

## PROTOCOL

# Proof-of-principle antimicrobial resistance routine diagnostics surveillance project (PoP project)



**Version 2.0**



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## Abstract

The Central Asian and Eastern European Surveillance of Antimicrobial Resistance Network (CAESAR) aims to address the lack of antimicrobial resistance (AMR) surveillance data in parts of the WHO European Region. Implementation of the Proof-of-Principle routine diagnostics project for antimicrobial resistance surveillance (PoP project) assists a country in building capacity to routinely perform antibiotic susceptibility testing at local hospital laboratories and at national reference laboratory level, by stimulating the utilization of blood culture diagnostics. This protocol provides the project coordination team with detailed information for the implementation of the project, including methods, standard operating procedures, and expected outputs of the project. As a result of the project, baseline data on the main pathogens causing blood stream infections and their antimicrobial susceptibility patterns will become available, providing clinicians with information needed to make antibiotic treatment choices. The collaboration between hospital laboratories and the national reference laboratory developed during the project lays down the foundation for a national AMR surveillance network.

### Keywords

DRUG RESISTANCE, MICROBIAL  
ANTI-INFECTIVE AGENTS  
DIAGNOSTIC TESTS, ROUTINE - METHODS  
MICROBIOLOGICAL TECHNIQUES  
DATA COLLECTION  
GUIDELINE

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# Abbreviations

AMR	antimicrobial resistance
AST	antimicrobial susceptibility testing
BA	blood agar (plate)
BSI	bloodstream infection
CA	chocolate agar (plate)
CAESAR	Central Asian and Eastern European Surveillance of Antimicrobial Resistance Network
CLSI	Clinical and Laboratory Standards Institute
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ID	identification (of species)
MAC	MacConkey (plate)
PoP project	Proof-of-principle antimicrobial resistance routine diagnostics surveillance project
SIRS	systemic inflammatory response syndrome
SOP	standard operating procedure



# About this protocol

This document is a generic protocol, containing guidance, recommendations and suggestions for the implementation of the Proof-of-Principle project. Based on continuous evaluation of ongoing and past PoP projects, adaptations and improvements are made to the generic protocol<sup>1</sup>.

The protocol provides guidance on active case finding, aseptic venipuncture procedures, and laboratory procedures, including timely feedback of results. In addition, it provides guidance on species identification and the use of EUCAST for antimicrobial susceptibility testing. The minimum set of antibiotics to be tested is based on recommendations from the CAESAR manual (1).

It may be beneficial to adapt the protocol to fit the financial and organizational context, and to better embed the PoP protocol within locally used protocols and guidelines. All adaptations should be implemented in collaboration with the WHO Country Office and WHO Regional Office for Europe.

Detailed SOPs and sample forms are included as Annexes. It may be beneficial to adapt these prior to implementation of the protocol.

Ethical approval of the project should be obtained at country level prior to implementation of the protocol.

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<sup>1</sup> Previous versions of the protocol have not been published online



# Summary

The WHO Global Action Plan on antimicrobial resistance (AMR) urges countries to develop a national surveillance system for antimicrobial resistance to strengthen their knowledge about AMR. The recent WHO global report on surveillance of antimicrobial resistance revealed that large gaps in information on antimicrobial resistance exist because many countries do not have a national surveillance system in place. An important limiting factor to conducting routine AMR surveillance in a country is the underutilization of bacteriological diagnostics in routine clinical practice. A recent assessment led by the WHO Regional Office for Europe identified the following constraints for the use of bacteriological diagnostics in routine clinical practice: (i) financial constraints such as lack of reimbursement for diagnostic testing, (ii) limited perceived benefit for performing such diagnostic tests by clinicians, and (iii) weak communication and linkages between hospital laboratories and clinical wards.

This project wants to demonstrate that use of routine bacteriological diagnostics to guide treatment decisions, and AMR surveillance provides essential information to care providers at the point of care. At the same time it can help to showcase how surveillance plays a key role in targeting infection prevention and control policies in clinical settings throughout the country. More specifically, this project (i) supports the collection of blood cultures from patients with a clinical suspicion for bloodstream infection (BSI) and (ii) facilitates bacteriological processing of microbiological samples, including species identification (ID) and antimicrobial susceptibility testing (AST).

A limited number of eligible hospitals, typically between three and five implement the project protocol, and are accompanied in that by a team of trained project coordinators, who provide follow-up and guidance throughout the process. In addition to technical guidance, material support is provided to clinicians and hospital laboratories, based on a needs assessment and process mapping before the start of project implementation. Samples collected as part of the project are processed twice – in parallel at the hospital laboratory and at the national reference laboratory for AMR – to (i) ensure process quality and (ii) to provide a training opportunity for the laboratories involved. As a result this project generates baseline data on the main pathogens causing BSIs and their antimicrobial susceptibility patterns. In the process of generating these data, the capacity for diagnostic bacteriological testing at the local hospital laboratory and at the national reference laboratory is strengthened. With a view on creating sustainable practice, the project also supports the creation of a sustainable structure for AMR data sharing and bacterial isolates collection, which builds the foundation for a national AMR surveillance network.



# Introduction

AMR is a threat to adequate treatment of infectious diseases in individual patients and has been associated with higher morbidity, higher mortality and longer hospital stays; consequently it has effects on both the individual and on wider society (2). Since resistance levels are currently rising worldwide (3–5), there is a need for better evidence to support health policy decision-making.

Local, national and international surveillance programmes that monitor the magnitude and trends in AMR can support the development of appropriate clinical guidelines, support empirical antibiotic therapy and guide infection control interventions. Information derived from surveillance also plays an important role in creating awareness for AMR among health care professionals, policy-makers and the general public (6).

The Central Asian and Eastern European Surveillance of Antimicrobial Resistance Network (CAESAR) aims to address the lack of surveillance data in parts of the WHO European Region by building capacity for surveillance, strengthening laboratory capacity and quality, and developing a network of national AMR surveillance systems for countries in the Region. CAESAR started in October 2012 as a joint initiative of the WHO Regional Office for Europe, the European Society of Clinical Microbiology and Infectious Diseases and the Netherlands National Institute for Public Health and the Environment (1).

By initiating collaboration with CAESAR, a country can identify a series of steps necessary to address the issue of AMR, including setting up a functional national AMR surveillance system. Underutilization of microbiological diagnostics, resulting in a low number of blood cultures being processed, tested for presence of bacterial pathogens and investigated for antimicrobial susceptibility or resistance, has been identified as an important obstacle to performing national AMR surveillance. This underuse of clinical diagnostics may furthermore have a negative effect on data quality as it leads to an overrepresentation of critically ill patients or patients in whom first-line antibiotic therapy did not work and it can bias surveillance results towards higher resistance numbers (2,7).

Most initial antibiotic prescribing is empirically based on the clinician's decision that a patient is likely to have a bacterial infection and on his or her knowledge of the most likely pathogens and their likely antibiotic susceptibility. Empirical choices must often be broad spectrum to cover many possible pathogens; however this approach increases the selective pressure for resistance. AMR threatens to undermine the efficacy of empirical choices, which results in poorer outcomes for individual patients. Appropriate use of bacteriological diagnostics should support clinical prescribing decisions for the individual patient, and provide information about the causative pathogen, and its pattern of susceptibility or resistance to antibiotics. The last can be used to guide therapy, confirming in some cases that the empirical choice was appropriate; identifying patients for whom the initial broad-spectrum therapy can be de-escalated while still providing adequate coverage; and identifying patients for whom the empirical choice was inappropriate because the infecting organism was resistant. Aggregating diagnostic data obtained over time for many individuals can provide an important source of surveillance information, which increases knowledge of pathogens dominating locally and their likeliest drug resistances; it can thus shape empirical prescribing policies for the benefit of future patients.

The objective of this project is identifying bacteria causing BSIs and their antibiotic susceptibility patterns. A BSI is defined as the presence of bacteria in the blood as demonstrated by blood culture. This is taken to include all clinical manifestations of BSIs, including sepsis (life-threatening organ dysfunction caused by a dysregulated host response to infection) and septic shock (sepsis with persistent hypotension despite fluid resuscitation plus cellular/metabolic abnormalities) (8). The systemic inflammatory response syndrome (SIRS) criteria proposed by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee (9) will be used as a means of early detection of patients with a BSI

and to indicate that a blood culture should be considered. Additional diagnostic criteria are required for children (10) and for identifying patients with sepsis, severe sepsis and septic shock (11).

The proposed project aims to provide insight into the current levels of resistance in a country by stimulating the appropriate collection of clinical samples (blood samples) at the hospital level and by routine surveillance at local and central laboratories of microorganisms causing BSIs. In the process, clinicians will receive timely feedback from microbiology laboratories as part of the diagnostic work-up of patients with infectious diseases, including those with hospital-acquired infections. It is expected that this feedback of individual results, together with the reports of average resistance in the whole population (cumulative antibiograms), will enhance the perceived utility of performing microbiological testing to guide treatment decisions among clinicians. Furthermore, the higher number of samples to be processed at the hospital and national reference laboratory levels will provide practical learning opportunities for microbiologists at both locations.

# Project set-up

## Goals and objectives

### Main goal:

The main overarching goal of the project is to contribute to improving clinical care for patients admitted with suspected BSIs, in accordance with evidence-based medicine.

Secondary goals of the project are:

- to demonstrate to clinicians the value of clinical microbiology as part of the diagnostic work-up of patients with suspected BSIs and to improve the clinical work-up and timely feedback of laboratory results to prescribers of antimicrobial drugs, thus allowing for optimization of antimicrobial therapy;
- to establish and support a surveillance network as a starting point for a functional national sentinel laboratory-based surveillance system for AMR;
- to provide insight into antibiotic resistance levels in a country.

### The projects objectives are:

Ad 1.

- improve active case finding practice;
- improve turnaround time at key steps of blood sample processing at the local laboratories;
- improve concordance between local and reference laboratory in reaching accurate test results;
- ensure multidisciplinary interaction around a patient.

Ad 2.

- strengthen the AMR reference and surveillance capacity at the national reference laboratory, by providing:
  - terms of reference;
  - training on international guidelines (EUCAST, laboratory quality stepwise implementation (LQSI));
  - training on how to train local laboratory personnel;
  - support to set up national external quality assessment scheme (NEQAS);
- strengthen capacity at the local laboratories, by providing
  - training on international guidelines (EUCAST, LQSI);
- ensure transport of samples, isolates, and data between local laboratories, reference laboratory and national AMR surveillance group.

Ad 2 and 3.

- increase the volume of clinical samples processed by local and reference laboratories, thus building laboratory capacity.

Ad 3.

- contribute local AMR data to a national database;
- improve the capacity to manage, analyse, interpret, and report on AMR data, by providing training;
- provide a top 5 ranking of organisms and a cumulative antibiogram;
- provide data for national and regional surveillance.

## Project design

### Type of project

This project is set up as a descriptive observational study.

### Research population

All consecutive patients with suspected BSI at time of admission or when already admitted to hospital during the course of the project will be asked to participate.

### Sampling frame

Three to five hospitals routinely treating patients with BSIs will be selected to participate in the project. Recruitment through active case finding will take place among patients admitted to hospital departments that take patients with suspected BSIs from the community (e.g. emergency departments) and wards where patients are at risk of developing hospital-acquired BSIs (e.g. intensive care, urology or surgical departments).

The hospitals participating in the project will be selected using an assessment questionnaire for participation in the PoP project (Annex 1). As a prerequisite, the hospitals need to routinely provide treatment to patients with BSIs, and have either on-site bacteriological laboratory diagnostic capacity (species ID and AST) or an agreement with an external laboratory capable of providing bacteriological diagnostic services (species ID and AST). Specific hospital departments or wards will be targeted to focus on active case finding and for capacity-building/technical support.

### Active case finding and inclusion of patients

Clinicians (medical doctors and nurses) will be trained to conduct active case finding (see Quality assurance). Sequential patients with clinical suspicion of BSI or sepsis will be included in the project. The SIRS criteria will be used for early recognition of patients with suspected BSI or sepsis (9). The SIRS criteria are sensitive criteria aimed at identifying as many patients with a potential BSI as possible (11).

**For adults**, the SIRS criteria are two or more ( $\geq 2$ ) of the following (9)

- core body temperature  $>38.3^{\circ}\text{C}$  (fever) or  $<36^{\circ}\text{C}$  (hypothermia)
- heart rate  $>90$  beats/min (tachycardia)
- respiratory rate  $>20$  breaths/min (tachypnoea)
- leukocytes  $>12 \times 10^9/\text{l}$  or  $<4 \times 10^9/\text{l}$  or  $>10\%$  immature neutrophils (band forms).

Age-appropriate criteria will be used for children as proposed by the International Consensus Conference on Paediatric Sepsis (10). For consistency with local practice, the age-specific criteria for tachycardia and tachypnoea used may be adapted to local guidelines.

**For children**, the SIRS criteria are two or more ( $\geq 2$ ) of the following (10):

- core body temperature  $>38.3^{\circ}\text{C}$  (fever) or  $<36^{\circ}\text{C}$  (hypothermia)
- tachycardia defined as a mean heart rate more than two standard deviations above normal for age (age-specific criteria; Table 1) or for children younger than 1 year of age, bradycardia defined as a mean heart rate  $<10$ th percentile for age
- tachypnoea defined as a respiratory rate more than two standard deviations above the normal for age (age-specific criteria; Table 1) or mechanical ventilation for an acute pulmonary process
- elevated or depressed leukocyte count (according to age-specific criteria; Table 1) or  $>10\%$  immature neutrophils (band forms).

