution in the presence of a pH indicator (solution of bromothymol blue).
- The technique of harvesting vomiting samples for bacterial culture is similar to spontaneous emission stool cultures. Sterile Petri dishes are used as containers.

- **Food** selection for examination in an epidemiological inquiry for food poisoning is based on the incubation period (only food consumed in the last 72 hours).

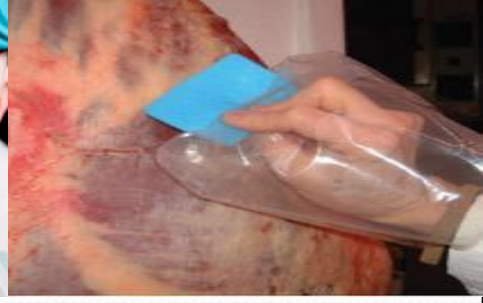
NR. .	FOODSTUFF	POSSIBLE ETIOLOGY
1.	Smoked food (meat, poultry, fish)	*Salmonella*, *Staphylococcus aureus* (and its enterotoxins), *Clostridium botulinum* (and its neurotoxins)
2.	Vacuum foods	*Clostridium botulinum* (and its neurotoxins), *Listeria monocytogenes*
3.	Cheese	*Salmonella*, *Staphylococcus aureus* (and its enterotoxins), *E.coli*
4.	Meat and meat products	*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 based food	*Bacillus cereus* (and its toxins), mycotoxins
7.	Soups and stews	*Bacillus cereus*, *Clostridium perfringens* (and its enterotoxin)
8.	House canned food	*Clostridium botulinum* (and its neurotoxins)
9.	Shellfish	*Vibrio parahaemolyticus*, *V.cholerae* 01
10.	Hamburger	*E.coli* 0157:H7, *Salmonella*
11.	Ice cream	*Salmonella*, *Staphylococcus aureus* (and its enterotoxins)
12.	Milk and milk products	*Salmonella*, *Staphylococcus aureus* (and its enterotoxins), *Campylobacte jejuni*, *Streptococcus pyogenes*, *Yersinia enterocolitica*

13.	Powder Milk	*Salmonella*, *Bacillus cereus*, *Staphylococcus aureus* (and its enterotoxins)
14.	Mayonnaise	*E.coli* 0157:H7
15.	Vegetables, peas	*Clostridium perfringens* (and its enterotoxin), *Bacillus cereus* (and its toxins)
16.	Rice	*Bacillus cereus* (and its toxins)
17.	Eggs, egg based products	*Salmonella*
18.	Pastry products with milk and eggs	*Staphylococcus aureus* (and its enterotoxins), *Salmonella*, *Bacillus cereus* (and its toxins)
19.	Fish and fish products	*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.

Taking samples from food

- Liquid foods are aseptically harvested 200 ml and 150-200 g for solids (multiple cubes from different regions and all layers) in sterile containers to be sealed, labeled and transported to the laboratory.
- The label must contain :
  - Name of the food;
  - Name of the seller;
  - Date of harvesting;
  - Production lot number;
  - The number of the harvesting report

Taking samples from food



- **Milk** - a package shall be harvested as such or if it exceeds 1 l, a sample of 200-500 ml;
- **Meat products** - 150 – 200 g aseptically cut from different portions or the whole packaging (in the case of concentrates);
- **House canned food** - an unopened package from the same batch shall be taken;
- **Consumed leftovers** - collect as much as possible using a sterile spatula;
- All of this will be kept in the refrigerator at + 4ºC and sent in an isothermal bag to the lab;
- **Frozen food** - an entire package is taken or drilled / chipped using sterile instruments for large carcasses, in such a way that there is both surface and depth evidence. These will be kept frozen until examination;
- **From rice, vegetables** - samples are taken from the surface, but also from the depth of the package and placed in sterile, sealed containers, sheltered from moisture.
- All samples must be taken to the laboratory in a maximum of 6 hours.

