

# Laboratory Procedure

## GonoGen™ II

### I. INTENDED USE

**GonoGen™ II** is a monoclonal antibody-based colorimetric test intended for the confirmatory identification of *Neisseria gonorrhoeae* from culture.

### II. SUMMARY AND EXPLANATION

Infection with *N. gonorrhoeae* requires different treatment than infections with other *Neisseria* species. Infections with nonpathogenic *Neisseria*, such as *N. flava*, *N. sicca* or *N. subflava*, usually require no treatment, whereas infections with pathogens such as *N. meningitidis* may require different antibiotic therapy than that for *N. 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 requires prolonged incubation and pure, viable isolates before unequivocal results can be obtained. The **GonoGen™ II** assay for the confirmation of *N. gonorrhoeae* is more rapid than sugar utilization tests and does not require pure or viable isolates.

### III. PRINCIPLE OF THE PROCEDURE

The **GonoGen™ II** test for *N. 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 *N. 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.<sup>1-3</sup> By including monoclonal antibodies to the various serotypes of *N. gonorrhoeae*, maximum specificity and sensitivity are achieved. These antibodies are absorbed 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).

### IV. REAGENTS

**Reagent 1,** **GonoGen II** solubilizing buffer. Organisms from a suspected isolate of *N. gonorrhoeae* are suspended in this buffer prior to addition of **GonoGen II** reagent. Contains 0.09% sodium azide (preservative).

**Reagent 2,** **GonoGen II** murine monoclonal antibodies to the Protein I antigens of *N. gonorrhoeae* that have been absorbed to metal sol particles. Contains 0.05% sodium azide (preservative).

**Control +,** **GonoGen II** heat-killed *N. gonorrhoeae*. When mixed with the **GonoGen II** reagent and applied to a well on the reaction device, a positive reaction is visible as a pink to red dot. Contains 0.05% sodium azide and 0.01% gentamicin sulfate (preservatives).

**Control -,** **GonoGen II** heat-killed *Neisseria* species other than *N. gonorrhoeae*. When 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 and 0.01% gentamicin sulfate (preservatives).

**Test Tray:** Consists of well with a special matrix and absorbent material. When the **GonoGen II** sample reactant is added, a positive (pink to red dot) or negative (white to pale pink dot) result is observed on the matrix.

**Precautions:** For *in vitro* Diagnostic Use.  
This product contains dry natural rubber.

**Reagents:** Do not use beyond the expiration date. Upon removal from the refrigerator, allow reagents to warm to room temperature before use. Do NOT mix reagents from different kit lot numbers. Prior to use reagents should be vigorously shaken or vortexed for 10 sec.

**Warning:** Reagents contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.

**Controls:** Do not use the kit if positive and negative controls do not yield appropriate results.

To assure proper drop delivery, the glass dropper rod assembly, plastic transfer pipet and reagent bottles should be held vertically, dispensing free-falling drops.

**Storage of Reagents:** Upon receipt, refrigerate reagents at 2 to 8°C. DO NOT FREEZE.

Reagents should be recapped and returned to refrigeration when not in use, taking care not to mix color-coded caps.

Pathogenic microorganisms may be present in specimens. “Universal Precautions” should be followed in handling all items.

## V. SAMPLE PREPARATION

Clinical samples should be processed as quickly as possible. Specimens should be grown on selective, enriched media such as **GC-Lect™**, modified Thayer-Martin or Martin - Lewis media to ensure growth of isolates. Inoculated media should be incubated at 35 to 37°C in a humid 3 to 10% CO<sub>2</sub> atmosphere for 18 to 48 h. Suspected organisms are inspected for typical colonial morphology, Gram stain appearance, and oxidase reaction (**BBL™** Oxidase Reagent Droppers, see “Availability”).

## VI. SPECIMEN COLLECTION

Do not use calcium alginate swabs to transfer suspected organisms from the culture media to the buffer.

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

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 to 48 h can be tested with equal confidence.

## VII. PROCEDURE

<b>Materials Provided:</b>	<b>#244002 (16 Det.)</b>	<b>#244003 (24 Det.)</b>	<b>#244004 (40 Det.)</b>	<b>#244005 (96 Det.)</b>
<b>Reagent 1, GonoGen II Buffer</b>	27 mL	27 mL	27 mL	2 x 27 mL
<b>Reagent 2 GonoGen II Reagent</b>	1 mL	1 mL	2 mL	2 x 2 mL
<b>Control +, GonoGen II Positive QC</b>	1 mL	1 mL	2 mL	2 mL
<b>Control -, GonoGen II Negative QC</b>	1 mL	1 mL	2 mL	2 mL
Test Tray	2	3	5	12

and accessories.

**Materials Required But Not Provided:** Test tubes (12 x 75 mm), test tube rack, cotton/polyester swab or loop, McFarland No. 1 turbidity standard.

Also required are the necessary equipment and labware used for preparation, storage and handling of clinical specimens.

**Performance of Test:** Review “Precautions,” “Sample Preparation” and “Specimen Collection” prior to performing procedures. The testing area, reagents and test components should be at room temperature when used.

