INSTRUCTIONS FOR USE

ChromArt

CRE SUPPLEMENT

Freeze-dried selective supplement

1 - INTENDED USE

In vitro diagnostics. Mixture of antimicrobials to be added to CRE-ESBL Base for the presumptive determination of *Enterobacteriaceae* resistant to carbapenems (CRE medium) in clinical specimens.

2 - COMPOSITION - VIAL CONTENTS (FOR 500 mL OF MEDIUM)

Antimicrobial mix	0,21 g

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

CRE Supplement added to CRE-ESBL Base, can be used for the preparation of CRE medium for the isolation and differentiation of carbapenem-resistant *Enterobacteriaceae* (CRE). The use of chromogenic media is the preferred option for the detection of CRE 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. 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

Dissolve the contents of one vial of CRE Supplement (42400082) with 5 mL of sterile purified water. Add to 500 mL of CRE-ESBL Base (REF 408025) autoclaved and cooled to 47-50°C under aseptic conditions. Mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

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
ChromArt CRE Supplement	Freeze-dried supplement	4240082	10 vials, each for 500 mL of medium base

7 - MATERIALS REQUIRED BUT NOT PROVIDED

CRE-ESBL Agar Base (REF 408025), 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

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.[3]
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 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.[1,2,4]

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, the end user can perform its own 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
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

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12- PERFORMANCES CHARACTERISTICS

The performances of CRE Medium prepared with CRE-ESBL Base and CRE Supplement were evaluated in a clinical study by a Clinical Microbiology Laboratory in northern Italy[5] 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 Chromart CRE Supplement added to dehydrated Chromart CRE-ESBL Base REF 408025, are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch. 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

- 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.[6]
- Culture-based methods may not be optimal for the detection of low-level carbapenemase production, which is important for epidemiological purposes.[6]
- 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.
- CRE Supplement and the complete medium are 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

- CRE Supplement is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- CRE Supplement is classified as dangerous according to current European legislation; consult the Safety Data Sheet before use.
- The supplement and the medium base shall be used in association according to the directions described above.
- Apply Good Manufacturing Practice in the preparation process of plated media.
- CRE Supplement is sterilized by membrane filtration.
- Be careful when opening the metal ring to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use CRE Supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product 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

Upon receipt, store the product in the original package at 2-8°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 plated media and the validation of their shelf life, according to the applied storage conditions (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. Public Health England. UK Standards for Microbiology Investigations (SMI) B 60: detection of bacteria with carbapenem hydrolysing β-lactamases (carbapenemases); September 2020
4. 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.
5. Comi C, Bracco S, Colombo L, Bartesaghi P, Barletta R, Silva M, Luzzaro F. Valutazione del terreno ChromArt ESBL (Biolife) per la rilevazione degli Enterobatteri produttori di ESBL in campioni clinici. XLIII Congresso AMCLI, Sezione Poster, 2014.
6. 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

4240082 CRE SUPPLEMENT
SDS 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.
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.

TABLE OF APPLICABLE SYMBOLS

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

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and contents	2021/12
Revision 3	Removal of obsolete classification	2023/04

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