SensiTest Colistin

System for colistin susceptibility testing with the broth microdilution method.

DESCRIPTION

Clinicians are re-thinking to colistin as therapeutic option for the treatment of severe infections caused by multidrug-resistant microorganisms, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and carbapenem-resistant Enterobacteriaceae. However, colistin-resistant strains are worldwide disseminated leading to the need to determine the value of minimum inhibitory concentration (MIC) before the drug is prescribed. Although several commercial tests based on different techniques have been developed, broth microdilution (BMD) is considered the best method for performing colistin susceptibility testing so far.

SensiTest Colistin is a 4-test panel containing the dried up antibiotic in 7 two-fold dilutions (0.25 - 16 µg/ml). The system is used to perform the broth microdilution (BMD) method for the antimicrobial susceptibility testing of colistin (polymyxin E) as recommended by international standards (i.e. CLSI, EUCAST, ISO) but in a simpler and less time-consuming way.

KIT CONTENT

- 4 Systems SensiTest Colistin
- 16 Vials of Mueller Hinton II Broth (3.6 ml)
- Sealing Film
- Instructions Sheet and Test Results Form

ITEMS NECESSARY BUT NOT INCLUDED IN THE KIT

- McFarland 0.5 Barium Sulphate Standard (ref.80400)
- Solution reservoir for multichannel pipette (ref. 96761)
- Physiological Solution (ref. 20095)
- Tips for multi.channel pipette (ref.96758)
- Multichannel pipette 30-300 µl (ref.96759)

CONFIGURATION

Test	Colistin Concentration (µg/ml)						
A Growth	0.25	0.5	1	2	4	8	16
B Growth	0.25	0.5	1	2	4	8	16
C Growth	0.25	0.5	1	2	4	8	16
D Growth	0.25	0.5	1	2	4	8	16

Growth indicates growth control: No antimicrobial agent in the well.

PRINCIPLE OF THE METHOD

A panel allows the susceptibility testing of 4 different isolates.
All wells in a single row (A, B, C or D) are rehydrated with a standardized microbial suspension.
After incubation in thermostat the result is read and interpreted.

COLLECTION AND STORAGE OF THE SAMPLE

SensiTest Colistin 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 envelop and leave it at room temperature for 10 min,
   (DO NOT DISCARD THE ENVELOP until all 4 tests have been carried out).
2. Prepare a suspension of the test organism using either the direct colony suspension or growth method.
3. Standardise the suspension to the density of a McFarland 0.5 standard.
4. Optimally within 15 min of preparation, dilute the adjusted suspension 1:20 in saline; this will be the **Solution A**.
5. Add 0.4 ml of Solution A to a tube of MH II Broth* provided in the kit to obtain the **Solution B**.
6. Dispense 100 µl of Solution B into each well in a row.
7. Cover the panel with the lid provided and incubate at 36 ± 2°C for 16-20 hours in ambient air.
8. Read the results.

* Mueller Hinton II Broth (g/l): Beef Extract 3.0 g; Acid Hydrolysate of Casein 17.5 g; Starch 1.5 g; Distilled Water 1000 ml; pH 7.3 ± 0.1
(adjusted with appropriate salts to provide 20-25 mg/l of calcium and 10-12.5 mg/l of magnesium)

INTERPRETATION OF THE RESULTS

At the end of the incubation period, observe the growth in the wells and establish the MIC value.

- Make sure the control well is positive for growth. If not, check the viability of the colonies picked and repeat the test using a new row in same panel or a new panel and a microbial culture of recent growth.
- The MIC obtained should be interpreted according to current EUCAST or CLSI interpretative criteria.
- Note the results on the test results form included in the kit (copy as many form as necessary).

NOTE: Each single panel can be used up to four times. If less than 4 tests have been performed, use the film provided in the kit to seal the inoculated rows. Once leakage of contaminated fluids is prevented, return the panel into its own desiccant envelop and then into the fridge.

USER QUALITY CONTROL

Quality control of SensiTest Colistin is performed using the following reference strains:

1. *Escherichia coli* ATCC® 25922
2. *Pseudomonas aeruginosa* ATCC® 27853
3. *Escherichia coli* NCTC 13846

Strain 1 and 2 are both susceptible to colistin and the acceptable MIC range is 0.25–2 µg/ml for *E. coli* and 0.5–4 µg/ml for *P. aeruginosa*. For *E. coli* NCTC 13846 (mcr-1 positive), which is instead resistant to colistin, the MIC target value is 4 µg/ml and should only on occasion be 2 or 8 µg/ml.

FACTORS THAT MAY INVALIDATE THE RESULTS

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

PRECAUTIONS

The product SensiTest Colistin does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. SensiTest Colistin is a disposable device to be used only for diagnostic use *in vitro*. The product must be used in the laboratory by properly trained personnel, using approved aseptic and safety methods for handling pathogenic agents.

STORAGE

Store SensiTest Colistin at 2-8°C in the original packaging. Keep away from sources of heat and avoid excessive changes in temperature. In such conditions the product will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.

DISPOSAL OF USED MATERIAL

After use, SensiTest Colistin 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. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 27th ed. CLSI Supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
2. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.0, 2017. http://www.eucast.org.
3. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 7.0, 2017. http://www.eucast.org.
4. CLSI. Methods for dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Tenth Edition. CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
5. ISO 20776-1:2006. Clinical laboratory testing and in vitro diagnostic test systems -- Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices -- Part 1: Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.

PRESENTATION

Product	µg/ml	Packaging	Ref.
SensiTest Colistin	0.25 - 16	4x4 tests	75001

TABLE OF SYMBOLS

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

CE IVD