RapID NH		
Doc #	**Section: Microbiology**	**Effective Date: 9/1/2022**

Scope: This policy applies to UPMC Hanover.

Keywords: Rapid NH, Neisseria i.d., Haemophilus i.d., Moraxella i.d. Eikenella i.d.

Purpose: RapID NH system is a qualitative micromethod using conventional and chromogenic substrates for the identification of medically important species of *Neisseria, Haemophilus,* and other bacteria isolated from human clinical specimens. A complete list of the organisms addressed by the RapID NH system is provided in the differential chart provided. The RapID NH system has been designed to definitively identify *N. gonorrhoeae, N. menigitidis,* and *Moraxella catarrhalis* and to differentiate these organisms from other species of *Neisseria, Moraxella,* and *Kingella*. It will also identify and differentiate *Haemophilus* sp. As well as biochemically type *H. influenzae* and *Haemophilus parainfluenzae.*

Equipment: RapID inoculation fluid (sold separately, stored at room temperature), RapID Nitrate A reagent (sold separately, store refrigerated and bring to room temperature before use), RapID Nitrate B reagent (sold separately, store refrigerated and bring to room temperature before use), RapID spot indole reagent (sold separately, store refrigerated and bring to room temperature before use), Oxidase reagent, swab, pipet, incubator, ERIC (electronic RapID compendium).

Quality Control: Performed upon receipt of new lot/shipment.

Control organisms: *Haemophilus influenzae* ATCC 9006, *Aggregatibacter aphrophilus* ATCC 7901, *Aggregatibacter aphrophilus* ATCC 49146, *Oligella urethralis* ATCC 17960, *Moraxella catarrhalis* ATCC 8176.

Specimen: Cultures should be 18-24 hours old. Slower growing isolates can be up to 48 hours old. Isolates can be tested from BAP, CHOC, Thayer-Martin and Martin-Lewis agar. Isolates are to be Gram stained and have oxidase performed prior to use in the system.

Procedure: There are two alternative procedures for the RapID NH system: the 1-hour procedure and the general procedure. The 1-hour procedure is only applicable to suspected gonococci obtained from urogenital specimens isolated on selective agars. The general procedure should be used for Neisseriaceae from all other body sites and isolated on all other media. *Haemophilus* and other bacteria should be tested using the general procedure.

1. Using a cotton swab or loop, suspend sufficient growth of pure culture in RapID inoculation fluid to achieve a turbidity equal to #3 McFarland standard. Mix thoroughly and use within 15 minutes of preparation.

2. Peel back the lid of the panel over the inoculation port by pulling the tab marked ‘peel to inoculate’.
3. Using a pipet, transfer the entire contents of the inoculation fluid tube into the upper right-hand corner of the panel. Reseal the inoculation port of the panel by pressing the peel back tab back in place.
4. While keeping the panel on a level surface, tilt the panel back away from the test cavities at a 45 ° angle.
5. While tilted back, gently rock the panel from side to side to evenly distribute the inoculum along the rear baffles.
6. While maintaining a level horizontal position, slowly tilt the panel forward toward the reaction cavities until the inoculum flows along the baffles into the reaction cavities. This should evacuate all of the inoculum from the rear portion of the panel.
7. Return the panel to a level position and gently tap to remove any air trapped in the cavities. Examine the test cavities which should appear bubble-free and uniformly filled.
8. When using the 1-hour procedure, incubate inoculated panels at 35-37^{o} C in non-CO_2 incubator for 1 hour. When using the general procedure, incubate for 4 hours. Incubation trays are provided with the kit for ease of handling.
9. After appropriate incubation period, remove panel from incubator. While firmly holding the panel on the benchtop, peel off the label lid over the reaction cavities by pulling the lower right-hand tab up and to the left.
10. Without the addition of any reagents, read and score cavities 1 through 10 from left to right using the interpretation guide provided. Record test scores in the appropriate boxes on the report form using the test code above the bar for bifunctional tests.
11. Add the following reagents to the cavities indicated:
    - Add 2 drops of RapID Nitrate A reagent to cavity 9.
    - Add 2 drops of RapID Nitrate B reagent to cavity 9.
    - Add 2 drops of RapID spot indole reagent to cavity 10.
12. Allow at least 1 minute but no more than 5 minutes for color development. Read and score cavities 9 and 10. Record the scores in the appropriate boxes of the report form using the test codes below the bar for bifunctional tests.
13. If the PRO test (cavity 1) is the only positive test and the isolate is a Gram-negative coccus (*Neisseria* sp.), perform a nitrate test in cavity 8 by adding 2 drops each of RapID Nitrate A and B reagents. Allow a full five-minute incubation for negative tests before scoring as positive.
14. Reference the microcode obtained on the report form in ERIC for the identification.

Interpretation of Results: Identifications are made using individual test scores form RapID NH panels in conjunction with other laboratory information (Gram stain, oxidase, growth on differential or selective media) to produce a pattern that statistically resembles known reactivity for taxa recorded in the RapID NH system database. These patterns are compared through the use of RapID NH differential chart or by derivation of a microcode and the use of ERIC.

Limitations: Specimen source, oxidase reaction, Gram stain characteristics, and growth on selective agars should be considered when using RapID NH system. It must be used with pure cultures of test organisms. It is designed for use with the taxa listed in the RapID NH differential chart. The us of organisms not specifically listed may lead to misidentifications. PRO-negative strains of *N. gonorrhoeae* have been reported. When referenced in ERIC, a microcode derived from PRO-negative *N. gonorrhoeae* will result in a probability overlap condition with *Kingella kingae*. However, such an overlap carries significant probability of *N. gonorrhoeae* as the first choice. Further testing is necessary to resolve the overlap condition. The 30% hydrogen peroxide test can be used to differentiate *N. gonorrhoeae* (positive), and *K. kingae* (negative). GGT negative strains of *N. meningitidis* have been reported. If suspected, additional testing such as carbohydrate acidification (maltose and glucose) is required to definitively identify PRO-positive, GGT-negative isolates that are otherwise characteristic of *N. meningitidis* or *N. gonorrhoeae.*

References: Remel RapID NH system package insert. Nov. 2021, Remel Inc., Santa Fe Trail Dr., Lenexa, KS, 66215, USA

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