

Antimicrobial Susceptibility Testing Discs

for the standardized *in vitro* antimicrobial disc-agar diffusion susceptibility test method

Description

Abtek Antimicrobial Susceptibility Testing Discs are recommended for use with internationally recognised standardised procedures such as the as those from CLSI, BSAC & EUCAST.

A standardized inoculum of a pure culture, isolated from a clinical sample, is streaked across a designated culture medium and allowed to dry. Antimicrobial disks of appropriate potency are then dispensed onto the culture medium. Following incubation, agar plates are examined and zones of inhibition surrounding the disks are measured. The minimum inhibitory concentration (MIC) of the antimicrobial appears at the edge of the zone of inhibition, and represents the interaction of a critical concentration of antimicrobial at a critical time on a critical microbial population. These results are compared with established zone ranges for individual antimicrobial agents to determine the most appropriate antibiotic for use *in vivo*.

The discs are 6mm in diameter and incorporate all routinely available antimicrobials at any concentration.

Discs are supplied in cartridges containing 50 discs and packed with silica gel desiccant. The cartridges fit Abtek disc dispensers and ejectors, or Dispenser and ejectors available from some other brands. Discs can also be supplied in glass vials of 100 discs, maintained dry by a silica-gel sachet.

Consultation with an infectious disease specialist is recommended for guidance in determining the need for susceptibility testing.

NOTE: Current editions of CLSI, BSAC or EUCAST standards should be consulted for the most recent recommendations.

Precautions

This product is for *in vitro* diagnostic use, and should be used by properly trained laboratory professionals.

Dispose of as clinical waste.

Storage

Store discs at 2-8°C in dry location. After cold storage allow discs to reach room temperature before opening storage containers.

Discs should not be used if packaging is damaged, if the expiry date has passed, or if there are other signs of deterioration. Do not use if the zones in quality assurance testing do not conform to recommended standards, if all other parameters in the test are correct.

Instructions for use

Specimen

Organisms used for testing must be isolated and not contain mixed flora. The discs are not to be used directly with clinical specimens.

Materials required but not included

- Media and other equipment for subculture.
- Sterile cotton swab.
- Sterile forceps.
- Incubator equipment to maintain 35-37°C.
- Agar plates of uniform depth. Refer to current CLSI, BSAC or EUCAST standards for appropriate media recommended for disk diffusion testing.

Procedure

1. Prepare a broth suspension of the organism to be tested, to a turbidity of ≥ 0.5 McFarland Standard (1.5×10^8 CFU / ml).
2. Within 15 minutes of turbidity adjustment, properly label the plate, and use the swab to evenly inoculate the agar surface.
3. Within 15 minutes of inoculation, aseptically place discs on the inoculated surface. To ensure complete contact with the agar surface, press them gently after application. Do not over dry the agar surface before applying the discs. No more than six discs should be placed on the agar surface of a 90mm dish. Do not relocate a disk once it has come in contact with the agar surface as the drug diffuses almost instantaneously.
4. Once the discs have been applied, plates should be placed in the incubator within 15 minutes, to prevent pre-diffusion of the antimicrobial at room temperature. Incubate plates, and incubate for 16-18 hours (20-24 hours for *N. gonorrhoeae* and streptococci), at 35-37°C. Agar plates should not be placed in high stacks because the middle plates will take longer to reach the incubator temperature and this delay could cause over-large zones. Incubate non-fastidious organisms in ambient air. Incubate *Haemophilus* spp., *N. gonorrhoeae*, and streptococci in a 5% CO₂ atmosphere.
5. Examine for the presence of zones of inhibition around the discs. If plate was properly inoculated, zones of inhibition will be uniformly circular along with a confluent lawn of growth. If

individual colonies are apparent, the inoculum was too light and the test should be repeated.

6. Measure the diameter of the zones of inhibition (including the diameter of the disc) to the nearest millimetre, using callipers or an automated zone reader. Discrete colonies growing within the clear zone of inhibition should be sub-cultured, re-identified, and re-tested. Swarming within the zone of inhibition may occur for some *Proteus* species, but zones are usually well defined and the thin veil of swarming should be ignored. When testing haemolytic streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of haemolysis. With trimethoprim and the sulphonamides, antagonists in the medium may allow slight growth. Therefore, with these drugs, slight growth (20% or less of the lawn of growth) should be disregarded and the margin of heavy growth measured to determine zone diameter.
7. Compare zone diameters with the zones provided with the standard procedure that is in use, interpreting as susceptible, intermediate, or resistant.

Interpretation

The "**Susceptible**" category implies that an infection due to the strain may be appropriately treated with the dosage of antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated.

The "**Intermediate**" category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The "Intermediate" category implies clinical applicability in body sites where the drugs are physiologically concentrated (e.g., quinolones and β -lactams in urine), or when a high dosage of a drug can be used (e.g., β -lactams). The "Intermediate" category also includes a "buffer zone", which should prevent small, uncontrolled technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

"**Resistant**" strains are not inhibited by the usually achievable systemic concentrations of the agent with normal dosage schedules and/or fall in the range where specific microbial resistance mechanisms are likely (e.g., β -lactamases) and clinical efficacy has not been reliable in treatment studies.

Quality Assurance

All lot numbers have been tested to confirm that the antimicrobial content lies within 80 – 140% of the stated content, using the following quality control organisms, as appropriate.

Staphylococcus aureus ATCC 25923
Escherichia coli ATCC 25922
Klebsiella pneumoniae NCTC 11228
Pseudomonas aeruginosa ATCC 27853
Micrococcus flavus NCTC 7343
Bacteroides fragilis ATCC 25285
Streptococcus pneumoniae ATCC 6305
 β -haemolytic Streptococcus pyogenes Group A ATCC 19615
Haemophilus parainfluenzae ATCC 10665
Haemophilus influenzae NCTC 10479
Bacillus subtilis ATCC 6633

If product fails to conform to specifications, please advise Abtek Biologicals (www.abtekbio.com).

For further information please see

www.clsi.org
www.bsac.org.uk
www.eucastr.org

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Date of revision 2014-03-21

