**Collection, Transport and Culturing of clinical samples**

In order for the results provided by the Microbiology Department to be accurate, significant and clinically relevant, itis required that all microbiological samples that arrive there be correctly selected, collected and transported, as this allows for an optimised analysis and interpretation. In recent years, automated equipment has been developed for the seeding of samples, the number of rapid diagnostic techniques of immunological type, and for the detection of antigens has expanded: viral, bacterial, fungal or parasitic, and there has been a deepening in the knowledge of molecular biology techniques and proteomics.

The clinical syndrome and the possible aetiological agents involved determine not only the type of sample to be sent, but also the procedure for obtaining, transporting, preserving and processing it. Likewise, clinical information is what allows the laboratory to apply the available diagnostic techniques in a more efficient manner. Therefore, it is essential that there is close communication between the microbiologists and the clinicians responsible for the patient, actively participating in the diagnostic process.

**Recommended clinical samples**

It is always necessary to choose the biological material in sufficient quantity that best represents the infectious process for which you want to determine the aetiological agent. The analysable substances are all the biological samples available, from sterile fluids, samples from different organs or systems, such as faeces, urine, sputum, bronchoalveolar lavage, aspirates, biopsies and exudates from different locations or superficial or deep lesions, and hospital devices, such as catheters and prostheses.

It is necessary to avoid contamination with the commensal microbiota to ensure that the sample is representative of the infectious process to be diagnosed and transportation to the laboratory must be as fast as possible.

**Containers used**

There is a great variety of containers in which microbiological samples can be collected, with a common characteristic to all of them being that they are sterile and with a leak-proof seal

The swabs can be made of different materials: dacron, rayon or nylon, cotton (not recommended for Chlamydia spp., Bordetella spp.and Neisseria gonorrhoeae), calcium alginate (may inhibit PCR techniques and be toxic to viruses) and may have the smooth absorption surface or be flocked; they can be used dry or use Amies transport medium in gel or liquid (recommended for automated work stations). The handle should be made of aluminium or plastic (rigid or flexible). The size and shape of the swab will vary depending on the anatomical location and the type of sample to be taken.

There are special transport systems, such as bags, vials or tubes with an anaerobic atmosphere, micro-haematocrit capillary tubes (Trypanosoma spp.), brushes in liquid transport medium for the papilloma virus, transport medium for universal virus (also valid for Chlamydia spp., Ureaplasma spp., and Mycoplasma spp.) or sterile tubes with fixatives for parasites or with preservative such as boric acid-sodium formate for the culture of urine.

Blood culture bottles, lysis-centrifugation bottles, tubes and bottles with screw closure, vacuum tubes with additives (EDTA, citrate) or separating gel (for serum samples), sterile petri dishes and syringes for obtaining aspirates can also be used.

**Sample collection**

The consequences of a poorly taken, poorly preserved or poorly transported sample, can result in a failure in the isolation of the aetiological agent or the isolation of possible contaminating microorganisms that can generate unnecessary or inappropriate treatments.

Since a large part of the determinations in Microbiology are based on the growth capacity of microorganisms, the conditions of collection and transport must ensure their viability at all times.

The Microbiology laboratory should prepare a clear and concise manual with the rules for collecting and transporting samples which is available to all professionals who may request samples for microbiological study and, if there are doubts about the suitability of the samples or how to obtain them, the Microbiology laboratory must always be contacted before proceeding with the collection.

***Collection of samples for study by conventional and automated methods***

The collection of samples for microbiological studies can vary depending on whether they are going to be processed using conventional methods or if they will be processed using automated methods.

Automated systems can only perform the cultivation from liquid samples directly or in liquid transport media.

The containers depend on the type of sample and sterile containers will be used for abscess samples, catheters, sterile fluids (except blood), prostheses, valves and other devices, tissues and biopsies, faeces, urine, semen, skin scraping, hair and nails, and all respiratory samples.

The swab will be used with gel or liquid transport medium (automated seeding and PCR techniques) in all genital exudates, conjunctival exudates, ENT (ear, nose and throat) exudates, wounds, burns and skin ulcers (if aspiration is not possible).