**Table 1. Age-specific criteria for SIRS**

Age group	Core body temperature ( $^{\circ}\text{C}$ )		Heart rate (beats/min)		Respiratory rate (breaths/min)	Leukocyte count ( $10^9/\text{l}$ )
	Fever	Hypothermia	Tachycardia	Bradycardia		
Newborn (0 days to 1 week)	$>38.3$	$<36.0$	$>180$	100	$>50$	$>34$
Neonate (1 week to 1 month)	$>38.3$	$<36.0$	$>180$	$<100$	$>40$	$>19.5$ or $<5$
Infant (1 month to 1 year)	$>38.3$	$<36.0$	$>180$	$<90$	$>34$	$>17.5$ or $<5$
Toddler and preschool ( $>1$ to 5 years)	$>38.3$	$<36.0$	$>140$	NA	$>22$	$>15.5$ or $<6$
School age ( $>5$ to 12 years)	$>38.3$	$<36.0$	$>130$	NA	$>18$	$>13.5$ or $<4.5$
Adolescent ( $>12$ to 18 years)	$>38.3$	$<36.0$	$>110$	NA	$>14$	$>11$ or $<4.5$
Adult ( $>18$ years)	$>38.3$	$<36.0$	$>90$	NA	$>20$	$>12$ or $<4$

Source: Bone et al (9) and Goldstein et al (10).

All patients meeting these SIRS criteria will be clinically evaluated by a medical doctor. *If the doctor's clinical judgement is that the patient may have a BSI or sepsis (any stage), blood for culturing will be taken prior to initiation of antibiotic therapy.* For clinical diagnostic criteria for suspected BSI or sepsis, prevailing clinical guidelines (12,13) or WHO guidelines (14,15) are used. A practical approach could be to take a sample for blood culture from all patients with a clinical suspicion of a serious bacterial infection in whom (intravenous) antibiotic therapy is considered.

The clinical utility of the SIRS criteria for detecting patients with sepsis has recently been called into question because of its low specificity and imperfect sensitivity (8). A recent large-scale retrospective study in the United States has identified alternative clinical criteria (altered mentation, low systolic blood pressure and elevated respiratory rate) to identify adults with sepsis (16). However, these criteria have not yet been validated prospectively in a wide range of clinical settings, including low-resource settings, and were not adopted in the 2016 international guidelines for clinical management of sepsis (17). Until

there is a clear consensus about an alternative, this project will use SIRS for early detection of patients with suspected BSI in whom a blood culture should be considered.

### Exclusion criteria

- Patients not admitted to one of the selected wards
- Patients not consenting to inclusion.

### Withdrawal criteria

Patients will only be subject to project procedures once they have agreed to inclusion. Information about patients following the initial blood sample will be secondary on collection of clinical information from the treating physician. A patient can withdraw from the project at any time.

### Sample size calculation

Having an adequate sample size is important to ensure the objectives of a study are attained. The main endpoint for estimating required sample size in this project uses the weighted average susceptibility for any one of the antibiotic choices for (empirical) treatment of a BSI caused by a Gram-negative bacterium. The weighted average is calculated as shown in Table 2. Importantly, the denominator for calculating the weighted average is the total number of relevant microorganisms isolated. Based on data collected by CAESAR in 2015, a rough expectation of rank-ordering and proportions non-susceptibility is made; approximately two thirds of all isolates are expected to be Gram negative.

**Table 2. Calculation of weighted average antibiotic susceptibility for BSI caused by Gram-negative bacteria**

	Proportion (%)	No. <sup>a</sup>	NS (%) <sup>b</sup>	Absolute NS <sup>c</sup>	Weighted average NS
<i>Escherichia coli</i>	40	80	40%	32	
<i>Klebsiella pneumoniae</i>	25	50	60%	30	
<i>Pseudomonas aeruginosa</i>	15	30	20%	6	
<i>Acinetobacter</i> spp.	20	40	IR	40	
<b>Total</b>		<b>200</b>		<b>108</b>	<b>54% (108/200)</b>

Notes: NS: Isolates non-susceptible (intermediate or resistant) to third-generation cephalosporins; IR: Intrinsic resistance (assumed that 100% of the species are NS);

<sup>a</sup> Total number of isolates for a given bacterial species assuming a total sample size of 200.

<sup>b</sup> Percentage of isolates not susceptible to third-generation cephalosporins.

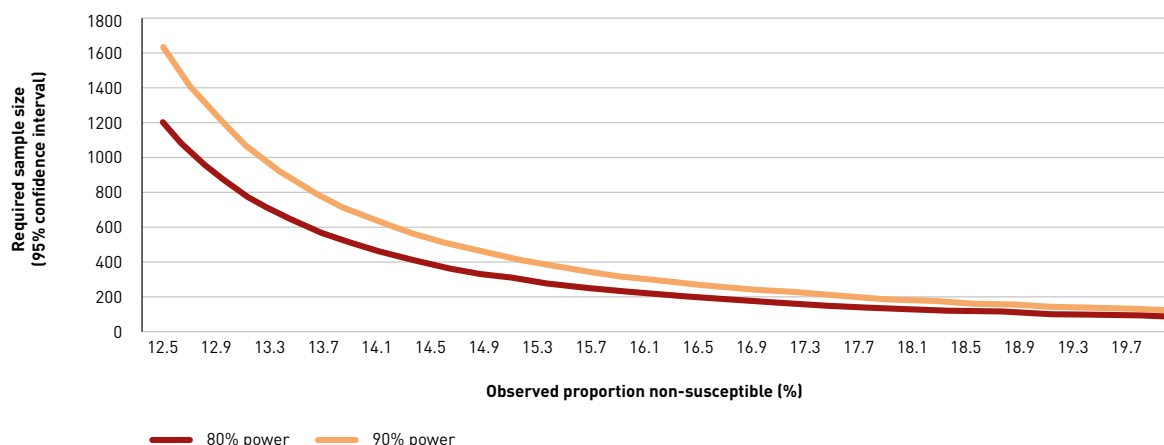
<sup>c</sup> Total number of isolates not susceptible to third-generation cephalosporins.

Source: Based on data collected by CAESAR in 2015 (1).

Sample sizes in Fig. 1 are estimated using the formula for one-sample comparison of a proportion to a prespecified proportion (18). More specifically, we want to assess whether susceptibility to a particular antibiotic or combination of antibiotics is significantly higher than 10%, which is often used as the critical threshold of non-susceptibility for which a certain treatment option is deemed not fit for empirical treatment of patients with life-threatening disease.



**Fig. 1. Required sample sizes for finding a statistically significant difference between observed proportion and a critical threshold of 10% based on a one-sample test for comparison of proportions for given confidence level and power**



Notes: Confidence interval is 100% minus the chance of a type I error (false-positive finding), or 100% minus the chance of incorrectly concluding the observed proportion is different from 5%; Power is 1 minus the type II error (false-negative finding) or 1 minus the chance of incorrectly concluding there is no difference between the observed proportion and 10%.

A total sample of 200 Gram-negative blood culture isolates (one per episode per patient) would be sufficient that an observed proportion of non-susceptibility of  $\geq 16.5\%$  would be statistically different from the threshold proportion of non-susceptibility of 10% (80% power and 95% confidence interval). Assuming that two thirds of bloodstream isolates are Gram negative and a 15% positivity rate of blood cultures, a total of 2000 blood cultures would need to be taken and processed.

**Expected duration of the project**

The expected duration of the project is six months. After the six months, the attained sample size will be assessed and a decision will be made either to stop or to continue the project until the required sample size has been attained (Table 3).

**Table 3. Planned project duration**

	Year 1												Year 2											
	Q1			Q2			Q3			Q4			Q1			Q2			Q3			Q4		
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Preparations	[Active]												[Active]											
Training	[Active]												[Active]											
Start	[Active]												[Active]											
Analysis	[Active]												[Active]											
Review	[Active]												[Active]											

## Coordination and technical expertise for the project

A country project will require the following participation:

### **Project initiator**

WHO Collaborating Centre for Antimicrobial Resistance Epidemiology and Surveillance, National Institute for Public Health and the Environment, the Netherlands.

Principal investigator: Tjalling Leenstra

### **Technical support team**

WHO Collaborative Centre for Antimicrobial Resistance Epidemiology and Surveillance, National Institute for Public Health and the Environment, the Netherlands

Department of Clinical Microbiology, University Hospital of Infectious Disease, Zagreb, Croatia

Programme Control of Antimicrobial Resistance, WHO Regional Office for Europe, Copenhagen, Denmark

WHO country office

### **Project coordination team**

National AMR focal point

Coordinator and data manager

Research coordinator and quality assurance specialist

Epidemiologist bacteriologist

Clinician

Support personnel (laboratory technician)

Support personnel (communication, public health/Ministry of Health)

Support personnel (procurement support, short term)

Terms of references for a national coordination team for the PoP project are listed in Annex 2.

### **Project sites**

Three to five research sites with each providing:

- clinical epidemiologist
- clinical bacteriologist
- clinician

### **Clinical laboratories**

Three to five linked laboratories will be involved in the project.

### **Reference laboratory**

A national reference laboratory for checking local results and creating a database of the information generated.

# Methodology

## Project procedures

The project procedures (work flow) are depicted in Fig. 2. The dotted lines reflect feedback of laboratory test results from the local laboratory to the clinic for updating patient therapy and from the reference laboratory to the local laboratory for quality assurance.

Throughout the duration of the project, data are collected to monitor the progress and evaluate the results of the project. The following data collection forms are included in the Annex: a clinical data form (Annex 3), a laboratory results form (Annex 4), a feedback form (Annex 5), and an evaluation form for weekly meetings (Annex 6).

### Active case finding

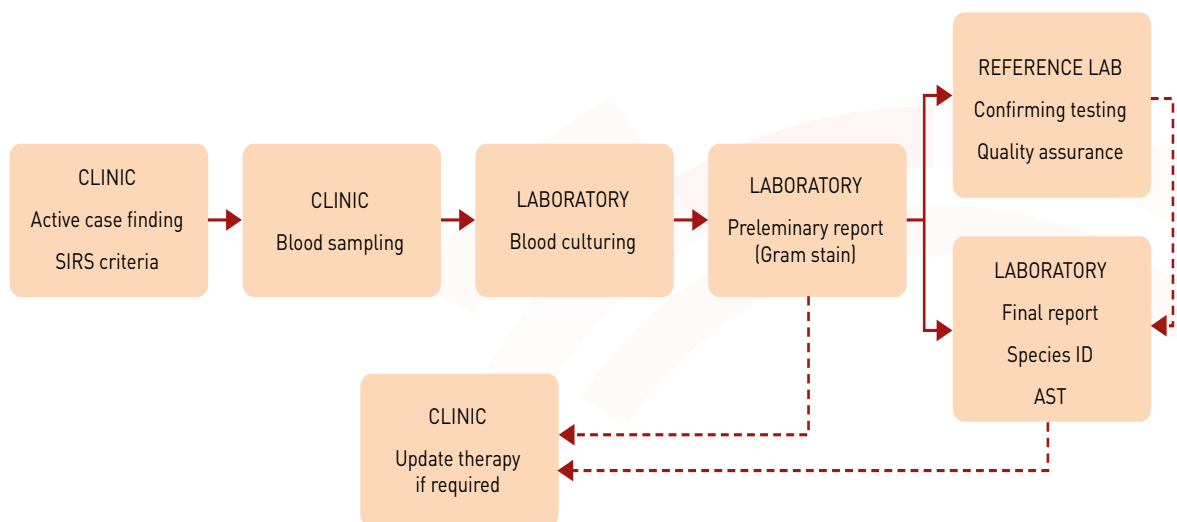
All clinicians, including nursing staff, working on selected departments/wards will be asked to support the project and the collection of samples for blood culturing. All clinicians will be trained in recognizing patients fulfilling the SIRS criteria. This approach will raise awareness about the project being done and maximize inclusion of patients meeting inclusion criteria. Patients should be included whenever they develop signs of a BSI or sepsis. All patients will be actively screened at least twice a day to increase timely recognition and inclusion of patients.

### Blood sampling

Annex 7 outlines the SOP for blood sampling procedures. Clinicians taking the blood culture samples from patients will receive training in proper sampling techniques at the start of the project and will be provided with SOPs.

- Samples for blood culture should be taken from all patients with suspected BSI, after evaluation and confirmation of the indication for blood culture by a physician.

Fig. 2. Project procedures



- Since antibiotic therapy may lead to selection of resistant pathogens, blood samples should, whenever possible, be taken prior to starting antibiotic therapy. However, because resistance proportions should reflect resistance levels at admission to the hospital and during hospital stay, antibiotic therapy initiated prior to admission to hospital will not be a reason for exclusion. Additionally, patients already admitted who are suspected of having a BSI developing during antibiotic treatment or who have a recurrence of symptoms after a period of remission or quiescence following initial antibiotic treatment will not be excluded. Distinction between patients with or without prior treatment will be made using the information collected on the clinical data form (Annex 3).
- Taking blood for culture should not delay the initiation of antibiotic therapy. To ensure this, sampling materials should be available at all selected departments/wards.
- Puncture sites will be carefully disinfected; the site is cleaned with alcohol followed by chlorhexidine and allowed to dry. Note. If possible, sampling from indwelling catheters should be avoided.
- Tops for blood culture bottles will be disinfected with 70% isopropyl alcohol (propan-2-ol; alcohol pad).
- For adults, two blood sample sets will be taken by venipuncture from separate sites with a maximum of 30 minutes apart, provided that the situation of the patient allows this. Each set contains two blood culture bottles of 10 ml blood each.
- For paediatric patients, the blood volumes taken vary with patient weight (see Annex 7 for details).
- The blood withdrawn should be immediately inoculated directly into the appropriate blood culture vial(s) without changing the needle on the syringe.

