BD™ Iso-Res Agar

INTENDED USE

BD Iso-Res Agar is a semi-defined medium for the susceptibility testing of clinical isolates with the disc diffusion (Kirby-Bauer) method.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

While Mueller Hinton II Agar has been recommended in many standards for susceptibility testing, several issues have been raised against its use, such as insufficient reproducibility between different lots and problems with the testing of tetracyclines and trimethoprim.[1-3] Iso-Res Agar (also described as Iso-Sensitest Medium) has been specially developed to obviate these problems.[4-6] The ingredients have been carefully selected to yield sharp and reproducible inhibition zones. The medium contains only low levels of thymine or thymidine, so that the testing of sulfonamides and trimethoprim are not influenced by these antagonists. Therefore, **BD Iso-Res Agar** is an excellent alternative to Mueller Hinton II Agar for disc diffusion susceptibility testing. The inhibition zone sizes described in the CLSI Standard M2 are valid for Mueller Hinton Agar. Therefore, they may differ from those determined on **BD Iso-Res Agar**.[8]

This medium is recommended by the Dutch WRG system.[9]

Casein and specially selected peptones provide basic nutrients, while many of the defined compounds of the medium supply nutrients, vitamins and trace minerals.

REAGENTS

BD Iso-Res Agar

Formula* Per Liter Purified Water

Hydrolysed Casein	11.0 g	Menadione (Vitamin K3)	0.001 g
Peptones	3.0	Cyanocobalamine (Vitamin B12)	0.001
Glucose	2.0	L-Cystein	0.02
Sodium Chloride	3.0	L-Tryptophan	0.02
Starch	1.0	Pyridoxin (Vitamin B6)	0.003
Disodium Hydrogen Phosphate	2.0	Pantothenate	0.003
Sodium Acetate	1.0	Nicotinamide	0.003
Magnesium Glycerophosphate	0.2	Biotin	0.0003
Calcium Gluconate	0.1	Thiamine	0.00004
Cobalt-II-Sulfate	0.001	Adenine	0.01
Copper-II-Sulfate	0.001	Guanine	0.01
Zinc Sulfate	0.001	Xanthine	0.01
Ferrous Sulfate	0.001	Uracil	0.01
Manganese-II-Chloride	0.002	Agar	8.0

pH 7.4 +/- 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE
On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.
Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL
For details of inoculation and reading of the results, see **Test Procedure** and **Results** sections.
BD Iso-Res Agar plates and the antimicrobial disks routinely used should be tested at least twice weekly for proper performance, using the test strains mentioned in the **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE** section.
Appearance of uninoculated medium: colorless to light amber.

PROCEDURE
Materials Provided
BD Iso-Res Agar (90 mm **Stacker™** plates)
Microbiologically controlled.

Materials Not Provided
1. Tubed inoculum broth, such as **BD Trypticase™ Soy Broth** (Soybean-Casein Digest Broth) or Mueller Hinton II Broth (cation-adjusted), for preparation of a standard inoculum, and sterile broth or saline for dilution of inoculum.
2. Barium sulfate comparison standard (0.5 ml of 0.048 M $BaCl_2$ [1.175% w/v $BaCl_2 \cdot 2H_2O$] to 99.5 ml of 0.18 M [0.36 N] H_2SO_4 [1% v/v]).
3. A photometric device for adjusting the turbidity of the inoculum suspension to be equivalent to the 0.5 McFarland standard.
4. As an alternative to the above materials (1-3), the **BD Prompt™ Inoculation System** (volumetric inoculum preparation device) can be used.[6,18]
5. Paper discs impregnated with specified amounts of antimicrobial agents,[6] such as **BD Sensi-Disc™** susceptibility test discs.
6. Dispensing device, such as the **BD Sensi-Disc** 6-, 8- or 12-place dispenser. A suitable dispenser is also available for Mueller Hinton II Agar Square plates.
7. Ruler or another device for measuring zone sizes in millimeters.
8. Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types
This product is used for susceptibility testing of pure cultures only and is not intended to be used directly with clinical specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure
Suspend the test strain in Trypticase Soy Broth™ or saline and adjust the turbidity to match the McFarland 0.5 standard. Within 15 min after adjusting the turbidity of the inoculum, immerse a sterile swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid. Alternatively, the **BD Prompt Inoculation System** may be used.
Inoculate the entire agar surface of the plate three times, rotating the plate 60° between streakings to obtain even inoculation.
Apply the antimicrobial discs by means of an antimicrobial disc dispenser, using aseptic precautions. Deposit discs so that the centers are at least 24 mm apart. After discs have been placed on the agar, tamp them with a sterile needle or forceps to provide complete contact with the medium surface. This step is not necessary if the discs are deposited using the **BD Sensi-Disc** 6-, 8-, or 12-place self-tamping dispenser.
Within 15 minutes after the discs are applied, invert the plates and incubate them in an aerobic atmosphere at 35°to 37° C for 24 hours.

Results
After incubation, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disc, to the nearest whole millimeter, using sliding callipers, a ruler, or a template prepared for this purpose. The measuring device is held on the bottom of the inverted plate over a black, non-reflecting background, and illuminated from above.
The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies which can be detected only with difficulty near the edge of the obvious zone of inhibition.
Results are reported as resistant, intermediate or susceptible. Note that zone sizes are not always comparable to those found on Mueller Hinton II Agar (see **Limitations of the Procedure**).

