MIC-Strip

Only for *in vitro* diagnostic

english **Release 2014-09-03**

MIC-Strip for manual susceptibility testing of bacteria. MIC-Strip is available in different product versions. For antibiotic composition of the strips, evaluation and interpretation of the results please refer to the evaluation protocol.

TEST PRINCIPLE

The susceptibility testing with MIC-Strip is based on the rehydration of antibiotics by adding a standardized bacteria suspension. After incubation of 18 - 48 hours (depending upon type of bacteria) at 35 - 37°C the result is read visually and interpreted.

REAGENTS

Contents

40 susceptibility tests can be performed with one packaging unit. The kit contains:

- 5 plates MIC-Strip
- each plate include 8 strips with 12 wells per strip
- Cover plate and frame
- Knife

Required additional reagents and materials:

- Mueller-Hinton II broth or H-broth
- Wilkins-Chalgren broth with NAD
- NaCl 0,9 % pH 5,5 bis 6,5
- Air-tight container to create a humid atmosphere.

Supplement for specific bacteria groups:

Lysed horse blood for *Streptococcus pneumoniae* Company Oxoid article no.: SR 0048C.
For sufficient growth of pneumococci lysed horse blood (at least 2%) should be added to the Mueller-Hinton II broth. Then the Mueller-Hinton II Bouillon broth is processed as usual.

For manual inoculation:

- 8 channel pipette (100-1200µl) (# M/BH3-880-001 or M/L3-880-001 or other) incl. pipet tips (# M/BH3-487-500 or. # M/L3-487-500)

(All products are available at sifin diagnostics gmbh)

Laboratory materials

- McFarland standard 0,5
- Blood agar plate (without additives)
- Incubator 37°C
- Inoculation loops
- Marking pen
- Anaerobic jar

COMPOSITION OF THE MEDIA

Media	Components
NaCl 0.9 % 1l	sodium chloride
Mueller-Hinton II broth	beef extract acid casein-hydrolyzate starch
H-broth	hematin NaOH Tween 80 pyridoxal ß-nicotinamide adenindinucleotide Columbia broth base glucose yeast extract neopeptone agarose type II A
Wilkins Chalgren broth with NAD 20 tubes á 11 ml Fa. Heipha REF 271r	Trypton a. Gel. yeats extract Glucose NaCl Agar L-Arginin Na-pyruvat Menadione Haemin NAD

Please note: For particular bacteria groups it is advisable to add supplement to the broth.

STABILITY/ STORAGE

MIC-Strip as well as the media have to be stored in the original packaging at 15 - 25 °C and can be used up to the indicated expiration date **except** the H-broth which must be stored at 2 - 8°C. Otherwise follow the storage instructions indicated on the products.

PRECAUTIONARY MEASURES

- Only to be used for *in vitro* diagnostic.
- Do not pipette the reagents by mouth.
- Caution in the use of the knife, risk of injury (sharp blade). After using blade push back into the protective sleeve.
- Only for proper use.
- Samples, bacteria cultures and the inoculated test plates have to be considered as potentially infectious and must be treated properly and with respect to the corre-

sponding precautionary measures by qualified specialist staff. It is important to work aseptically during the whole test procedure. For information please refer to "BioSafety in Microbiological and Biomedical Laboratories, HHS Publication No. (CDC) 99-8395, 4th Edition (April 1999)", or to the corresponding national legal requirements.

- Upon reading and evaluation of the tests, all samples, inoculated and contaminated products (pipette tips, stripes) must be autoclaved, burnt or disinfected in a bactericidal solution before disposal.
- It is important to follow the instructions carefully, each deviation may influence the quality of the results.
- The test results should be interpreted by qualified staff with experience in microbiology. The clinical background, origin of the samples, colony and microscopic morphology, serology and the identification result must be taken into consideration when interpreting the results.

TEST PROCEDURE

Preparation of the samples

- Prepare a tube with 5 ml NaCl 0.9 % pH 5.5 to 6.5.
- Prepare a tube of the corresponding Mueller-Hinton II broth, H-broth or Wilkins-Chalgren broth with NAD.
- Pick several single colonies of an 18 - 24 hours aged pure culture from the blood agar (without additives).

Preparation of the inoculum

- Homogenize the colonies well in 5 ml NaCl 0.9 % until the turbidity matches a McFarland of 0.5.