### **Sample processing at the laboratory**

Annex 8 outlines the detailed sample processing steps at the laboratory.

After sampling, the blood cultures are stored at the hospital laboratory (or contracted external laboratory) for incubation for a maximum of seven days. Blind subcultures will be performed after 24 h, 48 h, and seven days incubation at the site. If bacterial growth is detected, species ID and AST will be conducted on site. For reasons of quality assurance, each positive blood culture isolate will be sent to the national reference laboratory for parallel ID and AST. In addition, reference laboratory will perform confirmation of any special resistance patterns and further characterization of resistance mechanisms.

### **Blood culturing**

Blood culturing procedures are described in detail in the SOP for sample processing (Annex 8).

### **Number of cultures**

The optimal number of blood cultures that should be obtained varies with the clinical condition, suspicion for underlying infection (e.g. the pretest probability), and the urgency of the need for treatment (19). The total volume of blood drawn may be more important than the number of sets taken at the same time. In this study by Patel et al, collection of two aerobic and one anaerobic blood culture bottles per set resulted in improved yield compared to two bottles per set (10 ml per bottle) (20). Among 134 patients in the same study, the yield from two blood culture sets (30 ml in three bottles per set) was the same as the yield from three blood culture sets (20 ml in two bottles per set) (20). Another study compared the yield of various numbers of blood cultures taken within 24 hours (20 ml in two bottles per culture set). The cumulative yield of true pathogens increased from 65% in the first culture to 80% including the second culture, 96% including the third culture, and 100% including the fourth culture (21).

## Species ID

Standard methods for species ID will be used. All isolates should be identified to genus level. The following bacterial species should be fully identified:

- *Streptococcus pneumoniae* (STRPNE)
- *Staphylococcus aureus* (STAAUR)
- *Enterococcus faecalis* (ENCFAE)
- *Enterococcus faecium* (ENCFAI)
- *Escherichia coli* (ESCCOL)
- *Klebsiella pneumoniae* (KLEPNE)
- *Pseudomonas aeruginosa* (PSEAER)
- *Acinetobacter* spp.

*Nota Bene:* Coagulase-negative staphylococci should be identified as such. Other isolates should also be recorded but could be identified to genus level.

## AST

AST will be done by disc diffusion according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards (22). Annex 9 contains a minimum set of antibiotics to be tested per microorganism under surveillance. These “bug–drug” combinations are based on the recommendations in the CAESAR manual (1), including indicator antibiotics for the main antibiotic groups plus some empirical treatment options not in the CAESAR manual.

## Isolate storage and shipment

Shipping and transportation of isolates will be performed according to the recommendation provided in SOPs of the national AMR reference laboratory. Positive isolates and accompanying clinical data forms will be collected on the same day by a driver from the national AMR reference laboratory. The local team (microbiologist) will contact the coordinator team (microbiologist) about the existence of a positive sample and the national AMR reference laboratory will organize transport in timely manner. All isolates cultured at the local laboratories will be shipped to the national AMR reference laboratory for duplicate testing at central level.

## Feedback of laboratory results

Preliminary and final laboratory results should be noted on the laboratory results form – see Annex 4.

Each clinical laboratory should appoint a (clinical) microbiologist responsible for providing feedback to clinicians on preliminary results (Gram stain of a positive blood culture) and final results of blood cultures (species ID and AST). Feedback should be timely and frequent (as soon as results become available) to establish a strong working relationship. It is expected that most results will be final within 72 hours. It is recommended that the microbiologist provides preliminary results to the clinician over the telephone. The local laboratory is responsible for informing the clinicians on species ID and AST results based on local testing without waiting for confirmation by national AMR reference laboratory. The process of communication and feedback of results between the microbiologist and clinician will be registered on the feedback form (Annex 5).

### **Weekly meeting**

A weekly meeting with clinicians, clinical epidemiologists and the clinical microbiologist will be held to discuss patients and the laboratory results and any implications for antibiotic treatment at each of the project sites. The project coordination team will be present at this meeting and will fill out an evaluation form to monitor progress of the project (Annex 6). In addition, timely and frequent feedback on results of confirmatory testing done at the national reference laboratory should be provided to hospital laboratories to allow timely trouble-shooting and adjustment of procedures in case of discrepant results.

### **Roles and responsibilities of the teams at the project sites**

- Clinical microbiologist: sample processing, blood culturing, species ID and AST; feedback of laboratory results to clinician; filling in laboratory forms; participating in weekly meetings
- Clinicians: active case finding, obtaining consent for patient participation (signed informed consent form), blood sampling, incorporating laboratory results for treatment adjustments, filling in clinical forms, participating in weekly meetings
- Clinical epidemiologists: active case finding, coordination of data collection and completion of forms, participating in weekly meetings.

## **Data management and statistical analysis**

### **Unique patient identifier**

The unique patient identifier (patient registration number) used in the hospital together with the date of sampling will be used for the unique identifier combination for the patient. Both are required for merging of the data from different sources. *This combination should be present on all forms and patient materials.*

### **Laboratory data**

Laboratory results will be entered on paper laboratory result forms (Annex 4) and isolate record forms. Copies of the laboratory results and isolate record forms will be collected by the study coordinator on a weekly basis and checked for completeness and consistency. Whenever necessary, data are completed and/or corrected in consultation with the hospital laboratory. Data will then be entered/compiled in a dedicated database. Data will be entered twice using predesigned data entry screens (23) and any discrepancies manually corrected.

### **Clinical data**

With each clinical episode (i.e. every time blood cultures are taken), the clinical data form should be filled in (Annex 3). Copies of the clinical data forms will be collected by the project coordinator on a weekly basis and checked for completeness and consistency. Where necessary, forms will be completed and/or corrected after consultation with the clinician. Data will then be entered into a dedicated database. Data will be entered twice using predesigned data entry screens (23) and any discrepancies manually corrected.

### **Confidentiality of patient data**

All data and diagnostic specimens transferred to the project coordinator and the reference laboratory will be devoid of any personal identifying information (name, date of birth, residential address). The original patient registration number will be used as the unique patient identifier, rather than a project-specific unique identifier, to allow discussion of laboratory results and clinical process during the weekly project meeting (i.e. to prevent any miscommunication, mixing up of results).

Original clinical and laboratory data forms will remain at the hospital laboratory and be archived according to local procedures. Copies will be stored in a locked filing cabinet at the reference laboratory. Data entered into databases will be password protected and stored on secure computers/servers.

## Analysis of AST data

### Main objective (antibiotic susceptibility):

- the weighted average susceptibility for each of the main antibiotic groups used for empirical treatment of a BSI will be calculated. For the calculation of the weighted average, the total number of resistant bacteria is used as the numerator. The denominator is the total number of relevant microorganisms isolated. (See also Table 2, page 6);
- cumulative antibiograms (% sensitive/intermediate/resistant) for main antibiotic–pathogen combinations will be produced according to standard procedures (24) and as described in detail in the CAESAR manual (1). Analysis may be stratified by patient characteristics; e.g. age, ward, source of infection (nosocomial or community-acquired).

### Secondary objectives (performance indicators):

- comparison of the results of species ID and AST performed in duplicate at local and central level, to ensure the quality of the data produced during the project;
- blood culturing practice
  - rate of blood culturing (number of BC sets per 1000 patient-days);
  - culture positivity rate (% BC with growth of pathogenic bacteria);
  - contamination rate (% BC with growth of a bacterium judged to be a contaminant);
  - proportion (%) of patients on antibiotics when BC is taken;
- timing of actionable laboratory results (median and interquartile range);
  - time to blood culture positivity (Gram stain result);
  - time to final blood culture results;
- proportions of adequate antibiotic treatment (percentages and 95% confidence intervals);
  - use of active and non-active therapy;
  - use of empirical therapy: unnecessary Gram-positive or Gram-negative coverage; and
- therapy change after laboratory result: de-escalation, escalation, expansion, optimization.

## Quality assurance

### Training

Prior to initiation of the project, microbiologists from participating laboratories will be trained in blood culture procedures and techniques, species ID and EUCAST AST methodology. Training will consist of a theoretical and a practical part. Training will be facilitated by licensed physician(s) from one of the sponsoring institutes/organizations.

At the start of the project, at each site, clinical staff will be trained (one or two days) in case identification and sampling procedures. In addition, physicians will be trained in principles of antibiotic treatment of

patients with sepsis and the incorporation of blood culture results into management to optimize antibiotic treatment (de-escalation, escalation, expansion of coverage).

### **Feedback and continuous evaluation**

A weekly project meeting to discuss clinical sampling and laboratory results will be held at the participating hospital with clinicians, (clinical) microbiologists, epidemiologists, any additional staff involved in the project and the project coordinator; this will use the evaluation form (Annex 6). The quality of sampling and data collection, sampling rates, balance of patients from community/hospital, laboratory results, good practices, barriers and any problems/bottlenecks will be discussed. Additionally, the microbiologist will give feedback on diagnostic findings.

### **Laboratory quality assurance**

Proficiency testing will be done throughout the project by testing all isolates in duplicate/parallel at local and central level. To ensure the quality of the data produced during the project and evaluate the impact of available blood culture results on clinical decision-making, the laboratory and clinical data forms will be reviewed by the infectious disease doctor/medical microbiologist of the technical support team at least once a month. In certain situations with unusual results (rare resistance phenotype), a subset of isolates can be tested at the facility of the infectious disease doctor/medical microbiologist of the technical support team. In addition, the laboratories will participate in the CAESAR external quality assessment, which will be organized via the WHO Regional Office for Europe. External quality assessment packages will be sent to the national reference laboratory for further distributed to the laboratories.

### **Project management**

The project will be coordinated by the project coordination team. Full terms of reference are included in Annex 2. In short the national coordination team is responsible for;

- monitoring progress of the project
- developing local versions of the project protocol and SOPs
- providing project materials (protocol, SOPs, project forms)
- providing laboratory supplies
- data management
- data quality assurance
- evaluating and improving procedures during weekly project meetings at hospitals
- answering practical questions about project procedures
- continuing education

The project coordinator will be supported at the technical level by the project initiators. The project coordinator will report back to the national AMR focal point on a monthly basis or whenever necessary to discuss the progress and any problems encountered.

### **Safety considerations**

Taking samples for blood culture from patients is considered standard care for patients with suspected BSI or sepsis (12). The associated risks are the same as for any type of venipuncture. Risk will be mitigated by ensuring that sampling is done by trained medical staff. Sterile equipment and instructions on hygienic



practice during sample taking will be provided. Sampling will be done in the hospital setting with a physician on call if adverse events occur. The sampling procedures (venepuncture) may result in haematoma, swelling, tenderness and inflammation at the puncture site; persistent bleeding; vasovagal response; and in rare cases thrombosis of the vein from trauma or infection, which can result in thrombophlebitis.

Staff in participating laboratories will be trained in blood culturing, species ID and state-of-the-art AST methods prior to initiation of the project and will be provided with quality controlled materials. In addition, blood culture isolates will also be tested at the national reference laboratory for quality assurance and confirmatory testing. Physicians will be trained in the principles of antibiotic treatment of patients with sepsis and in incorporating blood culture results to optimize antibiotic treatment (de-escalation, escalation, expansion of coverage). However, there is a risk that laboratory results may be misinterpreted (in particular false-negative results) and lead to incorrect clinical decisions, such as improper switching to a lower level of antibiotics.

Training will be given by a licensed infectious disease doctor/medical microbiologist from the European Union at the start of the project. To ensure the quality of the data produced during the project and evaluate the impact of available blood culture results on clinical decision making, the laboratory and clinical data forms will be reviewed by the infectious disease doctor/medical microbiologist of the technical support team at least once a month. The infectious disease doctor/ medical microbiologist will also be available for trouble-shooting throughout the project.

## Follow-up

Treatment and follow-up of patients providing blood samples for culture remains the responsibility of the treating physician. Results from bacteriological diagnostics will be provided to the treating physician by the local bacteriology laboratory (or the external laboratory providing microbiological diagnostic services) as soon as they become available. Adjusting treatment of the patient based on bacteriological results remains the responsibility of the treating physician. The process of communication and feedback of results between the microbiologist and clinician will be registered on the feedback form (Annex 5) and will be discussed during the weekly meeting. Importantly, parallel microbiological testing at the national AMR reference centre is primarily for the purpose of quality assurance and additional AST (confirmatory testing), as opposed to direct patient diagnostics.

## Ethics

Taking blood samples from patients with suspected BSI for microbiological diagnosis (blood cultures, species ID and AST) is considered standard care required to inform treatment decisions (12) in many European countries, and according to expert opinion is considered best practice. If a patient with suspected BSI declines participation in the PoP project, the option of taking a blood culture for further AMR diagnosis will still be offered to this patient, in accordance with the SOPs in the hospital.

This project provides technical and material support to allow the collection and processing of these samples. Patients are not subject to any additional blood sampling beyond that needed for microbiological diagnosis. Also, patients will not be treated with any experimental or unregistered drugs as part of this project. Treatment decisions remain at the discretion of treating clinician. Indeed, treatment decisions will be influenced by feedback of results from the microbiological work-up and, therefore, this need to be reliable. The adherence to quality standards and the assessment of the quality of the microbiological laboratory work is an integral part of this project, as described under quality assurance. After final confirmation at the national AMR reference laboratory, isolates will be stored at the repository for further characterization and molecular typing.

