chromID™ ESBL agar (ESBL)

IVD

Selective chromogenic medium for the screening of Extended Spectrum ß-Lactamase-producing enterobacteria (ESBL)

SUMMARY AND EXPLANATION

chromID™ ESBL agar is a selective chromogenic medium for the screening of Extended Spectrum ß-Lactamase-producing enterobacteria in chronic carrier patients or patients at risk (1, 2, 3, 4).
This medium is not a substitute for the conventional antimicrobial susceptibility test methods.
ESBL-producing enterobacteria are multi-resistant bacteria which can be responsible for nosocomial infections (5). The detection of ESBL-producing enterobacteria carriers is particularly important for the prevention and epidemiological monitoring of these infections. In this context, the use of chromID™ ESBL agar contributes to the active surveillance of ESBL-producing enterobacteria.

PRINCIPLE

chromID™ ESBL agar (patent pending) consists of a rich nutritive base including a variety of peptones. It contains:

- a mixture of antibiotics, including cefpodoxime (1), enabling the selective growth of ESBL-producing enterobacteria.
- two chromogenic substrates and one natural substrate enabling the direct identification of the most frequently encountered ESBL-producing enterobacteria.
  - *Escherichia coli*: spontaneous coloration (pink to burgundy) of ß-glucuronidase-producing strains (ß-GUR) (6).
  - *Klebsiella, Enterobacter, Serratia, Citrobacter* (KESC): spontaneous green, brownish-green or blue colouration of strains expressing a ß-glucosidase (ß-GLU).
  - *Proteeae* (*Proteus, Providencia, Morganella)*: spontaneous dark brown to light brown colouration of strains expressing a deaminase.

Whereas identification to the species or group level is direct, ESBL production must be confirmed by one or more additional tests.

CONTENT OF THE KIT

	Ready-to-use media:
REF 43 481	Pack of 20 plates (90 mm)
	ESBL *

* printed on each plate

COMPOSITION

Theoretical formula:

This medium can be adjusted and/or supplemented according to the performance criteria required:

Peptones (porcine or bovine)	17.2 g
L-Tryptophan	0.9 g
Hepes buffer	0.4 g
Mixture of chromogenic substrates	6.87 g
Selective mixture	0.38 g
Agar	18 g
Purified water	1 l

pH 7.3

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents:

- ID Indole-TDA (Ref. 56 541).
- JAMES (Ref. 70542)
- Oxidase reagent (Ref. 55 635)

Material:

- Bacteriology incubator.
- Non impregnated discs (diameter 6 mm) (Ref. 54 991).

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, microbial cultures and inoculated products should be considered as infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLSI® M29-A, *Protection of Laboratory Workers From occupationally Acquired Infections*; *Approved Guideline* – Current Revision". For additional information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH – Latest Edition " or the current regulations in the country of use.
- Culture media should not be used as manufacturing material or components.
- Do not use reagents after the expiry date.
- Do not use reagents if the packaging is damaged.
- Do not use contaminated plates or plates that exude moisture.
- Use of the medium may be difficult for people who have problems recognising colours.
- It is not recommended to perform the indole test directly on the colony as the colour change may be difficult to observe.
- Use only one sample per plate.
- Interpretation of the test results should be made taking into consideration the patient's history, the source of the specimen, colonial and microscopic morphology and, if necessary, the results of any other tests performed.
- The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS

- **Store the plates in their box at 2-8°C until the expiry date.**
- If not in the box, plates can be stored for 2 weeks at 2-8°C in the cellophane sachet **in the dark.**

SPECIMENS

All types of specimens may be used: rectal swabs, urine, respiratory secretions and other specimens. They should be inoculated directly onto the agar, without enrichment.
Good laboratory practices for collection and transport should be respected and adapted to the type of specimen.

INSTRUCTIONS FOR USE

1. **Allow plates to come to room temperature.**
2. Directly inoculate the chromID™ ESBL agar with the specimens.
3. Incubate with the cover bottom side at 37°C in aerobic conditions. The cultures are generally examined after 18-24 hours of incubation.
   If a negative result is obtained, an oxidase test can be performed on the colourless colonies (see Limitations of the method) or the medium can be incubated for a further 24 hours to optimise its sensitivity of detection.

