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Europe +800 135 79 135 US 1 855 2360 910
CA 1 855 805 8539 ROW +31 20 794 7071

NET

REF R211173 25

1. INTENDED USE

Remel NET is a test kit recommended for use in qualitative procedures for rapid, presumptive identification of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Neisseria lactamica*, and *Moraxella catarrhalis*.

2. SUMMARY AND EXPLANATION

In 1978, D'Amato et al. described a method for identification of *N. gonorrhoeae* and *N. meningitidis* using chromogenic substrates to determine enzyme profiles.¹ In further testing, Hoke et al. used a modification of his technique to characterize atypical strains of *N. gonorrhoeae* and *N. meningitidis*.² In 1982, Yajko et al. described a method for identifying *Neisseria* spp. using mixed chromogenic substrates.³

3. PRINCIPLE

NET employs three synthetic chromogenic substrates to detect preformed enzymes. The enzyme β -galactosidase hydrolyzes 5-bromo-4-chloro-indolyl- β -D-galactopyranoside to form a blue colored complex in the presence of oxygen and γ -glutamyl peptidase hydrolyzes γ -glutamyl- p -nitroanilide to form a yellow color. The third enzyme, prolylaminopeptidase, hydrolyzes L-Proline β -naphthylamide which is detected by a color change to pink-red after adding p -dimethylaminocinnamaldehyde.

4. REAGENTS (CLASSICAL FORMULA)

Reactive Ingredients:

5-bromo-4-chloro-indolyl- β -D-galactopyranoside
 γ -glutamyl- p -nitroanilide
L-Proline β -naphthylamide
PRO Reagent: p -dimethylaminocinnamaldehyde
Phosphate Buffered Saline (PBS)

5. PRECAUTIONS

This product is for *in vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

6. STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Do not store in a humid atmosphere or strong light. Allow product to equilibrate to room temperature (20-25°C) before use. A precipitate formed at storage temperatures or a color variation from straw to pink in the PRO reagent is common and does not affect performance.

7. PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of visible condensation in the tube, (2) the expiration date has passed, or (3) there are other signs of deterioration.

8. SPECIMEN COLLECTION, STORAGE, TRANSPORT

Specimens should be collected and handled following recommended guidelines.⁵

9. MATERIALS SUPPLIED

(1) NET tubes, (2) Phosphate Buffered Saline (PBS), (3) PRO Reagent, (4) Instructions for Use (IFU).

10. MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop,

needle, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Oxidase reagent, (7) Gram stain reagents, (8) Microscope slides, (9) Microscope, (10) Applicator sticks.

11. PROCEDURE

NET is intended for use with oxidase-positive, Gram-negative diplococci morphologically characteristic of *N. lactamica*, *N. gonorrhoeae*, or *N. meningitidis*, **only**. Test isolates should be in pure culture and grown on a selective medium such as Thayer-Martin, Martin-Lewis, or New York City Medium.

- The test isolate should be 18-48 hours old.
- Remove the cap from a NET tube and add 3 drops of PBS.
- Emulsify 5-10 colonies of the test isolate in the PBS using an applicator stick.
- Replace the cap on the tube and tap 3-4 times to ensure adequate elution of the substrate in the liquid.
- Incubate in ambient air at 35-37°C for 30 minutes.
- Observe for the formation of a blue or yellow color. (The yellow color may best be viewed by holding the tube against a white background.) **If no color develops, proceed to step 7.**
- Remove the cap and add 1 drop of PRO Reagent to the tube. Tap to mix. Observe for a pink-red color within one minute after adding reagent.

12. INTERPRETATION

Color: Interpretation:

Blue -	Positive for β -galactosidase
Yellow -	Positive for γ -glutamyl-aminopeptidase
Pink-red -	Positive for prolylaminopeptidase
No color or - slight yellow	Negative for all three enzymes

13. EXPECTED VALUES

	Blue	Yellow	Pink-red	No color
<i>Neisseria lactamica</i>	✓			
<i>Neisseria meningitidis</i>		✓		
<i>Neisseria gonorrhoeae</i> ^a			✓	
<i>Moraxella catarrhalis</i> ^b				✓

^a Prolylaminopeptidase-negative strains of *N. gonorrhoeae* have been reported.⁵

^b The identification of *M. catarrhalis* can be confirmed by a positive butyrate esterase test (Catarrhalis Test Disk, REF R21121).

14. QUALITY CONTROL

All lot numbers of NET have been tested using the following quality control organisms and have been found to be acceptable. Testing of a positive and negative control should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
<i>Neisseria lactamica</i> ATCC® 23970	Ambient, 30 min. @ 35-37°C	Blue color
<i>Neisseria meningitidis</i> ATCC® 13090	Ambient, 30 min. @ 35-37°C	Yellow color
<i>Neisseria gonorrhoeae</i> ATCC® 43069	Ambient, 30 min. @ 35-37°C	Pink-red color after adding PRO reagent
<i>Moraxella catarrhalis</i> ATCC® 25240	Ambient, 30 min. @ 35-37°C	Clear or pale- yellow color after adding PRO reagent

15. PERFORMANCE CHARACTERISTICS

In a study by Dillon et al. using the same substrates as NET, the sensitivity and specificity were 99% and 86.6% respectively for *N. gonorrhoeae* and 100% and 94.1% for *N. meningitidis*; the sensitivity for *N. lactamica* was 100%.⁶ In another study of the same product, the sensitivity and specificity was found to be 95% and 100% respectively for *N. gonorrhoeae*.⁷

16. LIMITATIONS

- Only oxidase-positive, gram-negative diplococci known to grow on Thayer Martin or similar selective media should be tested since some saprophytic *Neisseria* produce γ -glutamyl-aminopeptidase and/or prolylaminopeptidase.^{4,5}
- Certain strains of *Kingella* spp., *Neisseria cinerea*, and *Neisseria subflava* may provide reactions consistent with *N. gonorrhoeae*. Test only isolates recovered on selective media or those grown on chocolate agar, that also do not grow on nutrient agar.⁸
- For isolates recovered from extra-genital sites or from children, two confirmatory tests that use distinct identification mechanisms are required.⁸
- Prior to testing with NET, verify by Gram stain that the test isolate is gram-negative diplococci with flattened adjacent sides or gram-negative cocci in clusters. *Kingella* spp. are short gram-negative rods with square ends, lying in pairs or chains, and may grow on selective media. The catalase test may be used to further differentiate *Kingella* (catalase-negative) from *Neisseria* spp. and *M. catarrhalis* (catalase-positive).^{4,8}
- Strains of *N. gonorrhoeae* that are negative for prolylaminopeptidase have been reported. Gram-negative, oxidase-positive diplococci isolated on selective media that are negative for prolylaminopeptidase can not be confirmed as negative for *N. gonorrhoeae* without additional testing.^{5,8}

17. BIBLIOGRAPHY

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18. PACKAGING


REF R211173 25

19. SYMBOL LEGEND

	Catalogue Number
	In Vitro Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
	For Laboratory Use Only
	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

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 12076 Santa Fe Drive, Lenexa, KS 66215, USA