GONOGEN II

For Culture Confirmation of
Neisseria gonorrhoeae

GonoGen II: 89-102024 / 24 determination kit
89-102040 / 40 determination kit

In Vitro Diagnostic Use Only

Ver. 4 / May 2007

New Horizons

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INTENDED USE

GONOGEN II is a monoclonal antibody-based colorimetric test intended for the confirmatory identification of *Neisseria gonorrhoeae.*

GENERAL BACKGROUND

Infection with *Neisseria gonorrhoeae* requires different treatment than infections with other Neisseria species. Infections with non-pathogenic Neisseria such as *N. flava, N. sicca,* or *N. subflava* usually requires no treatment, whereas infections with pathogens such as *N. meningitides* may require different antibiotic therapy than *Neisseria gonorrhoeae.* In order to differentiate *N. gonorrhoeae* from other Neisseria species it is necessary to perform a confirmatory test. The sugar utilization test is the standard reference method, but it requires prolonged incubation and pure, viable isolates before unequivocal results can be obtained. The GONOGEN II assay for the confirmation of *N. gonorrhoeae,* on the other hand, is much more rapid than sugar utilization tests, and does not require pure or viable isolates.

PRINCIPLE

The GONOGEN II test kit for *Neisseria gonorrhoeae* is comprised of a specific anti-gonococcal reagent. The specific anti-gonococcal reagent is composed of a pool of murine monoclonal antibodies (IgG) that have been prepared against a purified outer membrane protein, Protein I, of *Neisseria gonorrhoeae.* Protein I is a major protein molecule that is exposed on the surface of the gonococcus and its epitopes are largely responsible for serotype specific reactions of the gonococcus (1, 2, 3). By including monoclonal antibodies to the various serotypes of *N. gonorrhoeae,* maximum specificity and sensitivity are achieved. These antibodies are adsorbed to suspended metal sol particles, which give the reagent its raspberry red color.

When a culture of *N. gonorrhoeae* is suspended in the solubilizing buffer, the outer membrane is stripped from the organism releasing Protein I containing complexes into solution, enabling these complexes to be bound by the antibody-sol particles. When the solution is then passed through the special matrix test device, the Protein I-sol particle complexes bind to the matrix resulting in a color change. Sol particles which have not bound Protein I will yield a negative test (white to pale pink spot).

MATERIALS PROVIDED
Each kit contains enough reagents to perform the listed number of determinations.

GONOGEN II Reagent: Consists of murine monoclonal antibodies to the Protein I antigens of *Neisseria gonorrhoeae* that have been adsorbed to metal sol particles. Contains 0.05% sodium azide.

SOLUBILIZING BUFFER – Organisms from a suspected isolate of *Neisseria gonorrhoeae* are suspended in this buffer prior to addition of GONOGEN II reagent. Contains 0.05% sodium azide.

POSITIVE CONTROL REAGENT – consists of a heat-killed *Neisseria gonorrhoeae*. When this is mixed with the GONOGEN II reagent and applied to a well on the Test Tray, a positive reaction is visible as a red dot. Contains 0.05% sodium azide.

NEGATIVE CONTROL REAGENTS – consists of a heat-killed *Neisseria* species other than *N. gonorrhoeae*. When this is mixed with the GONOGEN II reagent and applied to a well on the test Tray, no reaction will be visible. Contains 0.05% sodium azide.

TEST TRAY – consists of wells with a special matrix and absorbent material. When the GONOGEN II sample reactant is added, a positive (red dot) or negative (white to pale pink dot) result is observed on the matrix.

DROPPERS – for delivering controlled volumes of the test organism suspensions.

MATERIALS NOT PROVIDED
1. Test tubes (12 x 75 mm)
2. Test tube rack
3. Cotton/Dacron swab or loop
4. #1 MacFarland turbidity standard

PRECAUTIONS
This product is for In Vitro diagnostic use only.
All reagent vials should be allowed to warm to room temperature and vigorously shaken or vortexed for 10 seconds prior to use.

SHELF LIFE AND STORAGE
The expiration date and storage temperature is indicated on the outer package label.
CAUTION: DO NOT FREEZE!