## **Risks**

The risks of taking part in this project are the same as those for any type of blood sampling; these are described in more detail in the patient information sheet (Annex 10). Another potential risk is that mistakes made in the laboratories could lead to the wrong result and, thus, incorrect treatment. This risk is, however, reduced by parallel testing at both the hospital laboratory and the national reference laboratory to ensure good-quality data. The analysts in the hospital laboratory and the national reference laboratory will have also received training before the project, as have the physicians in correctly taking blood samples.

## **Benefits**

The participants in this project will benefit from the laboratory test results as these will provide additional information to optimize their antibiotic treatment, leading to a better chance of recovery, a decrease the number of hospital-days and a lower risk of side-effects from certain antibiotics. The project results also benefit the general population of a country, providing an overview of the bacteria causing BSIs and their resistance patterns in that country and forming a basis for national AMR surveillance. The objective of the project is to establish good clinical practice that would be implemented as a sustainable routine in the participating hospitals, which can be scaled-up to become routine practice in all hospitals in the country.

## **Informed consent forms**

Patients will be informed about the project goals and procedures by the clinician, as described in the informed consent forms given in Annex 11, and it will be made clear that participation in the project is voluntary. The informed consent form outlines the purpose, procedure, risks, benefits, confidentiality and sharing of results. If participants would like to receive additional information about the project, this can be found in the patient information sheet (Annex 10). All patients will be asked by the clinician to provide written consent before participation (Annex 11). For *minors*, the parent or caregiver will be asked to give the informed consent (Form 11.2 in Annex 11). As soon as the doctor has established that the patient has a suspected BSI, he or she will contact the parent or caregiver to inform them about the procedure. A blood culture will be taken after the parent has had the opportunity to sign the consent form. When the patient is an adolescent (12–17 years), he or she is asked to sign an assent form as a minor (Form 11.3 in Annex 11) in addition to the parental consent. The informed consent forms are then stored by the clinical epidemiologist.

# Expected outputs of the project

The project should provide:

- baseline AMR data for main pathogens causing BSI in selected hospitals in the country;

In addition, the project should:

- provide a basic infrastructure for a national sentinel laboratory-based AMR surveillance network;
- demonstrate the value of clinical microbiological diagnostics in routine patient care to clinicians;
- build care provider's capacity for taking clinical samples according to standard protocols and for using microbiological information to guide treatment decisions; and
- strengthen laboratory capacity for species ID and AST at the local laboratories and the national AMR reference laboratory (also including confirmatory testing); and
- allow to create cost estimates for blood culture sampling and routine AST in patients with sepsis, which can support planning for sustainable implementation.

## Problems anticipated

An important problem that may arise is a low number of blood samples taken during the course of the project. Throughout the duration of the project, strategies to expand the teams involved in active case finding and blood sampling (including additional wards) will be discussed during weekly evaluation meetings. If the number of blood samples is too low to attain the required sample size by the end of the project, a number of secondary objectives can still be achieved.

## Dissemination of results

It is expected that results will be shared in a number of ways:

- evaluation workshop for presentation and discussion of project outcomes with all participating clinicians and laboratory personnel;
- follow-up meeting with the Ministry of Health for presentation of the results and discussion of the sustainability of the best practice demonstrated during the project;
- publication of the results and project design in a peer-reviewed journal; and
- a summary of the results of the project being published in a newsletter with copies provided to the participating hospitals and used as promotion material in a national and international setting.

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# Assessment questionnaire for participation in the PoP project

## Project goals and objectives

### Main goal:

The main overarching goal of the project is to contribute to improving clinical care for patients admitted with suspected BSIs, in accordance with evidence-based medicine.

Secondary goals of the project are:

- to demonstrate to clinicians the value of clinical microbiology as part of the diagnostic work-up of patients with suspected BSIs and to improve the clinical work-up and timely feedback of laboratory results to prescribers of antimicrobial drugs, thus allowing for optimization of antimicrobial therapy;
- to establish and support a surveillance network as a starting point for a functional national sentinel laboratory-based surveillance system for AMR;
- to provide insight into antibiotic resistance levels in a country.

### The projects objectives are:

Ad 1.

- improve active case finding practice;
- improve turnaround time at key steps of blood sample processing at the local laboratories;
- improve concordance between local and reference laboratory in reaching accurate test results;
- ensure multidisciplinary interaction around a patient.

Ad 2.

- strengthen the AMR reference and surveillance capacity at the national reference laboratory, by providing:
  - terms of reference;
  - training on international guidelines (EUCAST, laboratory quality stepwise implementation (LQSI));
  - training on how to train local laboratory personnel;
  - support to set up national external quality assessment scheme (NEQAS);
- strengthen capacity at the local laboratories, by providing
  - training on international guidelines (EUCAST, LQSI);
- ensure transport of samples, isolates, and data between local laboratories, reference laboratory and national AMR surveillance group.

Ad 2 and 3.

- increase the volume of clinical samples processed by local and reference laboratories, thus building laboratory capacity.

Ad 3.

- contribute local AMR data to a national database;
- improve the capacity to manage, analyse, interpret, and report on AMR data, by providing training;
- provide a top 5 ranking of organisms and a cumulative antibiogram;
- provide data for national and regional surveillance.

In addition, the project should allow the estimation of costs involved in routine blood culture sampling and routine AST in patients with suspected BSI, which can support planning for sustainable implementation.

## Questionnaire

Please fill in this questionnaire to assess the possibility of participation in the project.

1. Name of hospital

---

2. Name of person filling in the questionnaire

---

3. Address

---

4. Contact details (telephone/email)

---

5. Date the hospital was opened

---

6. Hospital profile (general, specific, etc.)

---

7. Hospital structure

7.1. Subordination, owner of hospital (private, governmental, etc.):

---

7.2. Please provide total numbers for [year]

Admissions	Patients	Beds	Patient days	Doctors	Nurses	Epidemiologists

7.3. Please provide total numbers per department (emergency, intensive care, etc.) for [year]

Department	Admissions	Patients	Beds	Patient days	Doctors	Nurses	Epidemiologists

7.4. Please provide the total number of patients with suspected BSIs (sepsis, septicaemia and bacteraemia) in [year]:

---

7.5. What is the total number of blood cultures taken in [year]?

---

*If available, please add the departments (ICU, surgery, etc) at which blood cultures were taken:*

Department	Number of blood cultures taken
Total number	
Intensive care unit	
..	

7.6 What is the number of positive blood cultures in [year]? \_\_\_\_\_

7.7. Does a microbiology laboratory exist in the hospital? Yes/No \_\_\_\_\_  
(if no, please go to question 9)



## 8. Overview of bacteriology laboratory

### 8.1. Staff at the bacteriology laboratory

Staff	Number
Clinical microbiologists, MD	
Microbiologists/bacteriologists	
Analysts	
Other, please specify	

### 8.2. Microbiology laboratory equipment

Equipment	Year of issue	Specifications
Centrifuge		
Incubator		
Refrigerator		
Microscope		
Automated system for blood cultures		
Other, please specify		

### 8.3. Available material for bacterial species identification and antimicrobial susceptibility testing

Materials	Specifications
Antibiotic discs	
Antibiotic minimum inhibitory concentration testing	
Media	
Other, please specify	

8.4. Does the laboratory perform species identification of microorganisms? Yes/No \_\_\_\_\_

8.5. Does the laboratory perform antimicrobial susceptibility testing? Yes/No \_\_\_\_\_

8.6. Are SOPs and guidelines (e.g. from CLSI or EUCAST) used in the laboratory? Yes/No \_\_\_\_\_  
 If yes, please specify

SOP guidelines for	Yes/No	Specify
Bacterial species identification		
Antimicrobial susceptibility testing		
Other, please specify		

8.7. What were the main microorganisms isolated in blood cultures at the laboratory in [year]

Microorganisms	Total

8.8. Does the laboratory have an electronic laboratory information system where laboratory results are stored? Yes/No \_\_\_\_\_  
 If yes, what kind of system

\_\_\_\_\_

If no, are the results stored in a paper-based log book? Yes/No \_\_\_\_\_

9. Are guidelines for empiric antibiotic therapy used in the hospital? Yes/No \_\_\_\_\_  
 If yes, please specify

\_\_\_\_\_

10. Does the hospital have a pharmacy? Yes/No \_\_\_\_\_

11. Would the hospital like to participate in the PoP project and collaborate with the National Centre for Disease Control? Yes/No \_\_\_\_\_

12. Other information that is relevant to the project

\_\_\_\_\_

\_\_\_\_\_

*Thank you very much for your participation!*

# Terms of reference for a national coordination team for PoP project

## Tasks of the project coordination team during the preparatory phase

1. Selection of three hospitals for participation using the Assessment Questionnaire for Participation in the PoP project (Annex 1) with defined criteria
2. Forming the multidisciplinary teams in the hospitals
3. Introducing the hospital teams to the PoP project with support from WHO consultants
4. Procuring the needed consumables with WHO support
5. Adapting the PoP protocol and SOP to fit the country's clinical setting
6. Provide training/retraining on project principles and procedures to anyone involved in the project, with WHO support.

## Overall tasks of the national team during the project

1. Ensure that each team member has access to all available (clinical and laboratory) information collected during the project to allow the monitoring of progress and discussion quality issues within the project coordination team
2. Work towards achieving the project goals and objectives of the PoP project as described in the protocol
3. Ensure that the project is undertaken in accordance to the procedures and standards specified in the project protocol.
4. Monitor and support the hospital teams for the correct implementation of the project methodology, active case finding, blood taking for culture, sample processing in the laboratory (diagnostic testing, sample storage and shipment), feedback of laboratory results and collection of clinical and laboratory data
5. Take responsibility for planning weekly meetings at the participating hospitals throughout the project period and keep records of the discussed items and action points via the weekly meeting evaluation forms; follow up where needed.

## Specific roles and responsibilities of team members

### National AMR focal point

1. Responsible for the overall coordination of the project and communication with the Ministry of Health and directors of the participating hospitals

2. Responsible for the communication with the principal investigator, the team of consultants and WHO country office, providing monthly progress reports that include an overview of the data collected so far and any issues that need to be solved to ensure smooth continuation of the project

### **Clinician**

1. Support the hospital teams with active case finding, obtaining consent for patient participation (signed informed consent form), blood sampling procedures, filling clinical forms and interpreting laboratory results in relation to adjusting antibiotic therapy
2. Participate in weekly meetings at the hospitals and provide feedback and support to the hospital teams where needed
3. Together with the local and the national epidemiologist, make sure that the correct clinical information is collected at the hospital level and send to the national database.

### **Microbiologist**

1. Support the clinical bacteriologists at the participating hospital with sample processing (blood culturing, species ID and AST), providing feedback of laboratory results to clinician and completion of laboratory forms
2. Participate in weekly meetings at the hospitals and provide feedback and support to the hospital teams, specifically regarding the laboratory aspects and ensuring complete and correct collection of the requested laboratory information
3. Coordinate proper storage and timely transfer of positive blood culture isolates from the hospital to the national reference laboratory for retesting of the isolates, provide feedback on the comparability of results and discuss ways to improve the quality if required during the weekly meetings or through direct communication if required
4. Communicate directly with the microbiology consultant from the international team if any unusual results are found and for any other issues related to the laboratory work.
5. Make sure that materials are in stock and available throughout the project
6. With support of the microbiology consultant, ensure that the reference laboratory has a good quality management system (in collaboration with support provided/initiatives by other partners) and work towards improving the laboratory quality management in the hospital laboratories.

### **Epidemiologist**

1. Support the hospital teams with setting up logistics for active case finding, with coordination of data collection, including continuous monitoring of data completeness
2. Participate in weekly meetings at the hospitals and provide feedback and support to the hospital teams to ensure complete collection of the requested information
3. Together with the hospital teams make sure that all needed information is collected at the hospital level and sent to the national database
4. Ensure that the forms and related samples have a unique identifier to connect all the data collected per patient/isolate for data entry at national level and feedback to the hospitals
5. Take responsibility for timely digital data entry at national level and provide overviews of data collected by the hospitals to discuss during the weekly meetings and the monthly reports, with support of a data manager.

### **Data manager**

1. With the support of WHO consultants and the national epidemiologist, develop and maintain a database to collect all the data gathered at hospital level and the reference laboratory
2. Enter the project forms provided by the hospitals in the national database
3. Ensure that the collected data are kept safe (password encrypted) and that regular back-ups are made of the database
4. Support the epidemiologist with the data analyses to generate overviews of data collected by the hospitals for the weekly meetings, monthly and final reports.