The user is responsible for choosing the appropriate incubation temperature for the intended use, in accordance with current standards.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and the appearance of the colonies.

ESBL-producing enterobacteria show the following characteristic colorations:

- **Pink to burgundy** colonies or translucent colonies with a pink to burgundy centre: ***E. coli*** species.
- **Green, brownish-green and blue** colonies: **KESC** group.
  Identification of the micro-organism to the species level may be followed by biochemical tests.
- **Dark brown to light brown** colonies or bacterial growth: **P*roteeae*** tribe**.**
  Identification of the micro-organism to the species level may be followed by biochemical tests.

In any case, ESBL production must be confirmed.

QUALITY CONTROL

Protocol:

The nutrient capacity and the selectivity of the medium can be tested using the following strains:

- *Klebsiella pneumoniae* ATCC® 700603
- *Escherichia coli* CIP 105903
- *Escherichia coli* ATCC® 25922

Range of expected results:

Strain	Results at 33-37°C	
Klebsiella pneumoniae ATCC® 700603	Growth within 24 hours	Green colonies within 24 hours
Escherichia coli CIP 105903	Growth within 24 hours	Pink colonies within 24 hours
Escherichia coli ATCC® 25922	No growth within 48 hours	

Note:

It is the responsibility of the user to perform Quality Control taking into consideration the intended use of the medium, and in accordance with any local applicable regulations (frequency, number of strains, incubation temperature and time, etc.).

LIMITATIONS OF THE METHOD

- Some ESBL-producing enterobacteria produce colourless colonies, particularly *E. coli* strains which do not possess a ß-glucuronidase (7) and *P. mirabilis* strains showing weak growth associated with low expression of ESBL production. They can be suspected if a negative oxidase test is obtained using colourless colonies. ESBL production must therefore be confirmed.
- Some multi-resistant micro-organisms other than ESBL-producing enterobacteria may develop on the medium and produce typical colonies.
- Some *Pseudomonas* strains may produce a brown pigmentation. The oxidase test enables them to be differentiated from *Proteeae*.
- Some non ESBL-producing enterobacteria may develop on the medium. These are often strains hyper-productive in cephalosporinase (*E. coli*, *Enterobacter*, etc.) or *Klebsiella oxytoca* hyper-productive in penicillinase (K1).
- Some mostly atypical strains of certain species of enterobacteria other than *E. coli* (*Citrobacter freundii, Enterobacter cloacae, Salmonella* spp, etc.) may produce a pink to burgundy colour. If the local distribution is particular, with a high prevalence of this type of bacteria, an indole test can be performed on the pink to burgundy colonies:
  - If the indole test is positive (blue with the ID Indole TDA test or pink to red with the JAMES reagent), identification of *E. coli* is confirmed.
  - If the indole test is negative, additional identification is required.
- In the case of a polymicrobial culture, the indole test must be confirmed on a subculture.
- Growth depends on the requirements of each individual micro-organism. It is therefore possible that certain strains (notably *Proteeae*) which have specific requirements (substrate, temperature, incubation conditions, etc.) may not develop.

PERFORMANCE

Performance was evaluated at two sites in France (according to the same protocol) and 1 site in Belgium, using human clinical specimens (rectal swabs, urine, and bronchial secretions), from patients at risk and chronic carriers being screened for ESBL-producing enterobacteria. The specimens were directly inoculated on the agars. The readings were performed after 18-24 hrs and 48 hrs of incubation at 37°C in aerobic conditions.

The first evaluation (Belgium) was performed using 173 specimens. chromID™ ESBL bioMérieux was compared to another method associating a Mac Conkey agar and a ceftazidime disc. On this medium, any colonies which grow in the inhibition zone surrounding the antibiotic disc are suspect and should be confirmed (confirmation of the species and ESBL production).

19 specimens were found to be positive on at least one of the 2 media.