Blood cultures and bone marrow will be sent in blood culture bottles (sterile fluids are also possible, except for CSF). Blood smears for parasites in a tube with EDTA, for serological determinations in vacuum tubes with gel and, for nucleic acid detection tests, vacuum tubes with EDTA or specialised tubes.

The syringes used for aspiration of the samples can be used as containers.

Sampling for the study of viruses can be done with any type of swab except those made from calcium alginate or wooden stick.

The use of transport medium for viruses during the collection of samples depends to a large extent on the sample itself; liquid samples such as blood, CSF, urine and bronchoalveolar lavage fluid do not usually require it, so they must be transported and processed paying special attention to the optimal temperature and storage times.

Finally, there are some particular containers, such as the brush in special transport medium for the papilloma virus or a tube with heparin for the study of Leishmania spp. in the bone marrow or the swab without transport medium for antigen detection of Streptococcus pyogenes.

Regarding the volume of the sample, it is always recommended to obtain the maximum that can be obtained and from the most purulent area. The minimum volume for inoculating a plate or an enrichment broth is one drop (0.05 ml) and, as a general rule, for bacteriological culture at least 0.5 ml or 0.5 g of material is necessary.

As a general rule, in the liquid samples for each type of determination, a minimum of 1–2 ml and a maximum volume between 10 and 20 ml will be requested. The volume for each blood culture bottle is between 8 and 10 ml for adults and 1–3 ml for children and solid faeces >2 g.

The sampling procedure will be done as aseptically as possible and whenever feasible prior to taking antibiotics. There are samples for which peculiar methods are described that must be taken into account and in which the manual of sampling of the Microbiology Department should be consulted, such as the neutralisation of gastric juice with sodium carbonate for the study of mycobacteria.

***Collection of samples for study by rapid techniques***

The most commonly used rapid tests, excluding fresh examinations and staining are: immunofluorescence, agglutination, immunochromatography (ICT), enzyme immunoassay (EIA) and molecular microbiology techniques (real-time PCR and multiplex PCR).

By means of the latter, an isolated or several infectious agents can be detected simultaneously (bacteria, viruses, fungi, parasites), including antibiotic-resistant genes, in a short time, with little manipulation and preparation of the samples and with precise results. These techniques can present specific requirements (including the transport system and sample preservation) that will depend on the technique available in each laboratory.

There are, for example, panels marketed for diagnosis of respiratory infections from samples of nasopharyngeal aspirate or exudate or urine, gastrointestinal infections from fresh faeces or in **Cary-Blair medium**, blood or bone marrow infections or from direct blood with EDTA, serum, special media or positive blood culture bottle, central nervous system infections from CSF or urine, sexually transmitted infections from urethral exudate, endocervical exudate or urine, and skin and mucous infections from skin lesions.

PCR techniques are also available for rapid diagnosis of carrier status, such as detection of Staphylococcus aureus, Streptococcus agalactiae or bacteria that produce carbapenemases in nasal, pharyngeal or rectal exudates.

Each molecular detection test can be performed on specific samples, presenting specific requirements that will differ depending on the technique used, so that the collection system and storage conditions may vary depending on the manufacturer’s specifications.

***Preservation of the sample until its processing (culturing)***

***Available media, temperature and time***

In general, samples collected for microbiological studies should be sent as quickly as possible to the Microbiology laboratory. They can be transported at room temperature if their shipment is not delayed after they are obtained, although some will require ice for transport.

**In the event that they cannot be transported immediately, the following general recommendations may be followed-**

1. for studies of molecular microbiology, viruses or mycobacteria, refrigerate the samples (2–8 ◦C)
2. samples with request for parasites will be stored at room temperature, except the blood tube with EDTA that if not processed in 1 h will be stored in the refrigerator
3. for the rest of the studies, samples of urine, faeces, catheters, abscesses, wounds, burns, biopsies, tissues, recto-vaginal exudates of pregnant women, gastric aspirate, external ear, samples of respiratory origin and some types of exudates and biological
4. fluids (according to the request) will be kept refrigerated if the processing is not done in the first 2 h
5. and samples of blood, bone marrow, CSF, genital samples with suspected sexually transmitted bacterial infection, conjunctival and pharyngeal exudate, corneal scraping, vitreous humour, inner ear, nasopharyngeal aspirate, skin, hair and nails will be stored at room temperature.

