

Instructions for Use

NEISSERIA TEST

Cat. no. K9187	Neisseria Test	26 tablets/bottle
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PRINCIPLES/DISCUSSION

Neisseria spp. produce enzymes which hydrolyze various chromogenic substrates. When these substrates are bound to another base, the hydrolysis will produce a color reaction, some apparent immediately and others by the addition of color developers. Neisseria test tablets, by combining three non-interfering substrates (gamma-glutamyl nitroanalide, bromo-chloro-indol-beta-D galactopyranoside, and proline-naphthylamide) allow organism confirmation from a single tube.

The reaction will be yellow or blue: *Neisseria meningitidis* produces the enzyme gamma-glutamylaminopeptidase which releases the nitroanilide base which will turn the test yellow. *Neisseria lactamica* contains the enzyme beta-galactosidase which will turn the test blue.

The last reaction shows hydrolysis of proline releasing free naphthylamide which turns red after adding PEP Reagent. *Neisseria gonorrhoeae* is the only positive for this test.

SDS

Each tablet consists of approximately 0.5mg each of the applicable substrates with inert fillers and tableting compounds. None of the ingredients are hazardous in this form. Uninoculated tubes may be discarded in normal trash. The developer contains hydrochloric acid, which will stain surfaces and hands, and is corrosive.

STORAGE

Store all materials in the refrigerator between uses. It is not necessary to bring tablets or reagent to room temperature before use.

MATERIALS PROVIDED

Tablets are sold ready to use in test tubes, 26 tubes per bottle with PEP Reagent provided. Usage requires 24 hour growth on Chocolate, Thayer Martin or Martin Lewis media.

MATERIALS REQUIRED BUT NOT PROVIDED

Swabs for harvesting colonies (cotton preferred)
Distilled water, neutral pH

PROCEDURE

1. Add 3 drops of distilled water to the tube containing the tablet.

2. Using a swab harvest 5-10 colonies from fresh 24 hour growth on a plate or slant, mixing thoroughly. It is not necessary for the tablet to dissolve. Leave the swab in the tube during incubation.

Note: A loop or stick may be used for harvesting, however the proline reaction must be incubated for 2 hours in that case.

3. Incubate at 37°C for 30 minutes. Tests may be held as long as 2 hours but not longer as false-positives may occur.

INTERPRETATION

(Read and interpret in the exact sequence listed)

1. If the test is yellow the organism is *Neisseria meningitidis* . Results may be very light yellow or appear to be unchanged. In this case, *Neisseria meningitidis* may be confirmed by adding reagent at step 3.
2. If the test is blue, the organism is *Neisseria lactamica* .
3. If all of the above are negative, perform the aminopeptidase test by adding 1 drop of reagent to the swab and reincubating for 5 minutes. Positive orange/red results indicate the organism is *Neisseria gonorrhoeae* . If the tube was pale yellow before adding reagent (questionable gamma glutamyl) and turns blue or purple at this point, it indicates the organism is *Neisseria meningitidis* which had a positive gamma glutamyl reaction and a positive proline reaction.



Showing *Neisseria meningitidis* (ATCC® 13090) reaction in the Neisseria Test (Cat. no. K9187). Three drops of deionized water were added to the tube containing a tablet. Growth from a 24 hour culture was harvested with a loop and mixed with the reagents. The tube was incubated for two hours. The yellow color development was indicative as positive for *N. meningitidis*.



Showing *Neisseria lactamica* (ATCC® 23970) reaction in the Neisseria Test (Cat. no. K9187). The test was conducted in the same manner as indicated in the photo caption to the left. The blue color development was indicative as positive for *N. lactamica*.



Showing *Neisseria gonorrhoeae* (ATCC® 43069) reaction in the Neisseria Test (Cat. no. K9187). The test was conducted in the same manner as indicated in the photo caption above. At the end of the incubation period, no color reaction had occurred. One drop of developer reagent was added and the tube was reincubated for five minutes. The red color development was indicative as positive for *N. gonorrhoeae*.

LIMITATIONS

Only gram-negative, oxidase-positive diplococci which are able to grow on Thayer Martin or Martin Lewis Agar should be tested since some saprophytic *Neisseria* may be positive for some of the tests. If not using a primary culture, assure that saprophytic *Neisseria* have not grown through on the selective media by transferring the organism to nutrient agar to screen for growth. Saprophytic *Neisseria* will grow on nutrient agar. In the event of medico-legal cases, use another identification method to confirm results in addition to this system. Other useful rapid tests are nitrate discs and butyrate discs. *Moraxella catarrhalis* is butyrate-positive while *Neisseria* spp. are negative. Many of the saprophytic strains of *Neisseria* are nitrate-positive while *Neisseria gonorrhoeae* is nitrate-negative.

REFERENCES

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