1. Label a test tube (12 x 75 mm) for each specimen.
2. Using the glass dropper rod assembly provided, dispense 500 µL (demarcation line) of **Reagent 1** into each tube.
3. Using a cotton/polyester swab, make a suspension of test colonies (approximately 30 colonies) to match a McFarland No. 1 turbidity standard (barely visible turbidity).

**NOTE: When using an inoculating loop to remove bacteria, dispense 300 µL of Reagent 1 into a test tube, suspend bacteria to a McFarland No. 1 turbidity reference, vortex and perform the test as described, starting with step 6.**

4. Press the swab against the inside of the tube to express as much liquid as possible.
5. Discard the swab in disinfectant or appropriate biohazard container.
6. Vigorously shake or vortex **Reagent 2**, and add 1 drop into each of the tubes to be tested.
7. Mix well.
8. Allow tubes to set for at least 5 min. Longer incubation time increases clarity of the reaction.
9. With a plastic transfer pipet, add 2 drops of each test suspension to be tested into a well of the test tray, using a separate well for each test.
10. Using a clean plastic transfer pipet, add 1 drop of **Reagent 1** to each completed test well.
11. Interpret results: Pink to red dot in well of test tray = *N. gonorrhoeae*.  
White to pale pink dot in well of test tray - NOT *N. gonorrhoeae*.

NOTE: A color reaction more intense than the negative control should be interpreted as positive. If the color reaction is questionable, reincubate tube at room temperature 3 min and repeat test.

CAUTION: If specimen suspension is made too turbid, a faint background color will occur. This should not be interpreted as a positive reaction.

12. Properly dispose of all materials used.

NOTE: If all eight 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.

**User Quality Control:** Controls should be tested each day the **GonoGen II** test is performed for patients, to ensure the system is functioning properly. The controls may be performed along with the test specimen.

The **Control +** (Positive) and **Control -** (Negative) should be performed each day a test is used.

1. Label a small test tube (12 x 75 mm) with **Control +**.
2. Label a small test tube (12 x 75 mm) with **Control -**.
3. Dispense 500 µL of **Reagent 1** into each of these tubes.
4. Add 1 drop of well-mixed **Control +** into the tube marked **Control +**.
5. Add 1 drop of well-mixed **Control -** into the tube marked **Control -**.
6. Mix well by shaking vigorously.
7. Add 1 drop of **Reagent 2** into the **Control +** tube, and 1 drop into the **Control -** tube.
8. Mix and wait at least 5 min.
9. Add 2 drops of the **Control +** into a well in the test tray.

10. Add 2 drops of the **Control** - into a separate well in the test tray.
11. Using a clean plastic transfer pipet, add 1 drop of **Reagent 1** to each completed test well.

**Reading Results:**

Pink to red spot in the test well = **Positive**.

White to pale pink in test tray well = **Negative**.

**VIII. 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 an extremely weak or nonspecific reaction with **GonoGen™ II**, confirmation by other methods, such as carbohydrate utilization, may be necessary.

**IX. PERFORMANCE CHARACTERISTICS**

		<b>GonoGen II</b>	
		Positive	Negative
Culture	Positive	127	3
	Negative	0	60

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 freundii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (2 strains), *Flavobacterium* spp., *Enterococcus faecalis*, *Alkaligenes* spp., *Moraxella* spp., *Neisseria meningitidis* (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 kingae*.

**X. AVAILABILITY**

<b>Cat. No.</b>	<b>Description</b>
244002	<b>GonoGen™ II</b> , 16 Determinations Test Kit.
244003	<b>GonoGen™ II</b> , 24 Determinations Test Kit.

244004                    **GonoGen™II**, 40 Determinations Test Kit.  
244005                    **GonoGen™ II**, 96 Determinations Test Kit.  
261181                    **BBL®** Oxidase Reagent Droppers, Box of 50.

**XI.    REFERENCES**

1.        Buchanan, T. M., and J. F. Hildebrandt. 1981. Antigen-specific serotyping of *Neisseria gonorrhoeae*: characterization based upon principal outer membrane protein. *Infect. Immun.* 2:985-994.
2.        Sandstrom, E. G., J. S. Knapp, and T. M. Buchanan. 1982. Serology of *Neisseria gonorrhoeae*: W-antigen serogrouping by coagglutination and protein I serotyping by enzyme-linked immunosorbent assay both detect protein I antigens. *Infect. Immun.* 35:229-239.
3.        Sandstrom, E. G., K. C. S. Chen, and T. M. Buchanan. 1982. Serology of *Neisseria gonorrhoeae*: coagglutination serogroups WI and WII/WIII correspond to different outer membrane protein I molecules. *Infect. Immun.* 38:462-470.

**XII.   TECHNICAL INFORMATION:** In the United States, telephone BD Diagnostic Systems Technical Services, toll free (800) 638-8663.

Approved by:

Supervisor: \_\_\_\_\_ Date: \_\_\_\_\_

Director: \_\_\_\_\_ Date: \_\_\_\_\_

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