There are bacteria that are especially sensitive to environmental conditions: Shigella spp., N. gonorrhoeae, Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae and anaerobic bacteria; reliable detection of these species requires immediate processing.

**Transport**

The transport of samples of biological material within a hospital or centre, from a health centre to a hospital, from one laboratory to another, from one hospital to another within the same city or to another city must be managed by the hospital itself, by the health service or by any transport organisation or agency that has been authorised.

The laboratory must establish a system of prioritisation in the processing of samples, based on previously defined criteria (request of the clinician, care burden and continued care), although it is obvious to say that any sample required urgently has to be processed immediately, and that all samples must be processed in biosafety cabinets.

**Processing (culturing) of sample**

In general, CSF, fluid samples and sterile cavities, surgical specimens and rapid antigen detection tests should always be processed first (within 20 min after receipt). This group is followed by samples with a limited processing time, such as the culture of mycobacteria or tests for nucleic acid detection, tissue samples or recent aspirates (within 1 h after receipt) and respiratory samples (can remain 1 h at room temperature and 2 h if they are kept at 4ºC without the microorganisms losing viability, except bronchoalveolar lavage, which must be processed in the first 20 min after receipt).

Stool samples should be sent in transport medium if the processing cannot be done in a period of time between 30 min and 1 h. Finally, urine samples (they can be kept up to 8 h at 4ºC) and the swabs with transport medium will be seeded. Blood cultures can be maintained at room temperature up to 4 h after receipt.

**Conventional processing: cultivation**

Within the conventional processing of the samples that arrive at the Microbiology laboratory, four well differentiated phases canbe established that must be carried out following this order:

1. **Pre-treatment phase**: when this is necessary, there are different techniques such as centrifugation (sterile fluids), homogenization (biopsies and tissues) or sonication (prosthesis), which will be applied depending on the type of sample and the request made.

2. **Inoculation of culture media**: manual seeding will be done using the isolation technique for most samples and the counting technique for those that need a colony count (e.g. urine). It is necessary to first process the samples for anaerobes and it is advisable to first inoculate the less selective media to avoid carrying any inhibitory substance to another medium. The media will be selected according to the type of sample and the diagnostic suspicion of the possible causal agent to be detected (bacteria, anaerobes, fungi, etc.).

3. **Preparing the smears**: it is necessary to make smears for Gram staining of most samples, including respiratory samples, wound exudates, abscesses, samples from normally sterile origins and, upon request, urine and genital samples. In the case of sterile fluids, especially CSF, it is recommended to cytocentrifuge the sample to prepare the smears; in case of insufficient sample, culturing will be prioritised over staining.

4. **Finally, the incubation that can be done in different atmospheres**: aerobiosis, for the majority of microorganisms and blood agar and MacConkey agar media, enriched with 5–7% of CO2 for the chocolate agar and Thayer-Martin media, microaerophilic for the isolation of Campylobacter spp. and H. pylori and anaerobiosis to search for anaerobes and Brucella agar media, laked blood with kanamycin and vancomycin (LKV) agar and Bacteroides bile esculin (BBE) agar.

As for temperatures, although most bacterial cultures are incubated at 35–37ºC (temperature set in automated equipment). Some exceptions are the samples in which Mycobacterium marinum, Mycobacterium ulcerans, Mycobacterium chelonae and Mycobacterium haemophilum are suspected which grow between 35ºC and 33ºC. For the isolation of Campylobacter spp. an incubation of 42ºC is needed and 35ºC (Campylobacter fetus), and fungi are usually incubated at 30ºC.

In general, for a routine bacteriological culture, most samples in the laboratory must be maintained for at least 48 h, except urine samples, which are usually incubated for only 24 h. Some samples of wounds, sterile fluids or prosthetic material must be kept in incubation for up to 7–10 days to recover slow-growing microorganisms such as Propionibacterium spp., and even 12 weeks if M. ulcerans is suspected.

In the case of fungal cultures, the duration will vary depending on the clinical suspicion, when this is for the most common fungi such as Candida spp., Aspergillus spp., Cryptococcus spp. or Fusarium spp., they are maintained for seven days, but some dermatophyte and dimorphic fungi (Histoplasma spp.) require longer periods and are incubated up to 3–4 weeks.

Reference

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