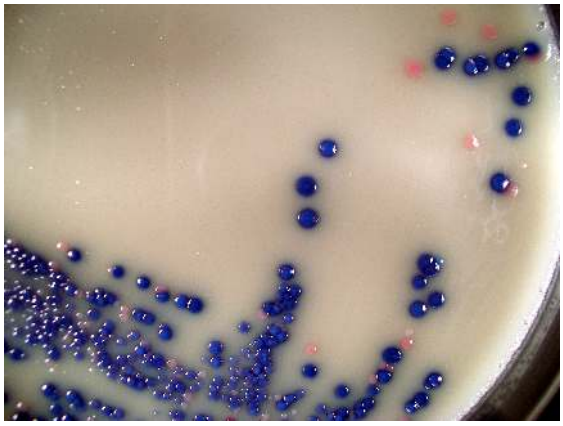
INSTRUCTIONS FOR USE

ChromArt ESBL

Ready-to-use plates



ChromArt ESBL:
ESBL-producing *K.pneumoniae* (blue colonies)
and *E.coli* (pink colonies)

1 - INTENDED USE

In vitro diagnostic device. Chromogenic medium for the presumptive detection of ESBL-producing *Enterobacteriaceae* in clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Peptones	16.0 g
Growth factors	5.0 g
Opacifying compound	10.0 g
Tryptophan	2.0 g
Chromogenic mix	0.4 g
Antimicrobials mix	0.21 g
Agar	16.0 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

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] The increased prevalence of ESBL-producing bacteria in acute care hospitals and the increased intestinal colonization by ESBL producing bacteria in healthy individuals and its association with community-acquired infections, are of great concern.
The use of chromogenic media is the preferred option for the detection of ESBL-producers in faecal screening.[1,2]
ChromArt ESBL 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. 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.

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 ESBL CND: W0104010404, EDMA: 14.01.04.04; RDM: 1403813/R	Ready-to-use plates	548020	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

Chromart ESBL 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] 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. In case of no growth, continue incubation for a further 24 hours.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
ESBL producing *Enterobacteriaceae* show the following characteristic colonies:

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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*
Enterobacteriaceae 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 SHV-18	ATCC	700603	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 ESBL were evaluated in a clinical study by a Clinical Microbiology Laboratory in northern Italy[4] on 2500 urine cultures and 38 samples from other body sites. The results are summarized in the tables below.

Tab. 1: summary of the results obtained with 2538 samples

		Enterobacteriaceae isolates	Isolates confirmed as ESBL producers*	Isolates confirmed as non-ESBL producers
N° of urinary specimens	2500	736	79	657
N° of other samples°	38	37	6	31
Total	2538	773	85	688
Growth on Chromart ESBL			84	12

*: confirmatory test: double disc. °: 37 blood cultures and 1 CSF

Tab. 2: sensitivity and specificity of ChromArt ESBL

	ESBL positive	ESBL negative
Growth on ChromArt ESBL	84 (true positive)	12 (false positive)*
No growth on ChromArt ESBL	1 (false negative)	688 (true negative

*: 9 out of 12 strains have been identified as hyperproducer of AmpC β-lactamase

Sensitivity: 98.82%
Specificity: 98.29%

The data demonstrate the capacity of ChromArt ESBL to detect ESBL-producing *Enterobacteriaceae* with high sensitivity and specificity.

Prior to release for sale a representative sample of all lots of ready-to-use plates of ChromArt ESBL and of the raw materials used for the production of prepared plates (dehydrated ChromArt CRE-ESBL Base REF 408025, supplemented with ChromArt ESBL Supplement REF 4240080) 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 700603, ESBL-producing clinical isolates of *E.coli, E.cloacae, C.freundii*. 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 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* and *S.aureus* is totally inhibited while the growth of non-target strains *E.coli* and *E.cloacae* 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 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 the medium or may show a delayed growth (e.g. *Proteus* spp.).
- 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 disease; 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. 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. Forsythe SJ *et al*. *Klebsiella* and selected Enterobacterales. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. 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.

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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