NEISSERIA SCREEN

PRINCIPLES/DISCUSSION:

Neisseria sp. 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* screen tablets, by combining three non-interfering substrates (gamma-glutamyl nitroanalide, bromo-chloro-indolyl-B-D galactopyranoside, and proline-naphthylamide) allow organism confirmation from a single tube.

The first 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 only positive for this test. This test is only a screen and is not meant to be used for legal cases.

MSDS / STORAGE

Each tablet consists of approximately 0.5 mgs. each of the applicable substrates with inert fillers and tableting compounds. None of the ingredients is hazardous in this form. Uninoculated tubes may be discarded in normal trash. The developer contains para-dimethyl amino-cinnamaldehyde in a weak hydrochloric acid solution, will stain surfaces and hands, and is mildly corrosive.

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

MATERIALS REQUIRED:

Tablets are sold ready to use in test tubes, 26 tubes per bottle with PEP reagent provided. Usage requires 24 hour growth on Thayer Martin or Martin Lewis media. The following items are also required but not provided:

swabs for harvesting colonies (cotton preferred)
Distilled water,

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 for 30 minutes at 35- 37C. 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 N. meningitidis. Results may be very light yellow or appear to be unchanged. In this case, *N. menigitidis* may be shown by adding reagent at step 3.

2) If the test is blue, the organism is *N. lactamica.*

3) If all of the above are negative, perform the aminopeptidase test by adding 1 drop of reagent to the swab. Positive orange/red results indicate the organism is *N. gonorrhoeae*. If the tube was pale yellow before adding reagent (questionable gamma glutamyl) and turns blue or purple upon addition of the reagent, it indicates the organism is *N. menigitidis* which has a positive gamma glutamyl reaction and a positive proline reaction.

CAUTION; the red color will develop quickly. Read the test within 2 minutes. Waiting longer will allow false positives to develop.

LIMITATIONS:

Only gram-negative, oxidase-positive diplococci which are able to grow on Thayer Martin or Martin Lewis agar should be tested since some saphrophitic *Neisseria,* as well as *K. denitrificans*, may be positive for some of the tests. You may assure that saphrophitic *Neisseria* have not grown through on the selective media by transferring the organism to a nutrient agar to screen for growth. Saphrophitic *Neisseria* will grow on nutrient agar. In the event of legal cases, use another identification method to confirm results in addition to this screen. Other useful rapid tests are KEY nitrate discs and KEY butyrate discs. *Moraxella catarrhalis* is butyrate positive while *Neisseria spp.* are negative. Some of the saphrophitic strains of *Neisseria* and *K. denitrificans* are nitrate positive while *N. gonorrhoeae* is nitrate negative.

References / Footnotes:

1. Chu, A.E., P.K. Chun, D.M. Yajko. 1984, Rapid Identification of Neisseria species, using mixed chromogenic substrates. Abstract of the annual meeting of the American Society for Microbiology N. C257, p. 279.
2. D'Amato, R.F., et al, 1978, Rapid identification of Neissera gonorrhoeoae and Neiserria meningitidis by using enzymatic profiles. J. Clin. Microbiolo. 7:77-81
3. Hoke, C. and N.A. Vedros, 1982. Taxonomy of the Nesseriae: Fatty acid analysis, aminopeptidase activity, and pigment extraction. Int. J. Syst. Bacteriol. 32:51-56.
4. A. Balows, W. Hauser, K. Herrmann, Henry Isenberg, H. J. Shadomy, 1991, Manual of Clinical Microbiology, 4th Ed. American Society for Microbiology, Washington, D.C.
5. Yajko, D.M., A. Chu, and W. K. Hadley, 1984. Rapid confirmatory identification of Neissera gonorrhoeae with lectins and chromogenic substrate. J. Clin. Microbiol. 19:380-382.
6. Dillon, R.L., et al, 1988. Evaluation of eight methods for identification of pathogenic Neisseria species...., J. Clin. Microbiol. 26:493-497a
7. Welborn. P/P/, et al, 1984 Evaluation of Gonocheck II as a Rapid Identificaiton Systme for Pathogenic Neisseria Species. J. Clin. Microbiol. 20:680-683.

QUALITY CONTROL

We recommend that each lot number be tested with organisms of known reactivity. We recommend the following:

ATCC#	DES.	GAMMA-GLU	BGAL	PRO
19424	N. gonorrhoea	-	-	+
13077	N. meningitidis	+	-	v
23970	N. lactamica	-	+	v
25240	B. catarrhalis	-	-	-

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