# Clinical data form

## Patient information

1. Hospital: \_\_\_\_\_
2. Patient ID: \_\_\_\_\_
3. Date of birth (dd-mm-yy): \_\_\_\_\_
4. Gender:      male      female      unknown

## Clinical data

5. Date of sampling (dd-mm-yy): \_\_\_\_\_
6. Date of admission to hospital (dd-mm-yy): \_\_\_\_\_
7. Primary diagnosis at admission: \_\_\_\_\_
8. Ward:
 

<input type="checkbox"/> paediatrics/ neonatal	<input type="checkbox"/> paediatrics/ neonatal intensive care unit
<input type="checkbox"/> intensive care unit	<input type="checkbox"/> haematology/oncology <input type="checkbox"/> obstetrics/ gynaecology
<input type="checkbox"/> emergency	<input type="checkbox"/> internal medicine <input type="checkbox"/> infectious disease ward
<input type="checkbox"/> surgery	<input type="checkbox"/> urology <input type="checkbox"/> dermatology
<input type="checkbox"/> other: _____	<input type="checkbox"/> unknown
9. If in intensive care unit, date of admission at intensive care unit (dd-mm-yy): \_\_\_\_\_
10. Which SIRS symptoms led to suspicion of blood stream infection:
 

<input type="checkbox"/> fever	<input type="checkbox"/> hypothermia	<input type="checkbox"/> increased heart rate
<input type="checkbox"/> decreased heart rate	<input type="checkbox"/> increased respiratory rate	<input type="checkbox"/> increased leukocyte count
<input type="checkbox"/> decreased leukocyte count	<input type="checkbox"/> increased immature neutrophils	
11. First day of SIRS symptoms (dd-mm-yy): \_\_\_\_\_

12. Suspected focus of infection:

- upper respiratory tract       lower respiratory tract       urinary tract
- indwelling vascular catheter       wound or burn       skin or soft tissue
- bone or joint       gastrointestinal       genital
- central nervous system       other: \_\_\_\_\_       unknown

13. Was the patient being treated with antibiotics at the time the blood culture was taken?

Yes/No \_\_\_\_\_

14. If yes, please specify the details for each antibiotic the patient is taking:

**Antibiotic 1**

Name of the drug: \_\_\_\_\_ Frequency: \_\_\_\_\_ times per day

Dosage: \_\_\_\_\_ Route of administration: \_\_\_\_\_

Date of start: \_\_\_\_\_

**Antibiotic 2**

Name of the drug: \_\_\_\_\_ Frequency: \_\_\_\_\_ times per day

Dosage: \_\_\_\_\_ Route of administration: \_\_\_\_\_

Date of start: \_\_\_\_\_

**Antibiotic 3**

Name of the drug: \_\_\_\_\_ Frequency: \_\_\_\_\_ times per day

Dosage: \_\_\_\_\_ Route of administration: \_\_\_\_\_

Date of start: \_\_\_\_\_

15. Has the patient been treated with antibiotics within 3 months prior to the blood culture? Yes/No

16. If yes, please specify the details for each antibiotic the patient has taken in the past 3 months.

**Antibiotic 1**

Name of the drug: \_\_\_\_\_ Frequency: \_\_\_\_\_ times per day

Dosage: \_\_\_\_\_ Route of administration: \_\_\_\_\_

Date of start: \_\_\_\_\_ Date of end: \_\_\_\_\_

**Antibiotic 2**

Name of the drug: \_\_\_\_\_ Frequency: \_\_\_\_\_ times per day

Dosage: \_\_\_\_\_ Route of administration: \_\_\_\_\_

Date of start: \_\_\_\_\_ Date of end: \_\_\_\_\_

**Antibiotic 3**

Name of the drug: \_\_\_\_\_ Frequency: \_\_\_\_\_ times per day

Dosage: \_\_\_\_\_ Route of administration: \_\_\_\_\_

Date of start: \_\_\_\_\_ Date of end: \_\_\_\_\_

17. Was patient admitted to a hospital within 3 months prior to current hospitalization?

Yes/No \_\_\_\_\_

18. Was the patient transferred from a different hospital or clinic?

Yes/No \_\_\_\_\_

19. If so, from which hospital or clinic: \_\_\_\_\_

20. Comments:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_



# Laboratory results form

## Local laboratory results form

### Sample information

1. Hospital: \_\_\_\_\_
2. Patient ID: \_\_\_\_\_
3. Date of birth (dd-mm-yy): \_\_\_\_\_
4. Date of sampling (dd-mm-yy): \_\_\_\_\_
5. Lab code: \_\_\_\_\_
6. Date sample received at the laboratory (dd-mm-yy): \_\_\_\_\_

### Preliminary result

7. Date (dd-mm-yy): \_\_\_\_\_
8. Gram stain       positive       negative       other: \_\_\_\_\_
9. Type               bacilli       cocci       other: \_\_\_\_\_
10. Reported to the clinic: yes /no \_\_\_\_\_  
Date (dd-mm-yy) \_\_\_\_\_

### Final result

11. Date (dd-mm-yy): \_\_\_\_\_
12. Isolate #1 ID \_\_\_\_\_      Isolate #2 ID \_\_\_\_\_      Isolate #3 ID \_\_\_\_\_
13. Species #1 \_\_\_\_\_      Species #2 \_\_\_\_\_      Species #3 \_\_\_\_\_
14. Reported to the clinic: yes /no \_\_\_\_\_  
Date (dd-mm-yy): \_\_\_\_\_

**Fill in isolate record form per isolate as provided in the CAESAR manual (1).**

## Reference laboratory results form

### Sample information

1. Hospital: \_\_\_\_\_
2. Patient ID: \_\_\_\_\_
3. Date of birth (dd-mm-yy): \_\_\_\_\_
4. Date of sampling (dd-mm-yy): \_\_\_\_\_
5. Date sample received at reference laboratory (dd-mm-yy): \_\_\_\_\_

### Result

6. Date (dd-mm-yy): \_\_\_\_\_
7. Isolate #1 ID \_\_\_\_\_ Isolate #2 ID \_\_\_\_\_ Isolate #3 ID \_\_\_\_\_
8. Species #1 \_\_\_\_\_ Species #2 \_\_\_\_\_ Species #3 \_\_\_\_\_
9. Reported to the local laboratory: yes /no \_\_\_\_\_  
Date (dd-mm-yy): \_\_\_\_\_

**Fill in isolate record form per isolate as provided in the CAESAR manual (1).**

### Reference

1. Central Asian and eastern European surveillance of antimicrobial resistance (CAESAR manual), version 2. Copenhagen: WHO Regional Office for Europe; 2015 (<http://www.euro.who.int/en/health-topics/disease-prevention/antimicrobial-resistance/publications/2015/central-asian-and-eastern-european-surveillance-of-antimicrobial-resistance.-caesar-manual,-version-2,-2015>, accessed 18 June 2017)

# Feedback form

## Patient information

1. Hospital: \_\_\_\_\_
2. Patient ID: \_\_\_\_\_
3. Date of birth (dd-mm-yy): \_\_\_\_\_
4. Date of sampling (dd-mm-yy): \_\_\_\_\_

## Feedback information

5. What was empiric antibiotic therapy? Specify name of drug, frequency, dosage, route of administration  
\_\_\_\_\_  
\_\_\_\_\_
6. Was therapy changed after preliminary result? Yes/no \_\_\_\_\_
7. If so what was therapy changed to? Specify name of drug, frequency, dosage, route of administration  
\_\_\_\_\_  
\_\_\_\_\_
8. Was therapy changed after AST results? Yes/no \_\_\_\_\_
9. If so what was therapy changed to? Specify name of drug, frequency, dosage, route of administration  
\_\_\_\_\_  
\_\_\_\_\_
10. Comments: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

# Evaluation form for weekly meetings

This form is filled out by the project coordinator/epidemiologist upon the meeting visit.

## Hospital information

1. Date of meeting (dd-mm-yy): \_\_\_\_\_
2. Hospital name: \_\_\_\_\_
3. Number of attendees: \_\_\_\_\_
4. Department(s) present at meeting represented: \_\_\_\_\_

## Sampling and data collection (agenda points)

- Discuss the clinical and laboratory results forms you have received and bring results from any parallel diagnostic testing done at the national AMR reference laboratory.
- Comment on the clinical and laboratory results forms you have received: whether they are being filled out appropriately and clearly, and if you are having any issues receiving them with the samples.
- Highlight any results (AST, species ID, Gram stain, etc.) from the reference laboratory that are different from those of the routine laboratory Highlight any areas for improvement of the isolates sent to the national AMR reference laboratory, mentioning any signs of possible sample contamination, mislabelling or lack of labelling, etc.
- State any other important findings with the isolates: any information or results that you want to discuss with the hospital and laboratory staff.

## Meeting notes

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## Barriers, bottlenecks and logistics

Please circle one response and fill in any details:

Any issues with the quality and/or usability of laboratory and hospital supplies provided?  
Explain:

Yes/No

Will any needed supplies be depleted soon and need replenishing?  
Explain:

Yes/No

Are participants having any issues with the SOPs, the forms and/or preparing the isolate shipment?  
Explain:

Yes/No

Are there any issues or a need for improvement of participating staff communication?  
Explain:

Yes/No

Please list any other issues that are affecting the progress and/or quality of the project

List any good practices, or positive findings

# SOP for sampling blood for culture

Blood culture is considered to be the gold standard investigation for the detection of microorganisms in blood and this SOP outlines procedures specific for taking blood cultures (based on York et al. (1)).

Two sets of blood cultures should be taken from different venipuncture sites (to help in distinguishing skin contamination from true bacteraemia) and preferably 30 min apart (to increase likelihood of taking a sample at the right moment in intermittent bacteraemia).

A blood culture set usually consists of blood from a single venipuncture inoculated into two separate bottles at the same time, one aerobic and one anaerobic (usually 20 ml for adults). A blood-to-broth ratio of 1:5 to 1:10 is optimal (e.g. 2–4 ml of blood added to a 20 ml paediatric culture bottle and 5–10 ml blood added to a 50 ml adult bottle). If separate aerobic and anaerobic blood culture bottles are not available, two aerobic bottles should be used in a set to increase the volume of blood taken in one sample (one set will be two 10 ml blood samples, 20 ml in total).

*Note.* Adequate volume is the most important factor to increase the likelihood of detecting microorganisms in the bloodstream.

## Timing of blood cultures

Ideally, blood culture should be taken prior to the start of antibiotic therapy and preferably 30 min before the peak of temperature or as soon as possible after the rise of temperature above 38.5°C.

When necessary to draw blood samples from patients on antimicrobial therapy, samples should be collected just before the next dose of antimicrobial drug, when its concentration in the blood will be at its lowest.

The collection of additional blood samples might be necessary under certain circumstances and should be individually evaluated for each patient. Additional information on the number of cultures can be found at the end of this Annex.

*Note.* Although drawing blood cultures before or during the fever spike is optimal for recovery, volume is more important than timing in the detection of agents of septicaemia.

## Disinfection of blood culture bottles

Following removal of the metal cap from the blood culture bottle, the rubber septum should be cleaned with 70% alcohol and allowed to dry.

The bottle should be labelled with the patient's name, hospital registration number and the date and time of sample collection.

## Skin antisepsis and collection of blood from venipuncture

1. Select a different site for each blood culture set.

2. Thoroughly clean the skin using 70% ethanol–2% chlorhexidine solution, allowing the skin to dry after cleaning.
3. Do not repalpate the skin after disinfection. This will minimize the chance of introducing contaminating skin organisms into the blood culture bottle.
4. Do not draw blood from the vein into which an intravenous solution is running.
5. If poor access requires blood culture to be drawn through a port in an indwelling catheter, the second culture must be from a peripheral site, because cultures drawn through catheters can indicate catheter colonization and may not be indicative of sepsis.
6. Immediately inoculate the blood withdrawn in the syringe directly into the appropriate blood culture vial(s) without changing the needle.

Blood should be inoculated into the blood culture bottle prior to inoculation of other blood tubes (e.g. EDTA (ethylenediamine tetraacetic acid) tube for complete blood count) to ensure enough blood is inoculated and to reduce the possibility of contamination.

Recommended total volume and numbers of blood cultures:

- For adults and children weighing > 36 kg: 40–60 ml divided between two blood culture sets (the minimal requirement is 20–30 ml of blood in two draws).
- For children, the recommended volume depends on the body weight of the patient, see table 7A.1.
- When 10 mL of blood or less is collected, it should be inoculated into a single aerobic blood culture bottle.

**Table 7A.1 Recommended volumes of blood for culture in paediatric patients**

Weight of patient (kg)	Recommended volume of blood for culture (mL)		Total volume for culture (mL)
	Culture set No. 1	Culture set No. 2	
≤1	2	...	2
1.1 – 2	2	2	4
2.1 – 12.7	4	2	6
12.8 – 36.3	10	10	20
>36.3	20 – 30	20 – 30	40 – 60

## Specimen transport

Blood cultures should be transported to the laboratory as soon as possible after collection.

If delays occur, the bottle should be kept at room temperature if processed in automated culture systems. If processed manually, bottles should be kept at 37°C. Blood cultures should not be refrigerated.

## References

1. York MK, Henry M, Gilligan P. Blood cultures. In: Isenberg HD, editor, Clinical microbiology procedures handbook, second edition: blood cultures, general detection and interpretation. Washington DC: ASM Press; 2004:3.4.1.1–3.4.1.4.
2. Baron EJ et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). Clin Infect Dis. 2013;57:c22-c121



# SOP for processing samples

This SOP has been adapted from the SOP for blood culture developed by Global Health Laboratories (1).

## Aim

To describe the processing of blood to identify invasive bacterial infection.

## Principle

Culture of blood is the gold standard investigation for detection of microorganisms in blood. Sensitivity of blood culture is poor (approximately 10–15% of blood cultures will be positive) but when positive this finding is of great clinical importance. Taking an adequate volume of blood is the most important factor to increase the likelihood of detecting microorganisms in blood. BSIs may be transient, intermittent or continuous, and may be either community acquired or health care acquired. Infecting organisms vary by patient age, immune status and presence of comorbidities as well as by site of primary infection. The bacterial load (in colony-forming units per litre) in paediatric infection is often greater than that in adult infections ( $>4 \times 10^3$  versus  $<1 \times 10^3$ ). BSIs can also be caused by certain fungi (fungaemia), most notably *Candida* spp.

