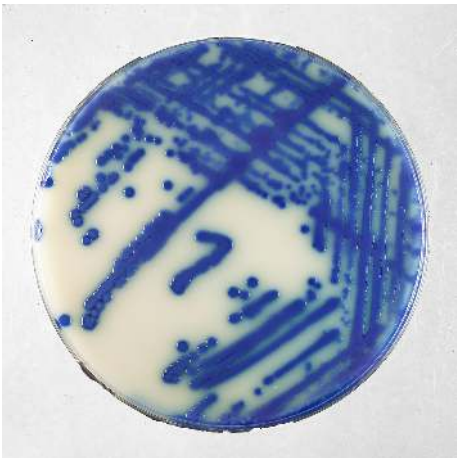
INSTRUCTIONS FOR USE

ChromArt CRE

Ready-to-use plates



Chromart CRE: carbapenem-resistant
Klebsiella pneumoniae

1 - INTENDED USE

In vitro diagnostic device. Chromogenic medium for the presumptive detection of carbapenem-resistant *Enterobacteriaceae* (CRE) in clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Peptones	16.00 g
Growth factors	5.00 g
Opacifying compound	10.00 g
Tryptophan	2.00 g
Chromogenic mix	0.40 g
Antimicrobials mix	0.21 g
Agar	16.00 g
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

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.[1] Carbapenemase production is the primary mechanism mediating increased carbapenem resistance among Gram-negative bacteria.[1] 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).[2]
Gastrointestinal colonisation of carbapenem-resistant organisms, is an important reservoir for transmission of these critical pathogens in the hospital setting.[1] Infections caused by carbapenem-resistant Gram-negative bacteria are a serious problem worldwide, because of the high spreading capacity of these microorganisms, the poor therapeutic options available, the high mortality. Their early identification 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.[1,3,4]
ChromArt CRE 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. Bacterial differentiation is obtained with a mixture of chromogenic compounds designed to detect specific enzymatic activities (β-galactosidase, β-glucosidase, tryptophanase), of *E.coli,* bacteria of the KESC group (*Klebsiella, Enterobacter, Serratia, Citrobacter*) and *Proteus-Morganella-Providencia* group. The grey and opaque background of the medium allows a better observation and colour reading of the colonies.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	Grey, opaque
Final pH at 20-25 °C	7.2 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
ChromArt CRE CND: W0104010404, EDMA: 14.01.04.04; RDM: 1403789/R	Ready-to-use plates	548015	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - 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.

8- 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.

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9 - 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.[1-3]

10 - 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
K.pneumoniae	ATCC BAA-1705	35-37°C / 18-24H / A	growth, 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

11- PERFORMANCES CHARACTERISTICS

The performances of ChromArt CRE 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 results are summarized in the tables below. Tables 1, 2, 3, 4 refer to 92 *Enterobacteriaceae* and 18 Gram-negative non-lactose fermenters resistant to carbapenems through different resistance mechanisms (including 13 resistant strains for porin loss) and in addition 40 carbapenem susceptible but beta-lactam antibiotics resistant *Enterobacteriaceae* (AmpC and ESBL strains).

Tab. 1: summary of results for carbapenemase producing strains (Ambler class A, B, D).

Resistance mechanism	n° of strains	Growth on ChromArt CRE*	Growth on Tryptic Soy Agar*
KPC	60	60	60
KPC +ESBL	1	1	1
OXA	12	12	12
VIM	15	15	15
NDM	3	3	3
IMP	4	4	4
MBL	2	2	2
TOTAL	**97**	**97**	**97**

*Inoculation of 100 µl bacterial suspensions with about 1.5 x10^4 and 1.5 x10^6 CFU's

Tab. 2: Summary of the results referred to carbapenem resistant strains for membrane impermeability.

Resistance mechanism	n° of strains	Growth on ChromArt CRE*	Growth on Tryptic Soy Agar*
AmpC + porin loss	5	3	5
ESBL + porin loss	8	8	8
TOTAL	**13**	**11**	**13**

*Inoculation of 100 µl bacterial suspensions with about 1.5 x10^4 and 1.5 x10^6 CFU

Tab 3: Summary of the results referred to carbapenems susceptible strains.

Resistance mechanism	n° of strains	Growth on ChromArt CRE*	Growth on Tryptic Soy Agar*
AmpC	10	0	10
ESBL	40	0	40
TOTALI	**50**	**0**	**50**

*Inoculation of 100 µl bacterial suspensions with about 1.5 x10^7 CFU

Tab 4: Sensitivity and specificity referred to the targets.

	Target: carbapenems-resistant strains	Growth on ChromArt CRE*
True positive	110	108
True negative	50	50
False negative		2

	Target: carbapenemase producing strains	Growth on ChromArt CRE*
True positive	97	97
True negative	63	11
False negative		0

False positive		0	False positive		11
Sensitivity	98.2%		100%		
Specificity	100%		85.1%		

The data demonstrate that ChromArt CRE medium detects carbapenem-resistant Gram-negative bacteria with high sensitivity 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 specificity is reduced as the medium allows the growth of carbapenem resistant strains caused by membrane impermeability due to porin loss.

Prior to release for sale a representative sample of all lots of ready-to-use plates of Chromart CRE and of the raw materials used for the production of prepared plates (dehydrated Chromart CRE-ESBL Base REF 408025, supplemented with Chromart CRE Supplement REF 4240082) 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 *E.cloacae, A.baumanni, P.aeruginosa.* 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, , ESBL producing *K.pneumoniae* ATCC 700603, a clinical isolate of AmpC producing *E.cloacae*. After incubation at 35-37°C for 18-24 hours, the growth of *P.aeruginosa,* and *S.aureus* 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 the medium.
- Multidrug resistant Gram-negative bacteria other than carbapemen-resistant *Enterobacteriaceae* (*Acinetobacte*r and *Pseudomonas*) may grow on the 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.[1]
- 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 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 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.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This 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 the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. 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.
2. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance Version 2.01, July 2017.
3. Public Health England. UK Standards for Microbiology Investigations (SMI) B 60: detection of bacteria with carbapenem hydrolysing β-lactamases (carbapenemases); September 2020.
4. 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.
5. Bracco S, Mauri C, Meroni E, Principe L, Pini B, Luzzaro F. Valutazione del terreno ChromArt CRE (Biolife) per la rilevazione di batteri Gram-negativi resistenti ai carbapenemi.. 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.

TABLE OF APPLICABLE SYMBOLS

REF or **REF** Catalogue number	**LOT** Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 0	First emission (in compliance with IVDR 2017/746)	2020/12

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

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