

Storage

Store **Gonochek-II** desiccated and in the dark at 2-8°C. This product should not be used past the expiration date. Do not use **Reagent Tube** if the substrate is visibly wet.

Specimen Collection and Processing

- Specimens to be tested with **Gonochek-II** must be isolated from Modified Thayer-Martin medium or an equivalent selective media. Do NOT use a non-selective medium, such as chocolate agar, as the primary isolation media. A list of recommended media and their manufacturers is available from EY Laboratories, Inc., Customer Service. (See also LIMITATIONS.)
- A GRAM STAIN, OXIDASE test and CATALASE test MUST be performed on the isolates, prior to testing with **Gonochek-II**. Only oxidase-POSITIVE, catalase-POSITIVE, and gram-NEGATIVE diplococci should be tested with **Gonochek-II**. (See NOTE below.)

Note: *Kingella* species may grow on Modified Thayer-Martin and other selective media. *Kingella* species are, catalase-negative and can be differentiated from catalase-positive *M. catarrhalis* and *Neisseria* species by a catalase test.

- Typical *N. gonorrhoeae* cultures should be tested after 24-48 hours. Atypical strains or AHU auxotypes of *N. gonorrhoeae* grow slower than normally expected. Examine culture plates daily for at least 72 hours.

Procedure

(Refer to Diagram)

Part A

- Allow **Gonochek-II Reagent Tubes** to come to room temperature (20°-28°C) before using.
- Remove paired caps from **Reagent Tube** and dispense 4 drops (approximately 0.2 ml) of phosphate buffered saline into the **Reagent Tube**.
- Remove at least 5-10 fresh medium to large sized colonies of similar morphology using an applicator stick or inoculation loop. Emulsify well into **Reagent Tube**. Recap tube with paired caps.
- Incubate the **Reagent Tube** at 37°C for 30 minutes.
- View for color formation. If solution is BLUE or YELLOW, do NOT continue. Formation of a BLUE color indicates a presumptive POSITIVE result for *N. lactamica*. A YELLOW color indicates a presumptive POSITIVE result for *N. meningitidis*. (See Note below.)

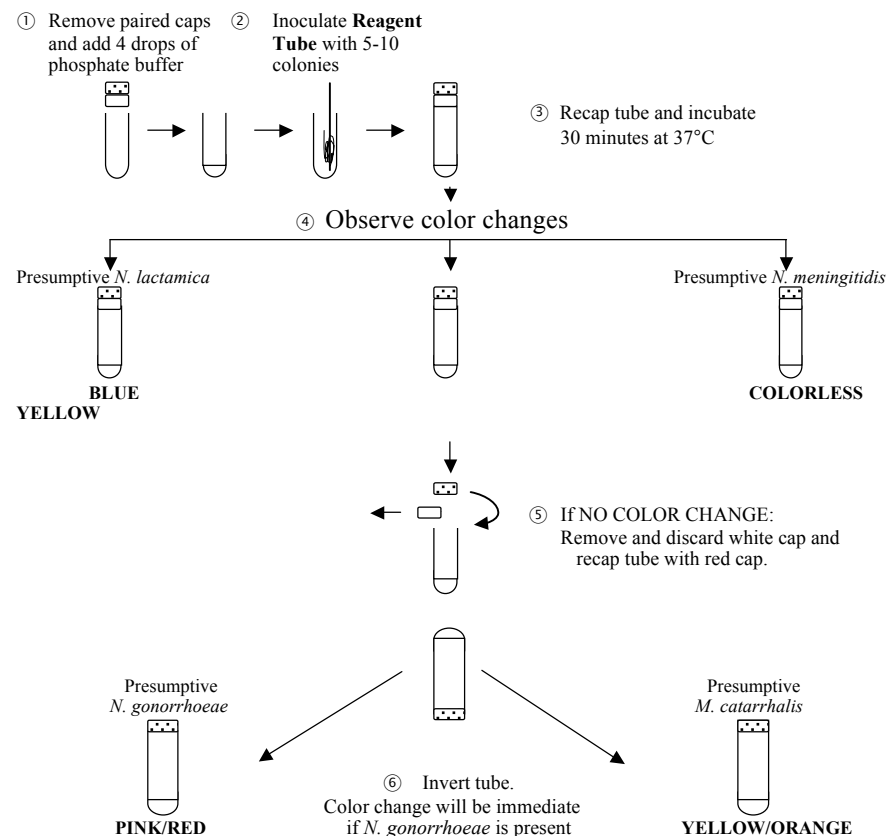
Note: The intensity of the YELLOW color in **Gonochek-II** with *N. meningitidis* may vary with size of inoculum, incubation time and temperature, and age or condition of the culture. ANY yellow color obtained with the sample after 30 minutes incubation at 37°C should be considered a POSITIVE result for the presumptive identification of *N. meningitidis*. If the yellow color is indistinct, extend incubation time to 60 minutes at 37°C, or, repeat the test with a larger inoculum on another **Reagent Tube**.