**Common Gram-positive pathogens** detected in blood cultures include:

*Staphylococcus aureus*  
*Streptococcus pneumoniae*  
*Streptococcus pyogenes* (group A)  
*Streptococcus agalactiae* (group B)  
*Enterococcus* spp.  
*Listeria monocytogenes*.

**Common Gram-negative pathogens** detected in blood cultures include:

*Escherichia coli*  
*Klebsiella* spp.  
*Enterobacter* spp.  
*Salmonella* spp. (including *S. typhi*)  
*Pseudomonas aeruginosa*  
*Neisseria meningitidis*  
*Haemophilus influenzae*  
*Burkholderia pseudomallei*  
*Acinetobacter baumannii*.

Contamination of the blood sample during sample collection may occur. Organisms that are often regarded as skin commensals and of unlikely significance include coagulase-negative staphylococci, diphtheroids (Gram-positive rods with morphology similar to *Corynebacterium* spp.), viridians streptococci and *Bacillus* spp. (excluding *B. anthracis*). Isolation of the same "contaminant" from multiple sets of blood cultures from the same patient should prompt re-evaluation of significance and further investigation. These organisms may frequently be involved in endocarditis or foreign body infection, most commonly infections associated with central venous catheters.

## Method

### Specimen transport

Blood cultures should be transported to the laboratory as soon as possible after collection.

If delays occur, the bottle should be kept at room temperature if processed in automated culture systems. If processed manually, bottles should be kept at 37°C. Blood cultures should not be refrigerated.

### Specimen processing

#### Reception

The specimen is logged in the appropriate specimen book and assigned a specimen number.

#### Rejection criteria for specimens

Unlabelled blood cultures and broken or cracked bottles should be discarded.

*Note.* Inform the clinician before rejecting the specimen. If not possible to substitute the samples, process the samples with an adequate remark.

#### Culture

Handling blood culture bottles poses a risk of generating aerosols or droplets and it is advised that all procedures are performed in a Class II biosafety cabinet. If this is not possible, use of a splash guard at the workstation is recommended (only when personal protective equipment is also used) (2).

If an automated system for blood culture processing is used, the manufacturer's instructions should be followed for insertion of the bottles into the incubator.

The alternative is manual processing. Positive bottles are either the ones detected by the automated incubator or ones detected as turbid by twice daily visual inspection. Positive blood culture bottles should be processed. The result of a preliminary Gram stain assessment on positive samples should be reported to the requesting clinician.

If manual blood culture processing is used, the bottles should be vented by:

- disinfecting the rubber septum with 70% ethanol and allowing it to dry; and
- venting the bottle by inserting a sterile BD sub-vent needle through the septum, leaving the cap in place.

The vented bottle is incubated at 35–37°C in air and inspected daily for seven days for signs of bacterial growth (turbidity or bowed septum). Each day, the bottle is moved to the appropriate tracking container (labelled day 1 to day 7) in the incubator.

Blind subcultures at 24 h, 48 h, and seven days are performed to detect early and late growth, respectively.

#### Blind subculture

If manual blood culture processing is used, blind subculture is carried out at 24 h, 48 h, and at seven days. The broth is gently mixed using a swirling motion (the bottle must not be vigorously shaken).

The cap of the sub-vent is removed being careful not to contaminate the vent.

The bottle is inverted to allow a single drop of broth to inoculate the subculture media:

at 24 h and at 48 h, a blood agar (BA), a chocolate agar (CA) and a MacConkey (MAC) plate are inoculated; and at seven days, just a CA plate is inoculated.

The 24 h and 48 h subculture plates may be divided into quarters. The inoculum is streaked out using a sterile microbiological loop to obtain single colonies.

Plates are incubated for up to 48 h at 35–37°C in air (BA and MAC plates) or 5–10% CO<sub>2</sub> (CA plates), inspecting for growth daily.

Table 8A.1 lists the subculture media and method.

**Table 8A.1. Subculture media for positive blood culture broths**

Gram stain result	Media <sup>a</sup>	Incubation			Culture readings	Target organism(s)
		Temperature (°C)	Atmosphere	Time		
All	BA <sup>b</sup>	35–37	5–10% CO <sub>2</sub>	40–48 h	Daily	Any
<b>Additional plates depending on the Gram stain</b>						
Gram-negative cocci/coccobacilli	CA <sup>c</sup>	35–37	5–10% CO <sub>2</sub>	40–48 h	Daily	<i>Haemophilus</i> spp., <i>Neisseria</i> spp.
Gram-negative bacilli	MAC	35–37	Air	16–24 h	≥16 h	Enterobacteriaceae, non-fermenters, <i>Pseudomonas</i> spp.
Yeasts	Sabouraud agar	28–30	Air	5 days	2 and 5 days	Fungi
<b>Other considerations</b>						
No organisms seen	BA, CA	35–37	5–10% CO <sub>2</sub>	40–48 h	Daily	Any
Unclear whether Gram-negative or Gram-positive organism	Blood-CNA, <sup>d</sup> MAC	35–37	Air	16–24h	≥16h	To help differentiate organisms with equivocal Gram results
Possible anaerobic infection	BA + 5 mg metronidazole disc	35–37	Anaerobic	40–48 h	≥40 h	Anaerobes
Primary culture negative but positive growth signal or Gram stain	BA + <i>S. aureus</i> streak	35–37	5–10% CO <sub>2</sub>	40–48h	≥40h	<i>Abiotrophia</i> spp.
	MAC	35–37	Air	16–24 h	≥16 h	Cysteine-dependent organisms

Notes:

<sup>a</sup> BA: blood agar, CA: chocolate agar; MAC: MacConkey

<sup>b</sup> An optochin disc may be added if streptococci are seen on Gram stain;

<sup>c</sup> Instead of CA, BA + *S. aureus* streak may be used;

<sup>d</sup> Selective media containing colistin and naladixic acid.

## Interpretation

### Minimum level of identification in the laboratory

All blood cultures should be discussed on the daily board/bench round:

- clinically significant isolates should be identified to species level; and
- any organism considered to be a contaminant may not require full species level identification.

Significant isolates are stored at -80°C in a cryovial containing 1 ml STGG (skim milk, tryptone, glucose and glycerin) with the isolate details recorded in the freezer logbook/database. If a participating hospital laboratory does not have access to a -80°C freezer, the isolates should be stored at the national AMR reference laboratory.

For the purpose of this project, all positive blood isolates should be sent to the national reference laboratory together with the corresponding patient form. Findings should be reported to the clinician without waiting for the central laboratory retesting results.

### Antimicrobial susceptibility testing

All significant isolates should have antimicrobial susceptibilities assessed according to EUCAST guidelines and standards (3).

## Reporting

### Gram stain

The organism detected is reported (by telephone/urgent ward review + hard copy).

### Culture

Growth is reported with preliminary identification based on colony morphology, Gram stain, catalase and oxidase reactions (by telephone). Absence of growth is reported as:

- no growth at 24 h if broth is clear at 24 h of blind subculture (interim report);
- no growth at 48 h (based on negative blind subculture at 24 h *and* clear broth at 48 h inspection) (interim report); or
- no growth at seven days: final report of no growth.

After complete identification, all organisms isolated are reported (with comment if isolate(s) are of doubtful significance)

- do not use the terms “no significant growth” or “mixed growth of doubtful significance”.

### Antimicrobial susceptibility testing

AST is reported as clinically indicated. No AST is needed for organisms that are considered to be skin contaminants. Whether AST should or should not be done for organisms of dubious clinical significance should be discussed with a clinician and based on the patient’s clinical presentation.

## Quality assurance

Media and identification tests should be quality controlled according to the relevant SOP in your laboratory.

## Limitations

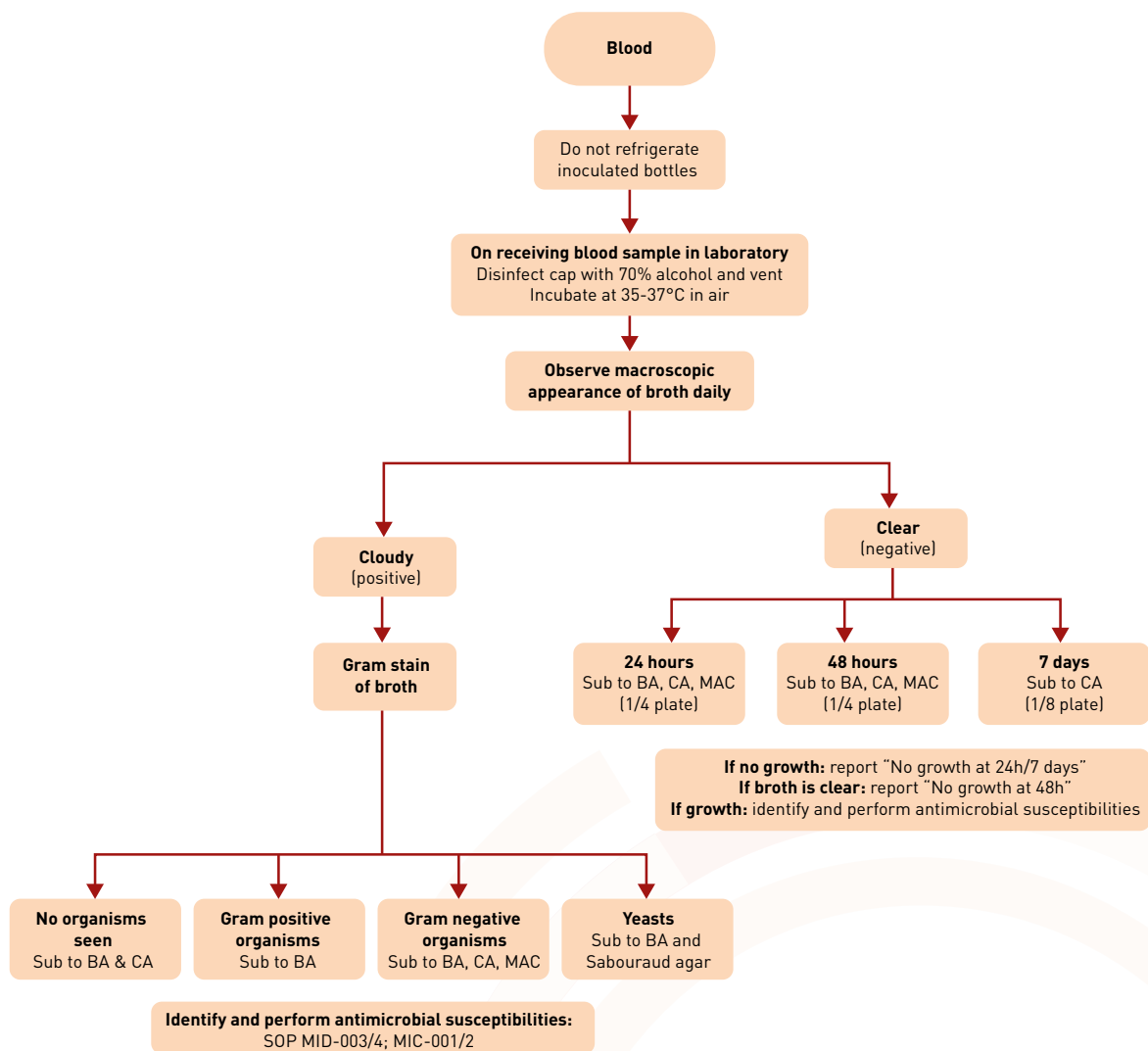
Several factors may result in a false-negative blood culture, including:

- exposure to antimicrobial agents prior to blood sampling;
- inoculation of an inadequate volume of blood; and
- intermittent bacteraemia sampled at the incorrect time point.

## Synopsis/bench aid

Fig. 8A.1 gives an algorithm for the assessment pathway.

Fig 8A.1. Assessment pathway



## Risk assessment

The University of Oxford provides an assessment form (4) for main hazards as covered by the Control of Substances Hazardous to Health Regulations 2002 (as amended; COSHH). Table 8A.2 is a risk assessment outline for culture of blood to identify pathogens.

**Table 8A.2. COSHH risk assessment for culture of blood to identify pathogens**

<p><b>Description of procedure</b> Culture of blood to identify bacterial and fungal pathogens</p>	<p><b>Substances used</b> Variable, depending on organism cultured: may include Gram stain reagents, 3% hydrogen peroxide (catalase test), <i>N,N,N',N'</i>-tetramethyl-1,4-phenylenediamine (oxidase test), sodium deoxycholate (bile solubility test), bioMerieux API reagents)</p>
<p><b>Quantities of chemicals used</b> Small</p>	<p><b>Frequency of SOP use</b> Daily</p>
<p><b>Hazards identified</b></p> <ol style="list-style-type: none"> <li>1. Autoclaved liquid</li> <li>2. Potentially infectious material in sample</li> <li>3. Potentially pathogenic bacteria</li> <li>4. Chemical exposure from bacterial identification tests</li> </ol>	<p><b>Could a less hazardous substance be used instead?</b> No</p>
<p><b>What measures have been taken to control risk?</b></p> <ol style="list-style-type: none"> <li>1. Training in GLP</li> <li>2. Appropriate personal protection equipment (laboratory coat, gloves, eye protection)</li> <li>3. Use of biosafety cabinet for reading plates until known not to be growing an HG3 (hazard group 3) organism/ follow-up of BSL-3 (biosafety level 3) organism (e.g. <i>Burkholderia pseudomallei</i>)</li> </ol>	
<p><b>Checks on control measures</b> Observation and supervision by senior staff</p>	
<p><b>Health surveillance</b> Not required</p>	<p>Training requirements GLP</p>
<p><b>Emergency procedures</b></p> <ol style="list-style-type: none"> <li>1. Report all incidents to safety adviser</li> <li>2. Use eyewash for splashes</li> <li>3. Clean up spills using 1% Virkon or chemical spill kit</li> </ol>	<p><b>Waste disposal procedures</b></p> <ol style="list-style-type: none"> <li>1. Sharps discarded into appropriate rigid containers for incineration</li> <li>2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration</li> <li>3. Chemical waste disposed of according to manufacturers' instructions</li> <li>4. Blood culture bottles disposed of by autoclaving and subsequent incineration</li> </ol>

Note: GLP: Good laboratory practice.

