AEROBIC BLOOD CULTURE

A specially designed excellent culturing capacity medium for the microbiological investigation of blood to detect aerob pathogens. In case of „Mini" Blood Culture 2 – 4 ml blood is enough to ensure the optimal rate of medium and blood (e.g. pediatric samples).

Prepared medium	
Aerobic Blood Culture:	60 ml: AEH30060
Aerobic Mini Blood Culture:	30 ml: AEH30030
Colour:	Amber
Appearance:	Transparent, precipitation free liquid
pH (25 °C):	7,2 – 7,4

Direction: The medium is ready to use.

Sample handling and evaluation: See below.

FORMULA in g/l

Nutrient substrate (peptones, extracts)	25,0
Glucose	5,0
Sodium chloride	5,0
Sodium polyanethole sulfonate	0,2
Buffers	3,0

Note: The typical formula can be adjusted to obtain optimal performance.

Storage conditions: Store the medium protected from light at room temperature. Use before the expiry date on the label.

Quality control:

Test strains	Incubation temp: 37 °C	**Growth**	Incubation time: 48 h
Klebsiella pneumoniae	ATCC 13883	Good	
Streptococcus pneumoniae	ATCC 49619	Good	

DRAWING AND PROCESSING OF BLOOD SAMPLES

The microbiological examination of blood samples drawn in a correct way and timing is essential in the diagnosis of bacteraemia and sepsis. Blood should be always drawn with strictly keeping the rules of asepsis. Keeping the rules serves for avoiding of contamination of Blood Cultures (BC).

Way and place of taking of samples

It is obligatory to wear rubber gloves to protect personnel.
After removing the protecting cap of Blood Culture bottle, the rubber stopper should be washed two times with 70 % alcohol.
To disinfect the spot of blood drawing, mostly Povidon Iodine (Betadine) solution is recommended, so that the chosen spot is better visible. After washing with Povidon Iodine, washing with 70 % alcohol could be used.
Blood drawing could be applied after drying of skin.
The spot of blood drawing should not be touched by hands after disinfection.
Blood drawing could be done by single-use needle and syringe, or by closed blood drawing system.
Intact peripheral veins are most suitable for blood drawing.
In case of intravascular device using patients, if the drawing is done from cannula, samples should be taken from every lumen and in parallel from peripheral, too. In case of suspected catheter infection and when the catheter is replaced, it is practical to send it to the laboratory to culture.

Time of sampling

Sampling should be done before antimicrobial treatment. In case patient receive antimicrobial treatment and his condition allow, blood could be taken after 1 – 2 days break, or if it is not possible, as late as possible from the last dose. Sample taking should not be done during the fever peak. In case of bacteraemia, number of germs is the highest before fever and beginning of shaking chill, so it is practical to take blood before expected temperature rise, or during shaking chill. In case of intravascular infections, e.g. infective endocarditis, bacteraemia is continuous. Therefore, in such cases blood taking is recommended at least three times within 24 hours, independently from fever.

Quantity of sample

In case of blood-stream infection, usually few number of microorganisms (10-21 colony forming unit/ ml blood) are present, especially at adults, therefore the optimal quantity is 20-30 ml at adults (it is practical to divide this quantity and inoculate into aerobe and anaerobe bottles), 1-2 ml at newborns, and 2-3 ml at small children. Inoculation of aerobe and anaerobe bottles in parallel is recommended because some pathogens (pl. Streptococci) grow faster in anaerobe bottles, which composition is more demanding.

With regard to quantity of blood, keep the optimal blood-medium ratio, according to the values given by the manufacturer. Blood taken in one time but divided to more bottles, should be considered as one sample.

The reason to give only the given quantity of blood in one bottle is that the own antibacterial materials of blood should be diluted.

Frequency of sampling

In a fever period (within 24 hours), blood taking should be done 2-3 times in 20-30 minutes intervals from different veins. In case of patients with severe conditions, when beginning of antimicrobial treatment is urgent, to keep time intervals is not necessary.

Taking only one sample is a mistake, because pathogen could not be detected safely, and contamination during sampling could be excluded with difficulties. Taking of more BC, it could be made likely that transitory, intermittent, or continuous bacteraemia is the case. Numerous literature data and our own experiences prove that in case of prolonged bacteraemia, pathogens could be found in 95-97 % with two BC examination. During antibiotic therapy, it is not necessary to take more than three blood samples, because there are no evidences that it would be more efficient. When pathogens could not be detected after 48 hours, it is practical to repeat blood drawing, if the clinician keeps his supposed diagnosis of sepsis.

Which type of BC bottle shall we use?

Because bacteraemia is caused by aerobe and facultative aerobe bacteria in the majority of cases, blood should be inoculated preferably to aerobe BC bottles. Anaerobe bottles should be used first of all in case of suspected anaerobe infections (sepsis connected to unknown origin intra-abdominal, colon, rectal, gynaecological infections, or foetid excretion in the spot of primer infection, etc.). In case of children, using of specially developed children bottle is recommended.

Processing of blood samples

BC bottles should be incubated at 37 °C, usually for 7-10 days in a normal thermostat and check the turbidity of media daily. In case of turbidity or other signs of microbial growth (e.g. lysis of blood cells) (see table), or in 1, 3, 7 days, or in the end of incubation, inoculation should be done from every BC bottle.

Microscopic signal	In case of microorganism
Haemolysis	Streptococcus spp., Staphylococcus spp., Listeria spp., Clostridium spp., Bacillus spp.
Turbidity	Aerobe, Gram-negative rod, Staphylococcus spp.
Gas forming	Aerobe, Gram-negative rod, Anaerobe
Surface membrane	Pseudomonas spp., Bacillus spp., yeast
Precipitation	S. aureus

Way of inoculation: wash the rubber stopper with iodine tincture, allow to dry and punching with a sterile needle and syringe aspirate 1-2 ml from BC. In case of positive BC bottles sometimes overpressure could arise due to gas forming during bacterial growth (this is more frequent in bottles with smaller volumes, designed for automates). To stop this, punch a sterile needle to the bottle just before inoculation.

Put 1-1 drops of aspirated BC onto slides carefully not to splash, or flow from the slide, and put 1-2 drops onto blood and chocolate agars (from anaerobe bottles onto aerobe and anaerobe blood agars) and spread carefully.

During these operations, wearing of rubber gloves is obligatory!

Smears should be tested native and/or with methylene blue by phase-contrast or dark-field microscope, if necessary (when bacteria are visible in the microscopic preparate) Gram-stain could be employed. If fluorescent microscope is available in the lab, an Acridin Orange staining of smears from positive bottles could give useful information.

Incubate the sub-cultures for further 21, or 48 hours in raised CO_2 atmosphere, respectively the anaerobe blood agar in anaerobe atmosphere for 48-72 hours. If the microscope indicates the presence Gram-negative rods, inoculation to Eosin methylene blue medium is recommended. If bacteria are visible in the smear from BC, direct antibiotic susceptibility testing should be done.