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**Table 1: Interpretation of the reactions** (+ = positive, - = negative, +/- = variable)

	Positive reaction	Negative reaction	<i>N. gonorrhoeae</i>	<i>N. meningitidis</i>	<i>N. lactamica</i>	<i>N. species</i>	<i>M. catarrhalis</i>
(A) $\beta$ -lactamase	Red	Yellow	+/-	-	-	+/-	+/-
(B) Glucose	Yellow	Red	+	+	+	+/-	-
(C) Maltose	Yellow	Red	-	+	+	+/-	-
(D) Saccharose	Yellow	Red	-	-	-	+/-	-
(E) $\beta$ -galactosidase	Yellow	White	-	-	+	-	-
(F) $\gamma$ -glutamylaminopeptidase	Yellow <sup>a</sup>	White	-	+	-	+/-	-
(G) Polysaccharase	Black/ brown <sup>b</sup>	Yellow <sup>a</sup>	-	-	-	+ <sup>c</sup>	-
(H) Tributyrinesterase	Yellow	Red	-	-	-	-	+

<sup>a</sup> The color might be pale yellow.

<sup>b</sup> Black/brown: The color might fade rapidly.

<sup>c</sup> *N. cinerea* negative and *N. subflava* variable. Exclusively negative test results are indicative of *N. cinerea*.

# MINIBACT-N

## NEISSERIA IDENTIFICATION KIT

## Intended use

Minibact-N is a ready-to-use kit for identification in 4 hours of pathogenic strains of oxidase-positive, Gram-negative diplococci (*Neisseria* species and *Moraxella catarrhalis*).

## Description

The test is performed in strips of 8 microwells. One kit is sufficient for 12 identifications. The following materials are included in the kit:

- Microtiter strips placed in frame
- Lid for the frame
- An empty frame
- 1 bottle iodine reagent (polysaccharase test)
- 1 bottle nitrocefin reagent ( $\beta$ -lactamase test)

## Principle

The method requires an 18-24 hours pure culture of oxidase-positive, Gram-negative diplococci. Since the technique is based on the detection of preformed enzymes, identification is possible in 4 hours.

Identification is based on the following 8 reactions in row A to H:

- (A)  $\beta$ -lactamase
- (B) Glucose
- (C) Maltose
- (D) Saccharose
- (E)  $\beta$ -galactosidase
- (F)  $\gamma$ -glutamylaminopeptidase
- (G) Polysaccharase
- (H) Tributyrinesterase

The results are read as changes in color and interpreted according to table 1. The results can be read by naked eye.

## Materials required but not provided

- Sterile saline
- McFarland no. 5 (0.05 mL 1% BaCl<sub>2</sub> + 9.95 mL 1% H<sub>2</sub>SO<sub>4</sub>)
- Pipette
- Inoculating loop
- Incubator (35-37°C)
- Whirly mixer

## Procedure

1. Place the required number of microtiter strips in the frame.
2. Be sure the strips are placed correctly:
  - (A)  $\beta$ -lactamase = white disc
  - (H) Tributyrinesterase = red disc
3. Prepare a dense suspension (equal to McFarland no. 5) of an 18-24 hours culture of the strain to be tested in 2 mL sterile saline. It is important that the suspension is homogeneous (stir on a whirly mixer for 10 seconds).
4. Add 200  $\mu$ L suspension to each of the 8 wells.
5. Cover the frame with the lid and incubate at 35-37°C without CO<sub>2</sub> for 4 hours.
6. Read wells B, C, D, E, F and H directly according to table 1.
7. Add 1 drop of nitrocefin reagent to well A and read after 5-10 minutes according to table 1.
8. Add 2-3 drops of iodine reagent to well G and read immediately according to table 1. Please note that the color indicating a positive polysaccharase test may fade quickly but can be restored by further addition of Iodine solution.

## Storage and shelf life

Store the kit between 2-8°C. Expiry date of the kit is printed on the label.

## References

1. Berthelsen, L., Afprøvning af Minibact-N, Nyt om mikrobiologi, nr. 21, 1990.
2. Ison, C. A. et al., Evaluation of a new rapid identification kit for *Neisseria* species and *Moraxella catarrhalis*. ISSTDR-Meeting 1995.
3. Lewis D.A., Identification of *Neisseria gonorrhoeae* from clinical specimens. 11<sup>th</sup> meeting of the International Society for STD Research, New Orleans, Louisiana, USA 1995.

## Information and ordering

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