If there is NO COLOR CHANGE in the solution sample then continue to **Part B**.

Part B

- Remove and discard white cap and recap **Reagent Tube** with red cap only.
- Invert tube and tap it gently several times to allow solution to come into contact with the color developer present on the cap. Return tube to the upright position.
- View for color formation. Formation of a PINK/RED color indicates a presumptive POSITIVE result for *N. gonorrhoeae*. If there is still NO COLOR CHANGE or the solution is yellow/orange, a presumptive positive result for *M. catarrhalis* should be reported. *M. catarrhalis* must be confirmed by another method such as a Butyrate test.

Note: The yellow/orange color which may develop after inversion of **Reagent Tube** with red cap is due to unreacted color developer and not to hydrolysis of the gamma-glutamyl substrate by *N. meningitidis*.



Interpretation of Results

ORGANISM (not supplied)	ATCC #	EXPECTED RESULTS
<i>Neisseria gonorrhoeae</i>	19424	PINK/RED after inverting tube
<i>Neisseria meningitidis</i>	13077	YELLOW color
<i>Neisseria lactamica</i>	23970	BLUE color
<i>Moraxella (Branhamella) catarrhalis</i>	25238	No color change after inverting tube

Limitations of Test

- It has been reported that some strains of *N. gonorrhoeae* may produce a false negative result with **Gonochek-II** when isolated on certain selective media, such as Improved Thayer-Martin. If the isolate was taken from a selective medium and it is an oxidase-positive, catalase-positive, and gram-negative diplococci, and produces a negative result with **Gonochek-II**, then sub-culture onto an enriched media such as chocolate agar and retest with **Gonochek-II**. If the result is again negative for *N. gonorrhoeae*, then the sample should be confirmed by another method such as carbohydrate fermentation. A list of recommended media and their manufactures is available from EY Laboratories, Inc., Customer Service.
- It must be emphasized that only pure cultures with characteristics listed in SPECIMEN COLLECTION should be tested with **Gonochek-II**. The source of the specimen and the clinical symptoms are important. Further biochemical and serological testing is necessary for definitive identification.
- Saprophytic *Neisseria* species such as *N. sicca*, *N. mucosa*, *N. cinera*, etc. may appear on selective media especially with non-urogenital specimens after 26-48 hours of incubation. Since these species possess gamma-glutamyl-aminopeptidase and proliminopeptidase, they may

generate erroneous or misleading results with **Gonochek-II**. If a saprophytic *Neisseria* species is suspected from, for example, a throat or another non-urogenital specimen, then colonies should be subcultured onto a nutrient media, such as Trypticase Soya Agar. *N. gonorrhoeae* and *N. meningitidis* will not grow on nutrient media when incubated at 35°-37°C overnight without carbon dioxide supplementation.

- Published literature has reported that *Moraxella (Branhamella) catarrhalis* may not produce sufficient enzyme to be detected by the three enzymes utilized in the **Gonochek-II Reagent Tube**. False positive reactions may occur when assaying some isolates with prolyliminopeptidase and γ -glutamylaminopeptidase.