(a) Mueller-Hinton II broth

- Gram-negative bacteria:
  Pipette 50 µl of the bacteria suspension into 11 ml Mueller-Hinton II broth and homogenize well.
- Gram-positive bacteria:
  Pipette 100 µl of the bacteria suspension into 11 ml Mueller-Hinton II broth and homogenize well.
- Fastidious bacteria (e. g.: Streptococci):
  Pipette 200 µl of the bacteria suspension into 11 ml Mueller-Hinton II broth (supplement with lysed horse blood) and homogenize well.

(b) H-broth

- Fastidious bacteria (e. g.: Streptococci, coryneform bacteria, Haemophilus, Neisseria): Pipette 200 µl of the bacteria suspension into 11 ml H-broth and homogenize well.
- Fastidious Nonfermenter (CF strains):
  Pipette 50 µl of the bacteria suspension into 11 ml H-broth and homogenize well.

(c) Wilkins-Chalgren broth with NAD

- Anaerobic bacteria:
  Pipette 200 µl of the bacteria suspension into 11 ml Wilkins-Chalgren broth with NAD and homogenize well.

Inoculation

- Remove the MIC-Strip from the packaging not more than 30 minutes before inoculation.
- Cut the protective sheet along the strips with a cutter and remove the MIC-Strip.
- Put back the remaining MIC-Strip immediately into the packaging and reseal it carefully.
- Stack the removed strips into the frame and label it.
- Inoculate the prepared suspension into MIC-Strip manually by using a pipette, 100 µl per well.

Sealing and incubation

(a) Mueller-Hinton II broth

- After the inoculation cover the strips with the cover plate.
- Place the strips in an incubator at 35 – 37°C for 18–48 hours (depending upon type of bacteria).

(b) H-broth

- After the inoculation cover the strips with the cover plate.
- Place the test plate in an incubator at 35 – 37°C for 22-48 hours (depending upon type of bacteria) in enriched CO_2–atmosphere if necessary.

(c) Wilkins-Chalgren broth with NAD

- After inoculation cover the strips with the cover plate.
- Place the test plate in an incubator at 35 – 37°C for 24-48 hours in an anaerobic atmosphere.

To avoid a drying up of the medium in the wells (especially when using ventilated incubators) incubate the system in a humid atmosphere (e.g. air-tight container with damp cloth).

Reading

- Remove the cover plate.
- Wipe off the bottom of the strips.
- Read the strips visually and document the results in the evaluation protocol.
- Turbid= growth/ positive, clear = no growth/ negative
- MIC-Strips with vacuum dried AST indicator indicate the growth by a colour change from blue to pink.
- The growth control must be covered (cloudy respectively pink) otherwise the test must be repeated.

INTERPRETATION OF THE RESULTS

MIC

Is the lowest concentration of an antibiotic with no detectable growth (minimum inhibitory concentration).

Evaluation and interpretation

Please document the results on attached evaluation protocol. The interpretation of the results is described likewise on this protocol.

QUALITY CONTROL

The MIC-Strip and reagents are subject to quality controls which are carried out systematically at different stages of the production. The bacteriological quality control can be carried out with the following strains.

Strains	ATCC No.	DSMZ No.
Staph. aureus	ATCC 29213	DSM 2569
E. coli	ATCC 25922	DSM 1103
Ps. aeruginosa	ATCC 27853	DSM 1117
Ecoc. faecalis	ATCC 29212	DSM 2570

ATCC = American Type Culture Collection
DSMZ= German Collection of Microorganisms and Cell Cultures Ltd.

QUALITY AND PERFORMANCE DATA

The general requirements apply to DIN EN ISO 20776-1 and following or CLSI. For monitoring the accuracy for minimal inhibitory concentrations (MICs) please refer to the acceptable limits for quality control strains according to DIN or CLSI.

GUARANTEE

The quality data of the MIC-Strip have been determined by strictly following the present instruction. Divergences or alterations of the test procedure may reduce the quality of the results. Any claims for damages are excluded in this case.

