INSTRUCTIONS FOR USE

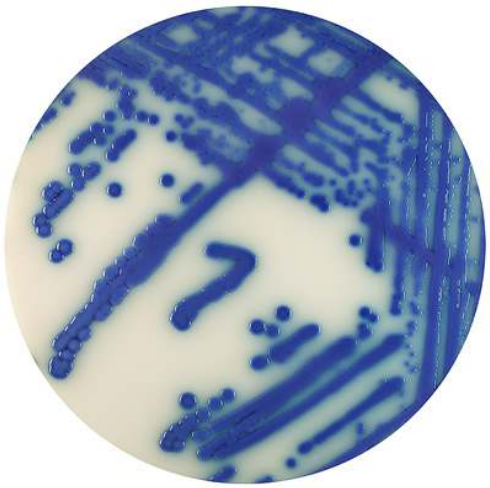
ChromArt

CRE-ESBL AGAR BASE

ESBL SUPPLEMENT - CRE SUPPLEMENT

Dehydrated culture medium and supplements

ESBL Medium:
E.coli



CRE Medium:
Klebsiella pneumoniae

1 - INTENDED USE

In vitro diagnostics. Chromogenic basal medium and selective supplements for the presumptive determination of *Enterobacteriaceae* resistant to carbapenems (CRE medium) and ESBL producing (ESBL medium), in clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

CRE-ESBL AGAR BASE (REF 4080252)

Peptones	16.0 g
Growth factors	5.0 g
Opacifier compounds	10.0 g
Tryptophan	2.0 g
Chromogenic mix	0.4 g
Agar	16.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

VIAL CONTENTS

ESBL Supplement (REF 4240080)

Antimicrobials mix	0.21 g

VIAL CONTENTS

CRE Supplement (REF 4240082)

Antimicrobial mix	0.21 g

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

CRE-ESBL Agar Base, with the addition of the suitable supplement, can be used for the preparation of CRE medium or ESBL medium. The use of chromogenic media is the preferred option for the detection of ESBL-producers or carbapenem resistant strains in faecal screening.[1,2] Bacterial differentiation is obtained with a mixture of chromogenic compounds designed to detect specific enzymatic activities (β-galactosidase, β-glucosidase, tryptophanase), of *E.coli,* of bacteria of the KESC group (*Klebsiella, Enterobacter, Serratia, Citrobacter*) and of the *Proteus-Morganella-Providencia* group. The grey and opaque background of the medium allows a better observation and colour reading of the colonies.

ESBL Medium

The Extended Spectrum Beta Lactamases (ESBLs) are acquired class A β-lactamases that hydrolyse and (usually) confer resistance to 2nd and 3rd generation cephalosporins, (e.g., cefuroxime, cefotaxime, ceftazidime and ceftriaxone), and 4th generation cephalosporins (e.g., cefepime, cefpirome), but not cephamycins (e.g., cefoxitin) or carbapenems.[1] ESBLs have become globally disseminated within species of *Enterobacteriaceae*.[2]

ESBL Medium is a chromogenic and selective screening medium for the isolation and differentiation of ESBL-producing *Enterobacteriaceae*. The selectivity of the medium is due to the presence of an inhibitory mixture of antibiotics against Gram-positive bacteria, fungi and Gram-negative bacteria susceptible to 3rd or 4th generation cephalosporins.

CRE Medium

Mechanisms of carbapenem-resistance among Gram-negative organisms are heterogeneous but are primarily broken down into two broad categories: carbapenemase-producing and non-carbapenemase-producing mechanisms. For the latter, carbapenem resistance is

mediated by porin mutations or efflux pumps or the combination of these with ESBL and/or AmpC production depending of the Gram-negative organism.[3] Carbapenemase production is the primary mechanism mediating increased carbapenem resistance among Gram-negative bacteria.[3] Carbapenemases are β-lactamases that hydrolyze penicillins, in most cases cephalosporins, and to various degrees carbapenems and monobactams (the latter are not hydrolyzed by metallo-β-lactamases).[4]
The early identification of carbapenem-resistant organisms in clinical samples is a determining factor in preventing or limiting their spread and preserving the therapeutic efficacy of carbapenems.
Chromogenic media are recommended for the detection of gastro-intestinal colonisation of carbapenem-resistant organisms. [3,5,6]
CRE Medium is a chromogenic and selective screening medium for the isolation and differentiation of carbapenem-resistant *Enterobacteriaceae* (CRE). The selectivity of the medium is due to the presence of an inhibitory mixture of antibiotics against Gram-positive bacteria, fungi and Gram-negative bacteria susceptible to carbapenems