Taking samples from water

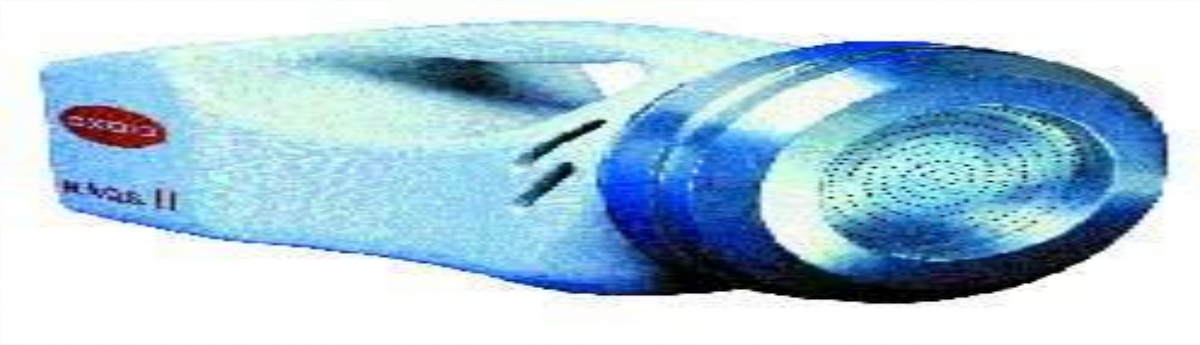
- **From central water installations** – sterilize the tap in flame, open completely and allow water to flow for 5-10 minutes; then adjust the flow so that there is a continuous water column with a maximum diameter of 1 cm, remove the plug from the sterile vial and fill up to 2 cm under the plug. The container is then closed and tagged. One sample contains 1-5 l of water;
- **From tanks and pools** - after removing the plug, the sterile vial is inserted into the tank / pool, filled up to 2 cm under the plug and closed;
- **From fountains, springs** - the sample is harvested directly from the well or pouring from the bucket;
- If the harvested water is chlorinated, before sterilizing the vial, introduce 10 mg of sodium thiosulphate for each 500 ml of test water;
- Labeling must contain the name of the harvesting point, the date / time of harvest and the sample number;
- The transport to the lab is done in isothermal casks within 2 hours (6 hours if a temperature of +4 ° C is ensured).

Microaeroflora sampling

- is used in epidemiological practice in the nosocomial environment, in rooms with high risk of infection for assisted patients - operating rooms, birth rooms, compartments of Anesthesia-Intensive Therapy, neonatology salons.
- It can be done by :
  - **Koch sedimentation method** - In each room are exposed 2 sets of Petri dishes, each set containing a blood-agar and one with nutrient agar. The first set is positioned in the middle of the room, on a table, and the other in a corner, on a bedside / shelf. Raise the caps of the Petri dishes, lay the caps with the opening downwards and leave for 10 minutes. After the interval has expired, they are closed and transported immediately to the laboratory.
  - **Suction method** - harvesting is carried out using apparatus such as the M.A.Q.S (Microbiological air quality sampler - Oxoid) analyzer. This apparatus involves attaching Petri dishes with culture medium to a special adapter, the air is aspirated at a rate of between 0.5-2 l / s and a volume of 1-999 liters is analyzed. Subsequent to incubation, the colonies are counted and the germ count is calculated according to a mathematical formula.

Microaeroflora sampling

- The total number of germs / m3 of air **should not exceed 500-1500 depending on the activity in the room, the beginning or the end of the working day.**
- In operating rooms (during work), in newborns and infants' salons, **up to 300 germs / m³ of air are admitted, with the absence of haemolytic flora.**
- **No coagulase-positive staphylococcal or β-hemolytic streptococcal colony per plate are admitted.**




M.A.G.S. II
OXOID


M.A.G.S. II
OXOID

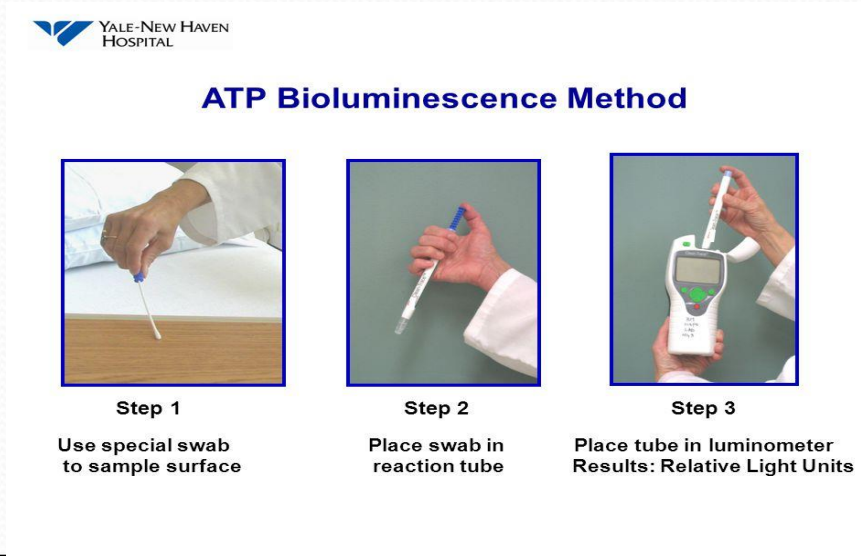
Microbiological control of surfaces and soft material

- is used for tables, bedside tables, bed sheets, wall tiles, linen aso.
- If the surfaces have been previously disinfected, harvesting will take place only after the contact time has elapsed.
- Wipe a surface of 25 cm square using a sterile buffer moistened in 1 ml of physiological serum;
- Wipe with the buffer both horizontally and vertically, with simultaneous rotation, then inserted into the protective tube, vigorously agitating to homogenize the microbial concentration, labeled and sent to the laboratory within 2 hours.
- The suture threads, catgut and compresses will be seeded in plain broth and thioglycolate broth.
- **see the movie 10 !**

Microbiological control of surfaces and soft material

- No coagulase-positive staphylococcal colony, *E. coli* enteropatogen or *Proteus* spp. per cm^2 is admitted.

