Instructions For Use

Rapid ESBL NP® test

System for detection of Extended-Spectrum-β-Lactamase-producing Enterobacteriaceae.

DESCRIPTION

Rapid ESBL NP® test is a 8-test panel used for the identification of ESBL producers among Enterobacteriaceae.

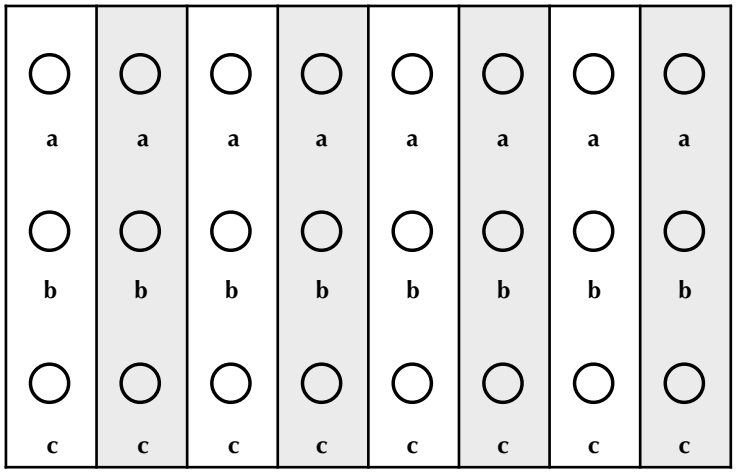
A panel allows the ESBL detection of 8 different isolates. The dried up substrates in the wells are rehydrated with a lysed microbial suspension. After a short incubation in thermostat the result is read and interpreted.

KIT CONTENT

- 2 Systems (panels) of Rapid ESBL NP® test
  (panels individually packed in foil with silica gel desiccant)
- 1 Vial of Lysis Buffer (8 ml)
- 16 Empty Vials
- Resealable Bag
- Sealing Film
- Instructions Sheet

CONFIGURATION

Tests are placed in columns: each test consists of three (3) wells, each indicated with a letter:



Well **a** does not contain any antimicrobial agent

Well **b** contains a cephalosporin antibiotic (cefotaxime)

Well **c** contains a cephalosporin antibiotic (cefotaxime) plus an ESBL inhibitor (tazobactam)

PRINCIPLE OF THE METHOD

β-Lactams are the most widely used class of antibiotics. Resistance to β-lactams is primarily because of bacterially produced β-lactamase enzymes that hydrolyze the β-lactam ring, thereby inactivating the drug. β-lactamases may confer resistance or reduced susceptibility to oxyimino-cephalosporins (i.e. cefotaxime, ceftriaxone, ceftazidime) and monobactams (i.e. aztreonam). ESBL activity is inhibited by several inhibitors such as clavulanic acid and tazobactam .

Rapid ESBL NP® test is based on a technique designed to identify the hydrolysis of the β-lactam ring of a cephalosporin (cefotaxime) generating a carboxyl group. This results in acidification of a culture medium displayed by the color change of a pH indicator (red phenol). Inhibition of ESBL activity is demonstrated in a complementary test reaction with tazobactam.

COLLECTION AND STORAGE OF THE SAMPLE

Rapid ESBL NP® test is not for use directly with clinical or other specimens. The microorganism to be tested must first be isolated on a suitable culture medium, either selective or non-selective. In case of mixed culture, selected colonies should be purified by subculturing.

TEST PROCEDURE

1. Take a panel from its envelope and leave it at room temperature for 10 min.
   NOTE Use scissors to cut off the envelope at one end in order to maintain its integrity and functioning. Do not discard the envelop until all the 8 tests have been carried out.
2. Add 400 µl of Lysis Buffer* to one of the empty vials provided in the kit.
3. Transfer 1-2 full calibrated loops (loop size 10 µl) of bacterial colonies into 400 µl Lysis Buffer. Check the colonies have been correctly resuspended. If necessary mix up and down with a pipette.
4. After 15 min, dispense 100 µl of the solution obtained into each well (a, b and c) in a single column.
5. Cover the panel with the lid provided and incubate at 36 ± 2°C for up to 20 min in ambient air.
   NOTE Do not extend incubation time.