4- DIRECTIONS FOR MEDIA PREPARATION

Suspend 49.4 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C.
ESBL Medium: dissolve the contents of one vial of ESBL Supplement (4240080) with 5 mL of sterile purified water. Add to 500 mL of medium base cooled to 47-50°C under aseptic conditions. Mix well and distribute into sterile Petri dishes.
CRE Medium: dissolve the contents of one vial of CRE Supplement (4240082) with 5 mL of sterile purified water. Add to 500 mL of medium base cooled to 47-50°C under aseptic conditions. Mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

CRE-ESBL Agar Base

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	greyish, opaque
Final pH at 20-25 °C	7.2 ± 0.2
ESBL SUPPLEMENT	
Appearance of the lyophilized	high, homogeneous, reddish pastille
Appearance of the solution	opalescent reddish
CRE SUPPLEMENT	
Appearance of the lyophilized	high, homogeneous, reddish pastille
Appearance of the solution	limpid or slightly opalescent, reddish

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
CRE-ESBL Agar Base CND W0104010101; EDMA: 14.01.01.01; RDM:1421712	Dehydrated medium	4080252	500 g (10,1 L)
ESBL Supplement CND W0104010104; EDMA: 14.01.01.04; RDM:1421723	Freeze-dried supplement	4240080	10 vials, each for 500 mL of medium base
CRE Supplement CND W0104010104; EDMA: 14.01.01.04; RDM:1421734	Freeze-dried supplement	4240082	10 vials, each for 500 mL of medium base

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

ESBL medium is intended for screening clinical specimens such as stools, rectal or peri-rectal swab and for processing other clinical specimens such as urine, wounds and respiratory secretions.[1]
CRE Medium: any sample type can be used; however, stool and rectal swab are the most sensitive for detecting CRE colonisation; if a rectal swab is not feasible or acceptable any clinical specimen such as blood, wound swab or urine is suitable. [5]
Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; collect specimens before antimicrobial therapy where possible.

9- TEST PROCEDURE

Allow plates to come to room temperature. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate in air at 35-37°C for 18-24 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
CRE isolates and ESBL producing *Enterobacteriaceae* show the following characteristic colonies:
Pink / red-magenta colonies: *E.coli*
Blue / green-blue / blue-violet / grey-violet colonies: *Klebsiella, Enterobacter, Serratia, Citrobacter*
Brown colonies with brown halo: *Proteus-Morganella-Providencia*
CRE isolates shall be subjected to confirmatory tests. Consult the listed references.[1-3]

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
ESBL Medium			
K. pneumoniae SHV-18	ATCC 700603	35-37°C / 18-24H / A	growth with blue colonies
E. coli	ATCC 25922	35-37°C / 18-24H / A	inhibited
C. albicans	ATCC 10231	35-37°C / 18-24H / A	inhibited
CRE Medium			
K.pneumoniae	ATCC BAA-1705	35-37°C / 18-24H / A	growth with blue colonies
E. coli	ATCC 25922	35-37°C / 18-24H / A	inhibited
C.albicans	ATCC 10231	35-37°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12- PERFORMANCES CHARACTERISTICS

ESBL Medium

The performances of ESBL medium were evaluated in a clinical study by a Clinical Microbiology Laboratory in northern Italy[7] on 2500 urine cultures and 38 samples from other body sites. The results are summarized in the tables below.
The data demonstrate the capacity of ESBL medium to detect ESBL-producing *Enterobacteriaceae* with high sensitivity (98.82%) and specificity (98.29%).