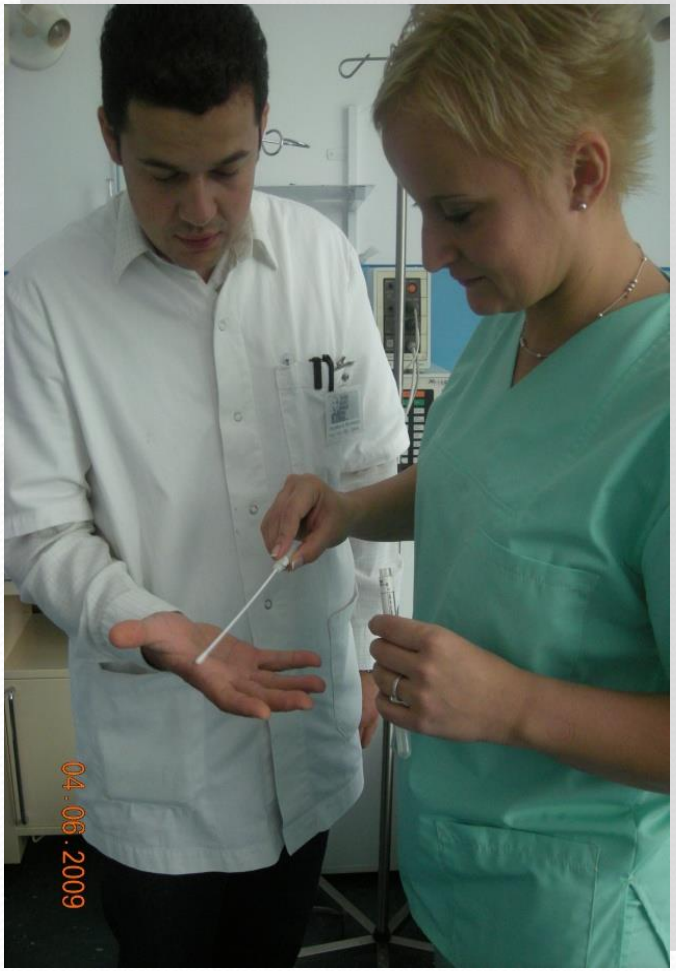
- Interpretation: Consider a clean surface / soft material, those that develops under 5 colonies / cm and no pathogenic germs.



Skin control of the medical staff

- hands are the most commonly targeted, known to be the most common way of transmitting germs in the nosocomial environment.

- With a sterile buffer moistened in 1 ml of physiological saline, the palm of the right hand, including the fingers, is wiped, insisting between fingers and around nails.



Skin control of the medical staff

- Colony-forming units are reported per sample.
- The presence of ***Escherichia coli* is reported; *Proteus; Staphylococcus aureus; Pseudomonas* spp .; *Klebsiella* spp .; *Acinetobacter spp.*; Vancomycin-resistant *Enterococcus*.**
- It is considered a clean hand the one that has:
  a) the microbial load **is not more than 100 CFU / ml** for carer and nursing staff;
  b) the microbial load **is not more than 40 CFU / ml** for the average and medical staff;
  c) the microbial load **is not more than 10 UFC / ml** for the staff that performs aseptic surgery;
  d) must not contain pathogenic germs;
  e) Isolated pathogenic germs shall be tested on antibiotic resistance on request.

Bibliography

- Roxana Moldovan and collaborators. Practical Guide, "Victor Babeș" University of Medicine and Pharmacy Timișoara, LITO 2002: 38-44

- D.Buiuc, G.Coman, M.Neguț. Clinical Microbiology, Teaching and Pedagogical Publishing House R.A., Bucharest 1998: 239-246; 392-399

- *** ORDER Nr. 961/02.09.2016 for the approval of the Technical Norms for cleaning, disinfection and sterilization in public and private sanitary units, the working and interpretation techniques for the tests for the evaluation of the cleanliness and disinfection procedure, the recommended procedures for hand disinfection, depending on the level of risk, chemical disinfectants depending on the support to be treated and the methods for assessing the performance and efficiency of the sterilization process