## References

1. Microbiology standard operating procedure: culture of blood. Amityville, NY: Global Health Laboratories; 2005 (<https://globalhealthlaboratories.tghn.org/articles/microbiology-clinical-laboratory-sops/>, accessed 17 June 2017).
2. Miller JM, Astles R, Baszler T, Chapin K, Carey R, Garcia L et al. Guidelines for safe work practices in human and animal medical diagnostic laboratories. MMWR. 2012;61(Suppl):1–101.
3. EUCAST guidance documents in susceptibility testing. Copenhagen: European Committee on Antimicrobial Susceptibility Testing; 2012 ([http://www.eucast.org/guidance\\_documents/](http://www.eucast.org/guidance_documents/), accessed 17 June 2017).

4. COSHH assessment form. Oxford: University of Oxford; 2013 ([http://www.tdi.ox.ac.uk/\\_asset/file/tdi-033-coshh-lead.pdf](http://www.tdi.ox.ac.uk/_asset/file/tdi-033-coshh-lead.pdf), accessed 17 June 2017).

### Other sources

Cheesbrough M. District laboratory practice in tropical countries, Part 2, second edition. Cambridge, UK: Cambridge University Press; 2006.

Investigation of blood cultures (for organisms other than *Mycobacterium* species). London: Public Health England; 2014 (UK Standards for Microbiology Investigations B37; [https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/372070/B\\_37i8.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/372070/B_37i8.pdf), accessed 22 June 2017).

Standard operating procedures from Lao–Oxford–Mahosot Hospital–Wellcome Trust Research Unit (LOMWRU; <http://www.tropmedres.ac/lomwru>), Shoklo Malaria Research Unit (SMRU; <http://www.shoklo-unit.com/>) and the Cambodia–Oxford Medical Research Unit at Angkor Hospital for Children (<http://www.tropmedres.ac/comru-cambodia>).

# Minimum set of antibiotics to be tested

Table 9A.1 outlines the minimum set of antibiotics to be tested per microorganism under surveillance. These “bug–drug” combinations are based on the recommendations in the CAESAR manual, including indicator antibiotics for the main antibiotic groups, plus some empiric treatment options not in the CAESAR manual (1).

**Table 9A.1. Minimum sets of antibiotics to be tested for a microorganism under surveillance**

Minimum set of antibiotics to be tested	Group name
<b><i>E. coli</i></b>	
<b>Regular surveillance</b>	
Ampicillin (AMP)	Aminopenicillin
<b><i>E. coli/Klebsiella pneumoniae</i></b>	
<b>Regular surveillance</b>	
Ceftriaxone (CRO) or cefotaxime (CTX), ceftazidime (CAZ)	Third-generation cephalosporins
Ciprofloxacin (CIP) or ofloxacin (OFX) or levofloxacin (LVX)	Fluoroquinolones
Amikacin (AMK), or gentamicin (GEN) or tobramycin (TOB) or netilmicin (NET)	Aminoglycosides
Ertapenem (ERT) or imipenem (IPM) or meropenem (MEM) or doripenem (DOR)	Carbapenems
<b>Extended monitoring</b>	
Amoxicillin–clavulanic acid (AMC)	Amoxicillin–clavulanic acid
Cefepime (FEP)	Fourth-generation cephalosporin
Norfloxacin (NOR)	Second-generation fluoroquinolone
Moxifloxacin (MFX)	Fourth-generation fluoroquinolone
Piperacillin–tazobactam (TZP)	Piperacillin–tazobactam
Polymyxin B (POL) or colistin (COL) MIC	Polymyxins
Tigecycline (TGC) (MIC or DD for <i>E.coli</i> / MIC for <i>K.pneumoniae</i> )	Glycylcycline
<b><i>P. aeruginosa</i></b>	
<b>Regular surveillance</b>	
Piperacillin (PIP) or piperacillin–tazobactam (TZP)	Piperacillin–tazobactam
Ceftazidime (CAZ)	Third-generation cephalosporin
Gentamicin (GEN) or tobramycin (TOB) or netilmicin (NET)	Aminoglycosides
Amikacin (AMK)	Aminoglycoside
Ciprofloxacin (CIP) or levofloxacin (LVX)	Fluoroquinolones
Imipenem (IPM) or meropenem (MEM) or doripenem (DOR)	Carbapenems
<b>Extended monitoring</b>	
Polymyxin B (POL) or colistin (COL) MIC	Polymyxins
<b><i>Acinetobacter spp.</i></b>	



**Table 9A.1. Minimum sets of antibiotics to be tested for a microorganism under surveillance (contd.)**

Minimum set of antibiotics to be tested	Group name
<b>Regular surveillance</b>	
Ciprofloxacin (CIP) or levofloxacin (LVX)	Fluoroquinolones
Gentamicin (GEN) or tobramycin (TOB) or netilmicin (NET)	Aminoglycosides
Imipenem (IPM) or meropenem (MEM) or doripenem (DOR)	Carbapenems
<b>Extended monitoring</b>	
Polymyxin B (POL) or colistin (COL) MIC	Polymyxins
Amikacin (AMK)	Aminoglycoside
<b><i>S. aureus</i></b>	
<b>Regular surveillance</b>	
Cefoxitin (FOX)	Detection of resistance to all semisynthetic penicillins, methicillin-resistant <i>S. aureus</i>
<b>Extended monitoring</b>	
Ciprofloxacin (CIP) or ofloxacin (OFX) or levofloxacin (LVX) or norfloxacin (NOR)	Fluoroquinolones
Rifampicin (RIF)	Rifampin
Linezolid (LNZ)	Oxazolidinone
Clindamycin (CC) and Erythromycin (ERY)	Lincosamide, Macrolide
Vancomycin (VAN) MIC	Glycopeptide
Daptomycin (DAP) MIC	Lipopeptide
<b><i>S. pneumoniae</i></b>	
<b>Regular surveillance</b>	
Oxacillin (OXA)	Detection of non-susceptibility to penicillins and cephalosporins
Penicillin (PEN) MIC	For oxacillin-resistant isolates
Erythromycin (ERY)	Macrolide
<b>Extended monitoring</b>	
Ceftriaxone (CRO) or cefotaxime (CTX) MIC	Third-generation cephalosporins
Ciprofloxacin (CIP) or ofloxacin (OFX) or levofloxacin (LVX) or norfloxacin (NOR) or moxifloxacin (MFX)	Fluoroquinolones
<b><i>E. faecalis/E. faecium</i></b>	
<b>Regular surveillance</b>	
Ampicillin (AMP)	Aminopenicillin
Gentamicin HL (GEN)	Aminoglycosides
Vancomycin (VAN)	Glycopeptide
Linezolid (LNZ)	Oxazolidinone
<b>Extended monitoring</b>	
Teicoplanin	Glycopeptide
Ciprofloxacin (CIP)	Fluoroquinolones
Levofloxacin (LVX)	Fluoroquinolones

MIC: minimal inhibitory concentration

## Reference

1. Central Asian and Eastern European surveillance of antimicrobial resistance (CAESAR manual), version 2. Copenhagen: WHO Regional Office for Europe; 2015 (<http://www.euro.who.int/en/health-topics/disease-prevention/antimicrobial-resistance/publications/2015/central-asian-and-eastern-european-surveillance-of-antimicrobial-resistance.-caesar-manual,-version-2,-2015>, accessed 18 June 2017).

# Patient information sheet

*This patient information sheet provides more detailed information in addition to the informed consent form for patients, and parents of patients with suspected bloodstream infections (Annex 11).*

*The researcher explaining the informed consent form will make the participants aware of this patient information sheet, in case the patients would like to receive additional more detailed information about the project.*

**Project title:** Proof-of-principle project to perform routine diagnostics for antimicrobial resistance surveillance

## Introduction

Together with the National Institute of Public Health and the Environment of the Netherlands, the World Health Organization Regional Office for Europe and the Ministry of Health of [insert country], we are conducting research to study the causes of blood infections and antibiotic resistance.

Blood infection can cause serious illness and can be life threatening. Your doctor suspects that you may have a blood infection. We invite you to take part in this project and provide some blood samples to test whether you do really have a blood infection. Taking blood samples from patients with suspected blood infection is a normal procedure in many countries. The results of testing your blood will give the doctor important information to help him/her decide which treatment is best for you and other patients like you.

The blood samples need to be taken as soon as possible but please feel free to ask the doctor or nurse to explain further before you decide to take part in the project. You are free to decide to take part or not to take part.

## Purpose of the project

Blood infection can be caused by different types of bacteria. Blood infection can in some cases lead to sepsis, which is a life-threatening condition. Quick and correct treatment is, therefore, very important. Blood infection can be treated with different types of antibiotic. Unfortunately, sometimes bacteria can become resistant to certain antibiotics. If a bacterium is resistant to an antibiotic, this antibiotic does no longer kill the bacterium and treat the infection. In this case, a different kind of antibiotic is needed. Only by taking blood samples and doing laboratory tests can the cause of the blood infection be found. Testing of bacteria found in blood can also tell which antibiotics work best to kill them.

The number of antibiotic-resistant bacteria is growing worldwide. This project provides doctors with information on the types of bacteria that cause blood stream infection in your country, and which antibiotics still work to treat these infections. This information helps doctors to treat their patients correctly.

## Type of intervention

If you take part in this project, two (2) blood samples will be taken, one from each of your arms. It is important that two separate blood samples are taken to have the best chance of the laboratory tests giving a useful result. The results of the laboratory tests will be shared with your doctor as soon as these are known. After receiving the test results, the doctor may decide to change your treatment. Taking blood samples from patients with suspected blood infection is a normal procedure in many countries.

## Participant selection

All patients with a suspected blood infection at any time during their stay in the hospital will be asked to take part in the project. Signs of blood infection include fever, chills, confusion, fast breathing and fast heartbeat.

## Voluntary participation

Your taking part in this project is entirely voluntary. Please be aware that taking blood samples from patients with suspected blood infection is a normal procedure in many countries and provides information that will help your doctor to better treat your illness.

## Procedures

If you take part in the project, you will receive the same starting treatment that you would get otherwise. Which treatment you get will be decided by your doctor according to national guidelines. Before starting antibiotic treatment, two (2) blood samples will be taken.

After cleaning your skin to prevent microorganisms from your skin contaminating the test, the doctor or nurse will take blood with a needle from a vein in your arm. This will be done twice, once from each arm. In total, four bottles of 10 ml each will be taken. Taking two separate blood samples is important because it gives the best chance of the laboratory tests giving a useful result.

The blood will be tested for the presence of bacteria. The bacteria will then be tested for resistance to different antibiotics. The results of the laboratory tests (type of bacteria and antibiotic resistance) will be discussed with your doctor. Your doctor may decide to change your treatment after receiving the test results. The blood sample will be destroyed after tests are finalized.

Any bacteria found in the blood will also be sent to the national reference laboratory, where the bacteria will be retested. The bacteria will also be stored in the freezer for five years to allow future studies.

## Risks

The project results will yield information to make sure you are receiving the right antibiotic treatment. The risks of taking part in this project are the same as those of taking any type of blood sample.

It is common for the site of the test to bleed after the blood sample has been taken; however, this should stop fairly quickly after a cotton wool pad or gauze patch has been placed on the wound. In some rare cases, the wound may bleed excessively. Mild bruising around the area where the needle went into the vein is fairly common after a blood test; however, in some rare cases, more severe bruising may develop. Some people may experience dizziness during or after a blood test; this is very common in people who have a fear of needles and injections.

A haematoma is a collection of blood under the skin; it is similar to bruising and is caused by the blood clotting to form a solid lump. It is fairly common to have a haematoma. In some rare cases, the site where the needle was passed into the vein may become infected; if this is the case, the wound may become red and swollen.

Another risk would be that mistakes are made in the laboratories, leading to the wrong result, which could lead to incorrect treatment. This risk is, however, minimized by dual testing done both by the hospital laboratory and the national reference laboratory, to ensure good quality of data. The analysts

in the hospital laboratory and the national reference laboratory have also received training before the project, as have the physicians to correctly take blood samples.

## Benefits

As a participant in this project you will benefit as the laboratory test results will give additional information to help your doctor to choose the right antibiotic treatment. Receiving the right antibiotic treatment will give you a better chance to recover, decrease the number of days you have to stay in the hospital and lower the risk of side-effects from certain antibiotics. The information that is collected through this project also benefits other patients like you. It is important for doctors in your country to know which bacteria are causing blood infections and which antibiotics work best against these infections. The results from your blood test and those from other participants helps to make sure that patient with blood infection will receive the most appropriate treatment.