CRE Medium

The performances of CRE Medium were evaluated in a clinical study by a Clinical Microbiology Laboratory in northern Italy[8] on 110 strains of carbapenem-resistant Gram-negative bacteria, 50 strains of 3rd generation cephalosporin-resistant Enterobacteria or ESBL-producing bacteria.
The published data demonstrate that CRE medium detects carbapenem-resistant Gram-negative bacteria with high sensitivity (98.2%) and specificity (100%) while it does not allow the growth of carbapenems susceptible organisms possessing other mechanisms that can cause resistance to beta-lactam antibiotics, such as ESBL or overproduction of AmpC.
If the research target is the determination of carbapenemase producing strains, the sensitivity is 100% and the specificity is reduced (85.1%) as the medium allows the growth of carbapenem resistant strains caused by membrane impermeability due to porin loss.

Prior to release for sale representative samples of dehydrated CRE-ESBL Agar Base REF 408025, and of supplements CRE Supplement (REF 4240082) and ESBL Supplement (REF 4240080) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

ESBL Medium (CRE-ESBL Agar base + ESBL Supplement)

Productivity is tested by semi-quantitative ecometric technique with the following target strains: *K.pneumoniae* ATCC 700603, ESBL-producing clinical isolates of *E.coli, E.cloacae, C.freundii*. and *C. koserii*. After incubation at 35-37°C for 18-24 hours all target strains show a good growth with typical chromatic characteristics.
Selectivity is evaluated by semi-quantitative ecometric technique by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target organisms *P.aeruginosa* ATCC 27853, *C.albicans* ATCC 10231, *S.aureus* (MR) ATCC 43300, *E.coli* ATCC 25922, *A.calcoaceticus* ATCC 19606, *E.fecium* (VRE) ATCC 700221, a clinical isolate of *E.cloacae* hyperproducer of AmpC and por+, a clinical isolate of *E.coli* hyperproducer of AmpC.
After incubation at 35-37°C for 18-24 hours, the growth of *P.aeruginosa, C.albicans* , *S.aureus, E coli* ATCC 25922 *and E faecium* is totally inhibited while the growth of hyperproducer of AmpC non-target strains *E.coli* and *E.cloacae* is partially inhibited.

CRE Medium ((CRE-ESBL Agar base + CRE Supplement)

Productivity is tested by semi-quantitative ecometric technique with the following target strains: *K.pneumoniae* ATCC BAA-1705, Carbapenem-resistant clinical isolates of *A.baumanni, P.aeruginosa, E.coli* and *K.pneumoniae*. After incubation at 35-37°C for 18-24 hours all target strains show a good growth with typical chromatic characteristics.
Selectivity is evaluated by semi-quantitative ecometric technique by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target organisms *P.aeruginosa* ATCC 27853, *C.albicans* ATCC 10231, *S.aureus* (MR) ATCC 43300, *A.calcoaceticus* ATCC 19606, *E.fecium* (VRE) ATCC 700221, ESBL producing *K.pneumoniae* ATCC 700603, a clinical isolate of AmpC producing *E.cloacae* and *E.coli*. After incubation at 35-37°C for 18-24 hours, the growth of *P.aeruginosa, S.aureus* and *E.faecium* is totally inhibited while the growth of other non-target strains is partially inhibited.