Performance Characteristics

In a study by Dillon, *et. al.*, of **Gonochek-II**, the sensitivity and specificity was 99% and 86.6% respectively for *Neisseria gonorrhoeae* and 100% and 91.4% for *N. meningitidis*; the sensitivity for *N. lactamica* was 100%. In another study, the sensitivity and specificity was 95% and 100% respectively for *N. gonorrhoeae*.

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Gonochek®-II Reagent Tube

Neisseria Species Differentiation Test
Cat. No.: 13-003-25

Intended Use

Gonochek-II is intended for the use in the detection of prolyliminopeptidase, gamma-glutamylaminopeptidase, and beta-galactosidase from colonies grown on selective media, to aid in the presumptive identification of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Neisseria lactamica* and *Moraxella (Branhamella) catarrhalis*.

Description

Gonochek-II consists of a reagent tube which contains three chromogenic substrates for use in the detection of three preformed enzymes, prolyliminopeptidase, gamma-glutamyl-aminopeptidase, and beta-galactosidase. These three enzymes have been shown to be accurate in identifying *N. gonorrhoeae*, *N. meningitidis* and *N. lactamica* respectively. The red cap of the **Reagent Tube** contains a color developer (diaz dye).

Chemical Principle

Each of the three chromogenic substrates contained in **Gonochek-II Reagent Tubes** produces a distinct color upon hydrolysis. Hydrolysis of 5-bromo-4-chloro-3-indolyl- β -galactoside by beta-galactosidase produces a BLUE color which signifies a presumptive positive result for *N. lactamica*. The gamma-glutamyl-aminopeptidase substrate hydrolysis yields a YELLOW color, which is a positive result for *N. meningitidis*. The hydrolysis of, L-prolyl-4-methoxynaphthylamide by prolyliminopeptidase, releases a free beta-naphthylamine derivative which complexes with the diazo dye (color developer) present on the red cap of the **Gonochek-II Reagent Tube**, to produce a PINK/RED color which is a presumptive positive result for *N. gonorrhoeae*.

Materials Supplied

25 Reagent Tubes and Reagent Caps	5-Bromo-4-chloro-3-indolyl- β -D-galactoside Gamma-glutamyl-para-nitroanilide L-Prolyl-4-methoxynaphthylamide Fast Garnet	50 µg/tube 50 µg/tube 50 µg/tube 10 µg/cap
25 Wooden Applicator Sticks		
1 Material Safety Data Sheet (MSDS)		

Materials needed but not Supplied

Gram stain reagents	10 mM Phosphate Buffered Saline pH 7.4 (Preservative free)
Swabzyme® Oxidase or oxidase test	8.5 mM Sodium phosphate (Dibasic)
Catalase test	1.5 mM Sodium phosphate (Monobasic)
	150 mM Sodium chloride

NOTE: Use only preservative-free phosphate buffered saline with **Gonochek-II Reagent Tubes**, preservatives may interfere with enzymatic reactions.

Recommended Quality Control Organisms and Expected Results

Good laboratory practices include the use of control specimens to ensure proper kit performance. Positive and negative organisms should be tested according to the laboratory's established Quality Control program.

Organism (not supplied)	ATCC #	Expected Results
<i>Neisseria gonorrhoeae</i>	19424	PINK/RED after inverting tube
<i>Neisseria meningitidis</i>	13077	YELLOW color
<i>Neisseria lactamica</i>	23970	BLUE color
<i>Moraxella (Branhamella) catarrhalis</i>	25238	No color change

Precautions

Gonochek-II is intended for *IN VITRO* DIAGNOSTIC USE only and should be used by properly trained, qualified laboratory personnel. Normal precautions should be taken against dangers of microbial hazards. Sterilization of all materials used during testing is recommended. The active ingredient in the red reagent cap, Fast Garnet, is a suspected carcinogen. Avoid contact with skin. Refer to enclosed Material Safety Data Sheet for further information. DO NOT use **Reagent Tube** if substrate is visibly wet.

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