* 20 mM Tris-HCl Lysis Buffer

READING THE RESULTS

At the end of the incubation period observe the color change in the wells.

The result can be read visually or automatically. Reading by the naked eye can be improved by use of bright indirect lighting against a dark background.

INTERPRETATION OF THE RESULTS

Interpret the results according to the scheme below.

Column	No ESBL	ESBL	Cephalosporinase or Cephalosporinase + ESBL or Carbapenemase with or without Cephalosporinase or/and ESBL	Non interpretable
a (no antibiotic)	red	red	red	yellow
b (cefotaxime)	red	orange/yellow	orange/yellow	yellow
c (cefotaxime + tazobactam)	red	red	orange/yellow	yellow

If all the wells in a column appear yellow the result cannot be reported for that particular test. In that case, check the viability of the colonies picked and repeat the test using a new columns in the same panel or a new panel and a microbial culture of recent growth.

NOTE If not all the 8 tests have been performed, use the film provided in the kit to seal the inoculated columns so to prevent any leakage of contaminated fluids. Then, return the panel into its own desiccant envelop and subsequently into the resealable bag provided with the kit. Store into the refrigerator until time of testing (see STORAGE).

USER QUALITY CONTROL

Quality control of Rapid ESBL NP® test is performed using the reference strains reported below:

	Strain		Result
1.	*Escherichia coli*	ATCC® 25922	No ESBL
2.	*Escherichia coli*	NCTC 13353	ESBL

PERFORMANCE CHARACTERISTICS

A total of 100 clinical gram-negative strains (40 ESBL producers with or without cephalosporinase, 43 carbapenemase producers with or without additional ESBLs, 8 AmpC-type producers, 6 penicillinase producers, and 3 non-β-lactamase producers) were tested by evaluating the reactions in the wells of the panel. The overall sensitivity and specificity were found to be 93.9% and 98.5%, respectively.

FACTORS THAT MAY INVALIDATE THE RESULTS

Contaminated culture; poor standardization of the inoculum; clinical material unsuitable; use of expired panels or expired supplementary reagents; non compliance with temperatures and times of incubation.

WARNINGS AND PRECAUTIONS

For *in-vitro* diagnostic use. For professional use only. The product must be used in the laboratory by properly trained personnel, using approved aseptic and safety methods for handling pathogenic agents. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

STORAGE

Store Rapid ESBL NP® test at 2-8°C in the original packaging. Once an envelope is opened the panel should be used within 7 days. Keep away from direct sunlight and direct heat. Do not use the panels beyond the expiry date indicated on the label. Eliminate without using if there are signs of deterioration.

DISPOSAL OF USED MATERIAL

After use, Rapid ESBL NP® test and material that has come into contact with the sample must be decontaminated and disposed of in accordance with guidelines used in the laboratory for decontamination and disposal of potentially infected material.

REFERENCES

1. Demord A, Poirel L, D'Emidio F, Pomponio S, and Nordmann P (2021) Rapid ESBL NP Test for Rapid Detection of Expanded-Spectrum β-Lactamase Producers in Enterobacterales. Microb Drug Resist. 27(8):1131-1135. DOI: 10.1089/mdr.2020.0391
2. Nordmann P, Dortet L, and Poirel L (2012) Rapid Detection of Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae. J Clin Microbiol. 50(9): 3016–3022. DOI: 10.1128/JCM.00859-12

Product	Packaging	Ref.
Rapid ESBL NP® test	2x8 tests	76036

TABLE OF SYMBOLS

LOT	Batch code	IVD	*In vitro* Diagnostic Medical Device		Manufacturer	Σ	Contains sufficient for <n> tests		Temperature limits
REF	Catalogue number		Fragile, handle with care		Use by		Caution, consult accompanying documents		Do not reuse

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