

BIOLOGICAL SAMPLES

All the sites are asked to locally process the blood and urine cultures at the times described in the schedule of visits, using standard microbiological techniques for the isolation and identification of bacteria; the Microbiology laboratories of these centers use the Quality Control system of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC). Susceptibility test are to be interpreted according to EUCAST recommendations. ESBL-EC isolated are to be sent to the central laboratory located in Hospital Universitario Virgen Macarena in Seville in order to confirm the identification, ESBL-production, susceptibility testing using reference techniques and ESBL characterization through PCR and sequencing.

Three hospitals (Hospital Universitario de Bellvitge, Hospital Universitario Vall d'Hebrón, both in Barcelona and Hospital Universitario Virgen Macarena, Seville) will participate in the study of the rectal carriage of ESBL- and carbapenemase-producing Gram negatives by taking rectal swabs from participants at different times as set out in the schedule of visits; 60 patients are expected to be included in the rectal carriage study. Additionally, fosfomycin serum concentrations will be measured in a sample size of 20 patients in only one site (Hospital Universitario Virgen Macarena).

All study samples will be anonymized, being identified only by the patient study code, in order to ensure that the association with personal data is not possible. Objective and management for these samples are included in the patient information sheet and informed consent form.

The study documentation includes a check list and specific instructions for samples management.

RECTAL SWAPS

Swab to be gathered are to be stored with tube transport medium, Amies, Stuart or Cary-Blair.

To obtain a rectal swab, the swab is inserted slightly surpassing the anal sphincter and gently rotated to take the sample from anal crypts. The swap has to be left between 10 and 30 seconds in order to the microorganisms to be absorbed and then, be removed (the presence of stool in the swab is to be checked).

Insert the swab immediately into the tube containing transport medium and clearly identify the samples with the study ID code.

Bring the sample to sample receiving area of the microbiology laboratory. If transport to the laboratory will be delayed more than two hours the sample should be maintained at 4 °C (refrigerator) until the arrival at the laboratory.

In the centres involved in the study of intestinal colonization (Hospital Virgen Macarena, Bellvitge and Vall d'Hebron) the presence of ESBL-producing enterobacteria or Carbapenemases, or gram-negative bacilli resistant to carbapenems (n = 60 patients) will be determined. Detection will be performed through Mc Conkey with cefotaxime (4 mg / l) or chromogenic medium for Enterobacteriaceae ESBL (ESBL ID Chrom. BioMerieux, France) means. The detected strains, once identified should be frozen at -80 °C following the usual procedure of each centre and sent to the reference by the method described afterwards.

DELIVERY OF MICROBIOLOGICAL ISOLATIONS

1. Pathogens once identified should be frozen at -80 °C following the usual procedure of each centre.
2. Delivery to the central laboratory will be performed every three months.
3. A blood agar plate subculture from the frozen strains has to be performed. Those will be incubated at 37 °C during 18-20h until an adequate bacterial growth is achieved.
4. Microorganisms are to be sent with swab in Amies transport medium or Stuart Cary-Blair medium at room temperature. In order to do so, abundance quantities of microorganisms on the surface of the agar plate subcultured are to be gathered with a sterile swab collect. The swab is to be inserted into the tube incorporating the means of transport. Fit the plug in the tube ensuring a good seal. Identify isolation clearly on the label of each tube using the reference study code.
 - Each isolate has to be properly identified and accompanied by the appropriate form (attached) Isolation and antibiogram report copy of the referring centre.
 - Samples has to be packed and transported as Category A infectious substances, according to the regulations by WHO (Guidance on regulations for the transport of infectious substances 2011-2012) and BOE # 63 (03/14/2013).
 - Contact with the courier services provided for the study.
 - Postal address for the central laboratory:
Dra. Inmaculada López Hernández
Servicio de Microbiología (primera planta)
Hospital Virgen Macarena
Avda. Dr. Fedriani s/n
41009 Sevilla

- Contact the referral centre via email (inlopezh@us.es) indicating in subject "FOREST_Name of the sender SHIPPING hospital" to confirm sending samples indicating in the text of the email:
 - a. Institution (Hospital) name.
 - b. Date of departure.
 - c. Name and contact details (phone number) of the responsible of the delivery.
 - d. Number of microorganisms delivered.
- 2. Confirmation of the specific form included in the delivery.

ISOLATION FORM

REFERENCE NUMBER <i>(to be filled in by the reference site)</i>	
Name of the Hospital (Sender)	
Date of isolation	
Reference number for the institution	
Unit/Department of origin	
Identification method in the reference centre	
Antibiogram method in the reference centre	
ANTIBIOTIC SENSITIVITY DATA <i>(copy of the report issued in the center of origin is recommended)</i>	

PHARMACOKINETICS

The pharmacokinetics parameters of fosfomycin will be evaluated solely in the coordinating center (Hospital Universitario Virgen Macarena, Seville). A total of four samples are collected from 20 patients with the evaluation times listed below:

Sample 1: just before the initiation of fosfomycin administration.

Sample 2: at the time of the initiation of fosfomycin administration.

Sample 3: after two hours of the initiation of fosfomycin administration.

Sample 4: after four hours of the initiation of fosfomycin administration.

Blood samples will be drawn into a tube with 3 ml EDTA. Each sample has to be centrifuged at 3500 rpm for 5 min, removing the plasma, which is to be frozen at -20°C until analysis (in <30 days).

Fosfomycin determination will be performed through high resolution liquid chromatography linked to a triple quadrupole mass spectrometer (LC-MS / MS) by Li et al (J Chromatography B 2007; 856: 171 -177). The method validation will be done according to the Bioanalytical Methods Validation Guide of the FDA.