## Confidentiality

The information that we collect in this project will be treated as confidential. Only the researchers and the hospital personnel will be able to see the results from the blood laboratory tests. The test results will not be shared with anyone outside the hospital or the researchers connected to the project. No information collected for the project will include your name, birth date or address. Only the hospital registration number will be included so the doctor knows which laboratory results belong to you.

## Sharing the results

The overall results of this project will be shared with doctors and the Ministry of Health and used to update national treatment guidelines. The results will also be shared more broadly through a project report, scientific publications and presentations at scientific conferences. Personal information will not be shared.

## Right to refuse or withdraw

You do not have to take part in this project if you do not wish to do so. You may also stop participating in the project at any time you choose. It is your choice and all of your rights will still be respected. Your treatment at this clinic will not be affected in any way. You will continue to receive medical treatment according to national standards.

## Who to contact

If you have any questions you may ask them now, later or even after the project has started. If you wish to ask questions later, you may contact [contact details].

This proposal has been reviewed and approved by Ethical Committee of [insert name of institution], which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find about more about this institutional review board, contact [contact details].

It has also been reviewed by the Ethics Review Committee of the World Health Organization, which is supporting the project.

# Informed consent forms

[Each form should carry the institutional letterhead.]

## 1.1.1. Informed consent form for patients with suspected bloodstream infections

Together with the National Institute of Public Health and the Environment of the Netherlands, the World Health Organization Regional Office for Europe and the Ministry of Health of [country] we are conducting research to study the causes of blood infections and antibiotic resistance.

### Introduction

Your doctor suspects that you may have a blood infection. We invite you to take part in this project and provide some blood samples to test whether you really do have a blood infection. Taking blood samples from patients with suspected blood infection is a normal procedure in many countries and provides information that will help your doctor to better treat your illness.

### Purpose of the project

Blood infection can in some cases lead to sepsis, which is a life-threatening condition. Quick and correct treatment is, therefore, very important. Blood infection can be caused by different types of bacteria and can be treated with different types of antibiotic. Unfortunately, sometimes bacteria can become resistant to certain antibiotics. Resistant means that an antibiotic no longer works to kill the bacteria and treat the infection. In this case, a different kind of antibiotic is needed. By taking blood samples and doing laboratory tests, we can find out which bacteria is causing the blood infection and which antibiotics will work best to kill the bacteria.

### Procedures

If you take part in this project, two (2) blood samples will be taken, one from each of your arms. The blood will be tested for the presence of bacteria. The bacteria will then be tested for resistance to different antibiotics. It is important that two separate blood samples are taken to have the best chance of the laboratory tests giving a useful result. The results will be shared with your doctor as soon as the test results are known. After receiving the test results, the doctor may decide to change your treatment.

### Voluntary participation and participant selection

All patients with a suspected blood infection at any time during their stay in the hospital will be asked to take part in the project. Signs of blood infection include fever, chills, confusion, fast breathing and fast heartbeat. Your taking part in this project is entirely voluntary; you have the right not to take part in the project or to withdraw at any point.

### Risks

The project results will yield information to make sure you are receiving the right antibiotic treatment. The risks of taking part in this project are the same risks as those of taking any type of blood sample.

### Benefits

Receiving the right antibiotic treatment will give you a better chance to recover, decrease the number of days you have to stay in the hospital and lower the risk of side-effects from certain antibiotics. The results from your blood test and those from other participants helps to make sure that patient with blood infection will receive the most appropriate treatment.

### **Confidentiality and sharing the results**

The information that we collect in this project will be treated as confidential. Only the project coordination team and the hospital personnel will be able to see the results from the blood laboratory tests.

The overall results of this project will be shared with doctors and the Ministry of Health and used to update national treatment guidelines. The results will also be shared more broadly through a project report, scientific publications and presentations at scientific conferences. Personal information will not be shared.

### **More detailed information and who to contact**

If you would like more detailed information about the project, please ask for the Patient Information Sheet. If you have any questions you may ask them now, later or even after the project has started. More details can be provided too by [contact details].

### **Project approval**

This proposal has been reviewed and approved by the Ethical Committee of [insert name of institution], which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find out more about this institutional review board, contact [contact details]. The project has also been reviewed by the Ethics Review Committee of the World Health Organization, which is supporting the project.

### **Certificate of consent**

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily as the patient or as the parent/caregiver of the patient to participate in this project.

Print name of participant: \_\_\_\_\_

Signature of participant: \_\_\_\_\_

Date (day/month/year): \_\_\_\_\_

### **Statement by the person taking consent**

I have accurately read out the informed consent form to the potential participant and have offered the patient information sheet with more detailed information. To the best of my ability, I made sure that the participant understands the project and what will be his/her contribution, as described above.

I confirm that the participant was given an opportunity to ask questions about the project, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

The patient was offered a copy of this informed consent form.

Print name of the person taking the consent: \_\_\_\_\_

Signature of the person taking the consent: \_\_\_\_\_

Date (day/month/year): \_\_\_\_\_

## 11.2. Informed consent form for parents of patients with suspected bloodstream infections

Together with the National Institute of Public Health and the Environment of the Netherlands, the World Health Organization Regional Office for Europe and the Ministry of Health of [country], we are conducting research to study the causes of blood infections and antibiotic resistance.

### Introduction

The doctor of your child suspects that your child may have a blood infection. Your child is invited to take part in this project and provide some blood samples to test whether he/she does really have a blood infection. Taking blood samples from patients with suspected blood infection is a normal procedure in many countries and provides information that will help your child's doctor to better treat his/her illness.

### Purpose of the project

Blood infection can in some cases lead to sepsis, which is a life-threatening condition. Quick and correct treatment is, therefore, very important. Blood infection can be caused by different types of bacteria and can be treated with different types of antibiotic. Unfortunately, sometimes bacteria can become resistant to certain antibiotics. Resistant means that an antibiotic no longer works to kill the bacteria and treat the infection. In this case, a different kind of antibiotic is needed. By taking blood samples and doing laboratory tests, we can find out which bacteria is causing the blood infection and which antibiotics will work best to kill the bacteria.

### Procedures

If your child takes part in this project, blood will be taken from his/her arm. When your child weighs less than 4 kilogram, one sample will be taken. From children weighing more than this, two (2) blood samples will be taken; one from each of his/her arms. The blood will be tested for the presence of bacteria. The bacteria will then be tested for resistance to different antibiotics. If the child weighs over 4 kg, it is important that two separate blood samples are taken to have the best chance of the laboratory tests giving a useful result. The results will be shared with your child's doctor as soon as the test results are known. After receiving the test results, the doctor may decide to change your child's treatment.

### Voluntary participation and participant selection

All patients with a suspected blood infection at any time during their stay in the hospital, or their parents when the patient is a minor, will be asked to take part in the project. Signs of blood infection include fever, chills, confusion, fast breathing and fast heartbeat. Your child's participation in this project is entirely voluntary; you have the right to decide that your child is not taking part in the project or to withdraw your consent and stop your child's participation in the project at any point.

### Risks

The project results will yield information to make sure your child is receiving the right antibiotic treatment. The risks of taking part in this project are the same risks as those of taking any type of blood sample.

### Benefits

Receiving the right antibiotic treatment will give your child a better chance to recover, decrease the number of days your child has to stay in the hospital and lower the risk of side-effects from certain antibiotics. The results from your child's blood test and those from other participants helps to make sure that patient with blood infection will receive the most appropriate treatment.

### Confidentiality and sharing the results

The information that we collect in this project will be treated as confidential. Only the project coordination team and the hospital personnel will be able to see the results from the blood laboratory tests.



The overall results of this project will be shared with doctors and the Ministry of Health and used to update national treatment guidelines. The results will also be shared more broadly through a project report, scientific publications and presentations at scientific conferences. Personal information will not be shared.

**More detailed information and who to contact**

If you would like more detailed information about the project, please ask for the Patient Information Sheet. If you have any questions you may ask them now, later or even after the project has started. More details can be provided by [contact details].

**Project approval**

This proposal has been reviewed and approved by the Ethical Committee of [insert name of institution], which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find out more about this institutional review board, contact [contact details]. The project has also been reviewed by the Ethics Review Committee of the World Health Organization, which is supporting the project.

**Certificate of consent**

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily as the parent or caregiver of the patient for the patient to participate.

Print name of participant: \_\_\_\_\_

Print name of parent or caregiver: \_\_\_\_\_

Signature of parent or caregiver: \_\_\_\_\_

Date (day/month/year): \_\_\_\_\_

**Statement by the person taking consent**

I have accurately read out the informed consent form to the parent or caregiver of the potential participant and have offered the patient information sheet with more detailed information. To the best of my ability, I made sure that the parent or caregiver of the participant understands the project and what will be his/her child's contribution, as described above.

I confirm that the parent or caregiver of the participant was given an opportunity to ask questions about the project, and all the questions asked by the parent or caregiver of the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

The parent or caregiver of the patient was offered a copy of this informed consent form.

Print name of the person taking the consent: \_\_\_\_\_

Signature of the person taking the consent: \_\_\_\_\_

Date (day/month/year): \_\_\_\_\_

### **11.3. Informed consent form for minors (12–17 years) with suspected bloodstream infections**

Together with the National Institute of Public Health and the Environment of the Netherlands, the World Health Organization Regional Office for Europe and the Ministry of Health of [country] we are conducting research to study the causes of blood infections and antibiotic resistance.

#### **Introduction**

Your doctor suspects that you may have a blood infection. We ask you to help us in the project described below and provide some blood samples to test whether you do really have a blood infection. Your parent or caregiver has already given his/her permission for you to participate, but you also get to decide if you want to participate in this project. If you do not understand what is explained to you, you can ask questions.

#### **What is the project about?**

When you have a blood infection, there are bacteria in your blood and this can be very dangerous. These bacteria can most often be killed by medicines, called antibiotics. Unfortunately, sometimes bacteria can become resistant to certain antibiotics. If a bacterium is resistant to an antibiotic, this antibiotic does no longer kill the bacterium and treat the infection. In this case, a different kind of antibiotic is needed. By taking blood samples and investigating them in the laboratory, we can see which bacteria is causing the blood infection and which antibiotics will work best to kill the bacteria. The results from your blood test and those from other participants helps to make sure that patients with blood infection will receive the most appropriate treatment.

#### **What will happen to me in this project?**

If you take part in this project, two (2) blood samples will be taken, one from each of your arms. The blood will be tested for the presence of bacteria. The bacteria will then be tested for resistance to different antibiotics. It is important that two separate blood samples are taken to have the best chance of the laboratory tests giving a useful result. The results will be shared with your doctor as soon as the test results are known. After receiving the test results, the doctor may decide to change your treatment. The risks of taking part in this project are the same risks as those of taking any type of blood sample.

#### **Do I have to participate?**

You do not have to take part in the project if you do not want to. If you are in the project, you can stop being in it at any time. If you say no, nothing will change, and the doctors and nurses will take care of you as they have in the past. We ask all patients with a suspected blood infection at any time during their stay in the hospital to take part in the project.

#### **What is the advantage for me to be in the project?**

Receiving the right antibiotic treatment will give you a better chance to recover, decrease the number of days you have to stay in the hospital and lower the risk of side-effects from certain antibiotics.

#### **What do you do with my information?**

The information that we collect in this project will be treated as confidential. Only the researchers and the hospital personnel will be able to see the results from the blood laboratory tests. When we have collected results from blood laboratory tests from many patients, we will write a report. This report may be used by doctors and by the Ministry of health. The report will not contain any of your personal information.

#### **If you have more questions**

If you would like more detailed information about the project, please ask for the Patient Information Sheet. If you have any questions you may ask them now, later or even after the project has started.

#### **Project approval**

This proposal has been reviewed and approved by the Ethical Committee of [insert name of institution], which is a committee whose task it is to make sure that research participants are protected from harm.

If you wish to find about more about this institutional review board, contact [name and contact details]. The project has also been reviewed by the Ethics Review Committee of the World Health Organization, which is supporting the project.

**Certificate of consent**

I have read this information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily; to participate as a participant in this project.

Print name of assenting minor: \_\_\_\_\_

Signature of assenting minor: \_\_\_\_\_

Date (day/month/year): \_\_\_\_\_

**Statement by the person taking consent**

I have accurately read out the informed consent form to the potential participant and have offered the patient information sheet with more detailed information. To the best of my ability, I made sure that the participant understands the project and what will be his/her contribution as described above.

I confirm that the participant was given an opportunity to ask questions about the project, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

The patient was offered a copy of this informed consent form.

Print name of the person taking the consent: \_\_\_\_\_

Signature of the person taking the consent: \_\_\_\_\_

Date (day/month/year): \_\_\_\_\_

Parent/guardian has signed an informed consent. Yes/No \_\_\_\_\_ (initialled by assistant) \_\_\_\_\_











The WHO Regional  
Office for Europe

The World Health Organization (WHO) is a specialized agency of the United Nations created in 1948 with the primary responsibility for international health matters and public health. The WHO Regional Office for Europe is one of six regional offices throughout the world, each with its own programme geared to the particular health conditions of the countries it serves.

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Austria  
Azerbaijan  
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Bosnia and Herzegovina  
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Cyprus  
Czechia  
Denmark  
Estonia  
Finland  
France  
Georgia  
Germany  
Greece  
Hungary  
Iceland  
Ireland  
Israel  
Italy  
Kazakhstan  
Kyrgyzstan  
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Montenegro  
Netherlands  
Norway  
Poland  
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