WARNING
All reagents contain 0.05% sodium azide. Sodium azide may react with lead and copper plumbing to form a highly explosive metal azide. On disposal, flush liberally with water.

Do Not Use Calcium Alginate Swabs to transfer suspected organism from the culture media to the buffer.

The solubilizing buffer has been found to inactivate microorganisms. However, no known test can guarantee 100% inactivation. Therefore, observe established precautions against microbiological hazards when performing this procedure and during disposal of reagents/tests.

SAMPLE PREPARATION
Clinical samples should be processed as quickly as possible. Specimens should be grown on selective, enriched media such as Modified Thayer-Martin or Martin-Lewis media to ensure growth of isolates. Inoculated media should be incubated at 35-37°C in a humid 5% CO_2 atmosphere for 18-48 hours. Suspected organisms are inspected for typical colonial morphology, Gram stain appearance and oxidase reactivity.

SPECIMEN
Colonies that have grown on selective or enriched plated media which are oxidase positive and appear as Gram negative diplococci and can be considered to be presumptively identified as *Neisseria* species. These are then tested to confirm them as *Neisseria gonorrhoeae* with the GONOGEN II test kit.

If there is sufficient presumptive growth, the primary culture may be used to perform the GONOGEN II test. Whether subcultures or primary subcultures are used for testing, viable cultures are not needed for testing. Any cultures incubated within 18-48 hours can be tested with equal confidence.

QUALITY CONTROL
Materials:

1. Positive Control Reagent
2. Negative Control Reagent
3. Solubilizing Buffer
4. GONOGEN II Reagent
5. Test Tray

Frequency:

Controls should be tested each day the GONOGEN II is used for patients to ensure the system is functioning properly. The controls may be run along with the test specimens.

QC Procedure:

The Positive and Negative Control are run each day a test is used.

1. Label a small test tube (12x75mm) for Positive Control (+).
2. Label a small test tube (12x75mm) for Negative Control (-).
3. Dispense 500µL of buffer into each of these + and – tubes.
4. Add 1 drop of well-mixed Positive Control into the tube marked Positive.
5. Add 1 drop of well-mixed Negative Control into the tube marked Negative.
6. Mix well by shaking vigorously.
7. Add 1 drop GONOGEN II Reagent into the Positive Control tube and 1 drop into the Negative Control tube.
8. Mix and wait at least 5 minutes.
9. Add 2 drops of the Positive Control into a well in the Test Tray.
10. Add 2 drops of the Negative Control into a separate well in the Test Tray.
11. Using a clean, plastic dropper, add 1 drop of buffer to each completed test well.
12. Read Reactions:

Positive: Dark Pink to Red spot in Test Tray well

Negative: White to Pale Pink spot in Test Tray well

CORRECTIVE ACTION:

If controls do not react as expected, do not use the Kit to test patient specimens. Please call New Horizons Diagnostics Corporation at (410) 992-9357 and ask for Technical Services or fax to (410) 992-0328.

LIMITATIONS OF THE PROCEDURE

No single diagnostic test result should be considered conclusive in diagnosing disease. The overall clinical and laboratory findings should be taken into consideration before a physician renders a diagnosis. Depending upon exposed antigenic sites and antigenic composition, some gonococci may not be identifiable with GONOGEN II Reagent and others may vary in color intensity. In the rare case of extremely weak or non-specific reaction with GONOGEN II, confirmation by other methods, such as carbohydrate utilization may be necessary.

PERFORMANCE CHARACTERISTICS

	TOTAL SAMPLES	CULTURE	GONOGEN II
Positive	130	130	127
Negative	60	60	63
Sensitivity			98%
Specificity			100%
Positive Predictive Value			100%
Negative Predictive Value			95%

The following organisms have been tested and found to be negative for GONOGEN II:
Lactobacillus casei, Proteus mirabilis, Klebsiella oxytoca, Citrobacter friend II, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus (2 strains), *Flavobacterium spp., Streptococcus faecalis, Alkaligenes spp., Moraxella spp., Neisseria meningitides* (24 strains), *Neisseria animalis, Neisseria canis, Neisseria caviae, Neisseria cineria, Neisseria cuniculi, Neisseria denitrificans, Neisseria elongata, Neisseria elongata subsp. glycolytica. Neisseria flava, Neisseria flavescens, Neisseria lactamica* (4 strains), *Neisseria mucosa, Neisseria ovis, Neisseria perflava, Neisseria sicca, Neisseria subflava, Branhamella catarrhalis, Kingella denitrificans,* and *Kingella kingelli.*

PRODUCT WARRANTY

NHD guarantees the quality of its reagents if used and stored as recommended. Any reagents found to be defective will be replaced free of charge upon return of the product. NHD disclaims any implied warranty of merchantability or fitness for a particular purpose, and in no event shall NHD be liable for consequential damage.