	Recovery rate for ESBL-producing enterobacteria	
	chromID™ ESBL (+ confirmation of ESBL status)	Mac Conkey + ceftazidime disc (+ confirmation of species and ESBL status)
18 - 24 hrs	19/19 without oxidase test (PPV = 67.9% [48.96% ; 82.29%])	13/19 (PPV = 43.3% [27.1% ; 61.13%])
48 hrs	19/19 without oxidase test (PPV = 57.6% [40.49% ; 73.03%])	13/19 (PPV = 38.2% [23.66% ; 55.29%])

CI: 95% confidence interval
PPV: Positive Predictive Value

The other two evaluations (France) were performed according to the same protocol and using 765 specimens. chromID™ ESBL bioMérieux was compared to a screening medium available on the market (Mac Conkey Bi-plate with ceftazidime and Drigalski with cefotaxime) on which all the colonies that develop are suspect and should be confirmed (confirmation of the species and ESBL production).

32 specimens were found to be positive on at least one of the 2 media.

	Recovery rate for ESBL-producing enterobacteria	
	chromID™ ESBL (+ confirmation of ESBL status)	Mac Conkey with ceftazidime / Drigalski with cefotaxime (+ confirmation of species and ESBL status)
18 - 24 hrs	29/32 without oxidase test 32/32 with oxidase test * (PPV = 41.4% [30.43% ; 53.35%])	27/32 (PPV = 17% [11.85% ; 23.73%])
48 hrs	31/32 without oxidase test 32/32 with oxidase test (PPV = 30.7% [22.4% ; 40.46%])	27/32 (PPV = 12,7% [8.79% ; 17.93%])

CI: 95% confidence interval
PPV: Positive Predictive Value

* With chromID™ ESBL agar, an oxidase test performed on the colourless colonies and the confirmation of ESBL production if a negative oxidase test is obtained, enable all the positive samples to be recovered after 24 hours.

Note: Performance may fluctuate depending on the local epidemiology (prevalence, species and type of ESBL, etc.)

WASTE DISPOSAL

Dispose of used and unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products. It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

1. PATERSON D., BONOMO R. – Extended-Spectrum B-lactamase: a clinical update – Clin. Microbiol. Rev. – 2005 – Vol. 18, N° 4 – p. 657-686.
2. JACOBY G.A., MEDEIROS A.A. – More extended-spectrum ß-lactamases – Antimicrob. Agents Chemother., 1991, vol. 35, p. 1697-1704.
3. GLUPCZYNSKI Y., BERHIN C., BAURAING C. and BOGAERTS P. - Novel chromogenic medium from bioMérieux for detection of ESBL-producing *Enterobacteriaceae* - Poster D-0457 - San Francisco (U.S), 2006 ICAAC.
4. GLUPCZYNSKI Y., BERHIN C., BAURAING C. and BOGAERTS P. - Evaluation of a new selective chromogenic medium for detection of Extended-spectrum beta-lactamases-producing Enterobacteriaceae – J Clin Microbiol – February 2007, Vol. 45, N°2, p. 501-505.
5. JARLIER V. – Bactéries multirésistantes dans les hôpitaux français : des premiers indicateurs au Réseau d'alerte d'investigation et de surveillance des infections nosocomiales (Raisin), BEH, 2004, vol. 32-33, p. 148-151.
6. KILIAN M., BULOW P. - Rapid identification of *Enterobacteriaceae* - II. Use of a β-glucuronidase detecting agar medium (PGUA Agar) for the identification of *E. coli* in primary cultures of urine samples. - *Acta Path. Microb.* Scand., 1979, vol. 87, p. 271-276.
7. RALOVICH B., IBRAHIM G.A.M., FABIAN A. and al. – "Beta-D-Glucuronidase (BDG) activity of Gram-negative bacteria" - *Acta Microbiol. Hung.,* 1991, vol. 38, p. 283-291.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Protect from light

WARRANTY

bioMérieux disclaims all warranties, express or implied, including any implied warranties of MERCHANTABILITY AND FITNESS FOR A PARTICULAR USE. bioMérieux shall not be liable for any incidental or consequential damages. IN NO EVENT SHALL BIOMERIEUX'S LIABLITY TO CUSTOMER UNDER ANY CLAIM EXCEED A REFUND OF THE AMOUNT PAID TO BIOMERIEUX FOR THE PRODUCT OR SERVICE WHICH IS THE SUBJECT OF THE CLAIM.

BIOMÉRIEUX

bioMérieux SA
RCS LYON 673 620 399

69280 Marcy-l'Etoile / France
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com

CE