TECHNICAL REMARKS

In order to obtain best results please follow the below listed points of the instructions carefully:

- Work with pure culture of blood agar (without additives) not older than 24 hours. Exception: Fastidious bacteria can be taken from a blood agar plate (without additives) after 48 hours of incubation at 35 - 37°C.
- If you do not use culture media ready for use follow the instruction carefully when producing the broth.
- When using Mueller-Hinton II broth check the correct concentration of the bivalent cations Ca 2+ and Mg 2+ (20 to 25 mg Ca 2+ /l and 10 to 12.5 mg Mg 2+ /l). Verify the pH-value of the Mueller-Hinton II broth, it should be between 7.2 and 7.4 at room temperature (25°C).
- Use NaCl 0.9 % pH 5.5 – 6.5.
- Preheat the medium before using it (1 hour in the incubator).
- Follow the correct McFarland 0.5 adjustment of the NaCl suspension. Homogenize the suspension sufficiently.
- Transfer the bacteria suspension on a blood agar plate (without additives) for purity control.
- Cut the protective sheet along the strips with a cutter. Take care not to damage the protective sheet of adjacent strips. Do not use strips with damaged protective sheets otherwise adverse effects may arise upon loss of drug activity.
- Follow the incubation times carefully, do not incubate aerobic bacteria less than 18 hours, fastidious bacteria less than 22 hours and anaerobic bacteria less than 24 hours.
- To avoid a drying up of the medium in the wells (especially when using ventilated incubators) incubate the system in a humid atmosphere (e.g. air-tight container with damp cloth).

EXPLANATION OF THE SYMBOLS ON THE LABELS

The symbols give information about:


Number of possible tests


Storage conditions


Follow instructions for use


Follow security advice on the safety data sheet

 Expiry date

CE marking in accordance with 98/79/EC (IVDD)

 Indication of the lot number

 In vitro diagnostics

 Article number

LITERATURE

- Kresken, Michael, Hafner, D. and the Study Group Bacterial Resistance of the Paul-Ehrlich-Society for Chemotherapy: Drug Resistance among Clinical Isolates of Frequently Encountered Bacterial Species in Central Europe during 1975 to 1995. Infection 27:2-8 (1999).
- Ingo Stock, Konstanze Machka, Arne Rodloff und Bernd Wiedemann. Qualitätssicherung und Qualitätskontrollen in der Antibiotika-Empfindlichkeitsprüfung von Bakterien mit der Mikrodilution. Chemotherapie Journal 10:78-91 (2001).
- Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That grow Aerobically; CLSI Document M7-A7 Vol. 26 No. 2 (2007).
- DIN EN ISO 20776-1 -Labormedizinische Untersuchungen und In-vitro-Diagnostika-Systeme- Empfindlichkeitsprüfung von Infektionserregern und Evaluation von Geräten zur antimikrobiellen Empfindlichkeitsprüfung Teil 1: Referenzmethode zur Testung der In-vitro-Aktivität von antimikrobiellen Substanzen gegen schnell wachsende aerobe Bakterien, die Infektionskrankheiten verursachen.
- Methods for Antimicrobial Susceptibility testing of Anaerobic Bacteria; CLSI Document M11-A7 Vol. 24 No. 2 (2007).
- DIN 58940-4 Bbl. 1: 2004 Medizinische Mikrobiologie. Empfindlichkeitsprüfung gegen Chemotherapeutika – Teil 4: Bewertungsstufen für die minimale Hemmkonzentration - MHK Grenzwerte von antimikrobiellen Wirkstoffen.
- DIN 58940-31 Empfindlichkeitsprüfung von mikrobiellen Krankheitserregern gegen Chemotherapeutika – Teil 31: Ergänzende Verfahren für die Empfindlichkeitsprüfung.

MIC-Strip Short instruction

MIC-Strip

1 strip = 1 test

preparation of the samples

insert the strips in the frame

bacteria

preparation of the inoculum

McFarland 0,5 in 5 ml NaCl

anaerobic bacteria	**gram negative** bacteria	**gram positive** bacteria	**1) fastidious** bacteria (Streptococci, coryneform bacteria, Haemophilus, Neisseria) **2) fastidious** Nonfermenter (CF strains)
1:50 dilution in Wilkins-Chalgren broth with NAD	1:200 dilution in Mueller-Hinton-broth	1:100 dilution in Mueller-Hinton- broth	**1)** 1:50 dilution in H-broth*1 **2)** 1:200 dilution in H-broth

inoculation

transfer 100 µl in each well of the test

covering

cover strips with the cover plate

incubation

incubate at 35 - 37 °C
18- 24 h (aerobic bacteria)
22 -24 h (fastidious bacteria if necessary in enriched CO_2 atmosphere)
24 - 48 h (anaerobic bacteria in anaerobic atmosphere

reading

visual reading and documentation on the evaluation protocol

*1 or in lysed horse blood supplemented Mueller-Hinton-broth

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