TEST PROCEDURE:

1. Allow all reagents to warm to Room Temperature.
2. Label a test tube (12x75mm) for each specimen.
3. Using the provided rubber bulb eye dropper, dispense 500 uL of buffer into each tube (up to scored mark).
4. a.*If using a cotton/Dacron swab, make a suspension of test colonies (approximately 30 colonies) to match a **#1 MacFarland turbidity standard (barely visible turbidity). Turbidity standard is critical, DO NOT EXCEED #1 MacFarland.**

   b. **If using an inoculating loop to remove bacteria, dispense 300 uL of solubilizing buffer into a test tube, suspend bacteria to a **#1 MacFarland turbidity reference**, vortex and perform the test with Step 7.
5. Press swab against inside of tube to express as much liquid as possible.
6. Discard the swab in disinfectant or appropriate biohazard container.
7. **Vigorously shake or vortex the GONOGEN II Reagent.**
8. Add 1 drop of GGE II Reagent into each of the tubes to be tested.
9. Mix well.
10. Allow tubes to sit for at least 5-15 minutes. Longer incubation time increases clarity of the reaction.. With very sticky samples wait at least 15 minutes.
11. Using provided dropper, add 2 drops of each specimen suspension into separate wells of the Test Tray.
12. Using a clean plastic dropper, add one drop of buffer to each completed test well.
13. INTERPRET RESULTS:

    Positive for *N. gonorrhoeae*: Dark Pink to Red dot in Test Tray well

    Negative for *N gonorrhoeae:* White or very light pink dot in well.

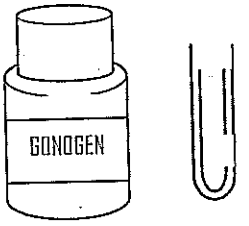
* If color reaction is questionable, incubate 5 additional minutes and repeat test.

CAUTION: **If specimen suspension is made too turbid, a faint background color will occur. Do not use greater than #1 MacFarland turbidity. Turbidity should not be interpreted as a Positive Reaction.

14. Properly dispose of all materials used.

NOTE: If all 8 wells of the Test Tray are not used during a given test period, the unused wells can be used at a later time. Reacted test trays may be saved as a permanent record.

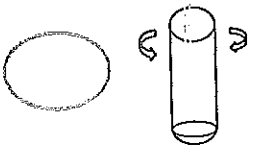
SIMPLE PROCEDURE



1

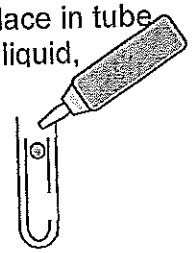
1. Dispense 500 uL Buffer.

2



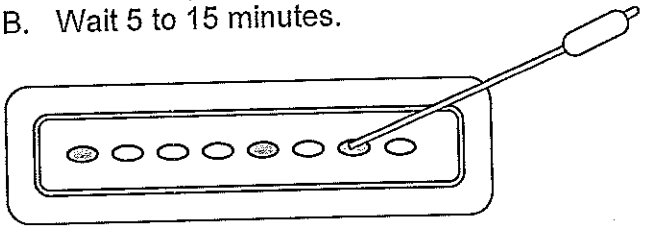
2.A. Swab plate & place in tube.
2.B. Roll to express liquid, hen discard swab.

3



3.A. Add 1 drop GONOGEN II Reagent to tube.
3.B. Wait 5 to 15 minutes.

4



4.A. Dispense 2 drops suspension in Reaction Tray Well.
4.B. Add 1 drop of Buffer
4.C. RESULTS: **Positive:** Dark Pink to Red
Negative: Clear to pale pink