12-LIMITATIONS OF THE METHOD

- ESBL Chromogenic agar media are likely to be less specific, particularly in areas where ESBL producers are common.[1]
- Some *Enterobacteriaceae* strains hyperproducing cephalosporinases, some multi drug resistant *Pseudomonas* spp. and *Acinetobacter* spp. may grow on the ESBL medium.
- Growth on the medium depends on the metabolic requirements of each microorganism and on the resistance to the antimicrobials present; some target strains may not be able to grow on ESBL medium or may show a delayed growth (e.g., *Proteus* spp.).
- Some Gram-negative bacteria resistant to carbapenem due to membrane impermeability mechanism may grow on CRE medium.
- Multidrug resistant Gram-negative bacteria other than carbapemen-resistant *Enterobacteriaceae* (*Acinetobacte*r and *Pseudomonas*) may grow on CRE medium.
- There is very little evidence that extended incubation enhances the sensitivity of chromogenic media for CRE, but there is evidence to show that specificity is decreased.[3]
- Screening for intestinal carriage of CRE is of significant importance for the development of infection control strategies. However, the optimal screening modality remains to be established for each location and for each specific purpose.[9]
- Culture-based methods may not be optimal for the detection of low-level carbapenemase production, which is important for epidemiological purposes.[9]
- Agar-based procedures always require confirmatory testing to detect the type of *bla* gene present after a potentially resistant isolate is detected.
- Growth on CRE medium depends on the metabolic requirements of each microorganism and on the resistance to the antimicrobials present; some target strains may not be able to grow on the medium or may show a delayed growth.

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. On the isolates, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of the microscopic and/or other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplements are qualitative *in vitro* diagnostics, for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements must be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- The supplements are classified as dangerous according to the EU regulations. Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before the use, consult the Material Safety Data Sheets.
- The culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- ESBL Supplement and CRE Supplement are sterilized by membrane filtration.
- Be careful when opening the metal ring of the vials to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium, supplements and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the products are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

CRE-ESBL Agar Base

Upon receipt, store at +2°C /+8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps.

ESBL Supplement - CRE Supplement

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles), the added supplements and the storage method applied (temperature and packaging).

16 - REFERENCES

1. Public Health England. UK Standards for Microbiology Investigations (SMI) B 59: Detection of *Enterobacteriaceae* producing extended spectrum β lactamases.2016
2. Perry JD. A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin Microbiol Rev. 2017; 30:449-479.
3. Simner PJ, Humphries R. Special phenotypic methods for detecting antibacterial resistance. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance Version 2.01, July 2017.
5. Public Health England. UK Standards for Microbiology Investigations (SMI) B 60: detection of bacteria with carbapenem hydrolysing β-lactamases (carbapenemases); September 2020.
6. Perry JD. A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin Microbiol Rev. 2017; 30:449-479.
7. Comi C, Bracco S, Colombo L, Bartesaghi P, Barletta R, Silva M, Luzzaro F. Valutazione del terreno ESBL (Biolife) per la rilevazione degli Enterobatteri produttori di ESBL in campioni clinici. XLIII Congresso AMCLI, Sezione Poster, 2014.
8. Bracco S, Mauri C, Meroni E, Principe L, Pini B, Luzzaro F. Valutazione del terreno CRE (Biolife) per la rilevazione di batteri Gram-negativi resistenti ai carbapenemi. XLIII Congresso AMCLI , Sezione Poster, 2014.
9. Viau R, Frank KM, Jacobs MR, Wilson B, Kaye K, Donskey CJ, Perez F, Endimiani A, Bonomo RA. Intestinal Carriage of Carbapenemase-Producing Organisms: Current Status of Surveillance Methods. Clin Microbiol Rev. 2016; 29:1-27

4240080 ESBL SUPPLEMENT
SDS rev 1
Regulation (EU) 2020/878

Contains:
CEFSULODINE
CLOXACILLIN SODIUM
CEFPODOXIME SODIUM

Classification

Respiratory sensitization, category 1	H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Skin sensitization, category 1	H317	May cause an allergic skin reaction.

Labelling
Hazard pictograms:

Signal words: Danger
Hazard statements:

H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H317	May cause an allergic skin reaction.

Precautionary statements:

P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P280	Wear protective gloves.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER / doctor / . . .
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

4240082 CRE SUPPLEMENT
SDS rev 1
Regulation (EU) 2020/878

Contains:
CLOXACILLIN SODIUM
TAZOBACTAM
ERTAPENEM SODIUM
CEFSULODINE

Classification

Respiratory sensitization, category 1	H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
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TABLE OF APPLICABLE SYMBOLS

REF or **REF** Catalogue number	**LOT** Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and contents	2021/12

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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