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

This medium has been evaluated internally. The zone size ranges are based on results from at least 3 different lots of **BD Iso-Res Agar**:

Strains	BD Sensi-Disc	Expected Range (mm)	Strains	BD Sensi-Disc	Expected Range (mm)
Escherichia coli **ATCC™ 25922**	Ampicillin AM-10	14 - 25	***Entero-coccus faecalis*** **ATCC 29212**	Ampicillin AM-10	24 - 28
	Mezlocillin MZ-30	20 - 29		Mezlocillin MZ-30	27 - 33
	Cephalothin CF-30	15 - 21		Erythromycin E-15	18 - 25
	Cefotaxim CTX-30	28 - 37		SXT	28 - 34
	Imipenem IPM-10	30 - 36			
	Gentamicin GM-10	19 - 26	***E. faecalis*** **ATCC 33186**	Ampicillin AM-10	24 - 30
	Amikacin AN-30	19 - 26		Mezlocillin MZ-30	27 - 33
	Ofloxacin OFX-5	29 - 33		Erythromycin E-15	18 - 25
	Ciprofloxacin CIP-5	30 - 40		SXT	24 - 32
	SXT	24 - 32			
Staphylo-coccus aureus **ATCC 25923**	Penicillin G P-10	30 - 40	***Pseudo-monas aeruginosa*** **ATCC 27853**	Ticarcillin TIC-75	20 - 28
	Oxacillin OX-1	18 - 24		Azlocillin AZ-75	21 - 29
	Cephalothin CF-30	33 - 44		Aztreonam ATM-30	26 - 31
	Cefotaxim CTX-30	27 - 34		Amikacin AN-30	18 - 28
	Tetracyclin TE-30	25 - 34		Gentamicin GM-10	18 - 25
	Gentamicin GM-10	23 - 30		Ceftazidim CAZ-30	23 - 31
	Erythromycin E-15	26 - 35			
	Lincomycin L-15	25 - 33			
	Ofloxacin OFX-5	24 - 30			
	SXT	24 - 32			

The disc diffusion must only be used with bacterial strains like *Enterobacteriaceae*, staphylococci, enterococci or other that produce well developed colonies within an incubation period of 18 to 24 hours on bloodfree media and that grow aerobically. For deteils, consult the CLSI standard. [7]
The test and the methods described above must only be used with pure cultures of bacteria. The method described here for inoculum preparation and inoculation is the one described in the CLSI Standard.[7] Other national standards have been developed for antimicrobial susceptibility testing according to the Bauer-Kirby procedure. In those standards, the inoculum densities and the method of inoculation may differ from the CLSI Standard. While common European standards for susceptibility testing do not exist, local national standards should be consulted if the CLSI Guidelines are not applicable.

Susceptibility test zone sizes obtained on this medium are not necessarily in agreement with those obtained on Mueller Hinton II Agar in the CLSI Standard M2.[7,8] It is therefore recommended to compare the zone sizes obtained on both media using the antimicrobial discs routinely tested before routinely using **BD Iso-Res Agar.**

Inappropriate inoculum concentration may produce incorrect results. Zones of inhibition may be too small if the inoculum is too heavy and they may be too large and difficult to measure if the inoculum is too light.
Improper storage of antimicrobial discs may cause a loss of potency and a falsely resistant result.
Bacteria requiring thymine or thymidine may be encountered. These organisms may not grow satisfactorily on this medium since it contains low levels of thymine or thymidine.
In vitro susceptibility of an organism to a specific antimicrobial agent does not necessarily mean that the antimicrobial will be effective in vivo.

REFERENCES

1. Ericsson, H.M, and J.C. Sherris 1971. Acta Pathol. Microbiol. Scand. Suppl. 217: 1-90.
2. Garrod, L.P., and P.M. Waterworth. 1971. J. Clin. Pathol. 24: 779-789.
3. Reller, L.B., et al. 1974. J. Infect. Dis. 130: 454-463.
4. Reynolds, A.V., et al. 1974. Brit. Med. J. II: 778.
5. Stewart, S.M., et al. 1975. J. Clin. Pathol. 28: 195-197.
6. Bell, S.M. 1975. Pathology 7, Suppl.: 1-48.
7. Clinical and Laboratory Standards Institute (NCCLS, formerly CLSI). Approved standard: M2. Performance standards for antimicrobial disk susceptibility tests, CLSI, Wayne, PA, USA.*Search for latest version at* **www.clsi.org**
8. Jorgensen, J.H., and J.D. Turnidge. 2003. Susceptibility test methods: dilution and disk diffusion methods. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C. USA.
9. Werkgroep Richtlijnen Gevoeligheidsbepalingen. 1981. Standaardisatie van Gevoeligheidsbepalingen. Bilthoven, The Netherlands.

PACKAGING/AVAILABILITY

BD Iso-Res Agar

Cat. No. 254021	Ready-to-use plated media, 20 plates
Cat. No. 254076	Ready-to-use plated media, 120 plates

FURTHER INFORMATION

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