CarboFerm Neisseria Kit Procedure

I. Principle

The majority of *Neisseria* species are considered normal flora of mucus membranes on humans. *Neisseria gonorrhoeae* is a common sexually-transmitted pathogen, and *Neisseria meningitidis* is often associated with meningitis as well as colonization of the nasopharynx. *Moraxella catarrhalis* can colonize the upper respiratory tract but may be associated with lower respiratory tract infections, typically in elderly populations or those with underlying pulmonary disease. *M. catarrhalis* may also be associated with otitis media and sinusitis, especially in children.

The CarboFerm Neisseria Kit is a rapid test for the identification of *Neisseria* species and *M. catarrhalis*. CarboFerm uses an acidimetric method for detection of carbohydrate utilization and butyrate esterase to differentiate and identify *Neisseria* species and *M. catarrhalis*. Each test strip consists of six microcupules that are rehydrated with a bacterial suspension prepared in the inoculation buffer provided. The carbohydrates included in the test strip are glucose, maltose, lactose, and sucrose. The carbohydrate and control wells contain the pH indicator phenol red. Organisms that utilize carbohydrates will produce an acid environment that exceeds the buffering capacity, resulting in a color change of the phenol red indicator. Organisms that possess butyrate esterase will hydrolyze the substrate Bromo-Chloro-Indolyl-Butyrate to produce a blue byproduct.

II. Specimen Information

Test isolates should be oxidase-positive, gram-negative diplococci. Inoculum may be taken from 24 – 48 h old cultures growing on Chocolate or Modified Thayer Martin agar. Sufficient inoculum is required to make a test suspension equal to a 4.0 McFarland. Care should be taken to ensure purity of the inoculum. If necessary, subcultures should be made to obtain additional growth in pure culture before using the CarboFerm test strip.

This test system may also be used for identifying fastidious gram-negative bacilli such as the HACEK organisms. However, there is limited data on the performance of these organisms in the CarboFerm kit. Any clinical isolates should be brought up for review during rounds and evaluated with consultation of the Microbiology director, supervisor, and/or the technical specialist.

III. Reagents & Equipment

A. Pure 24 – 48 h culture of test isolate growing on Chocolate or MTM agar
B. CarboFerm test strip (store at 2 – 8 °C protected from light)

C. Inoculation buffer
D. 4.0 McFarland standard
E. Sterile swabs
F. Sterile pipettes
G. Incubator: **non**-CO_2 at 35 °C

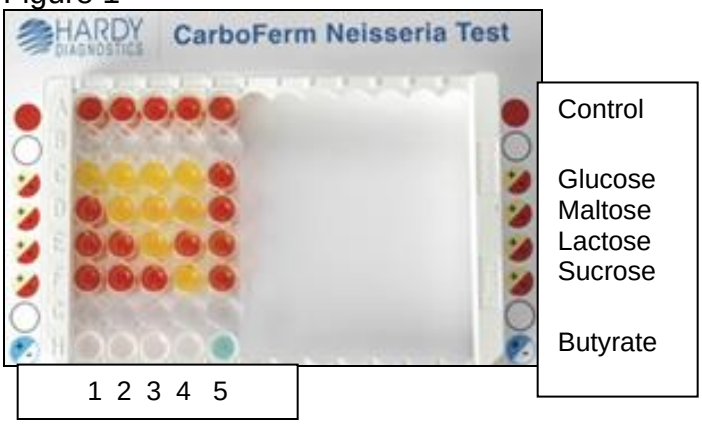
IV. Procedure

A. Remove caps from strips to be used. Leave strips in the base. Unused strips may be removed from the base and stored in the bag (with caps on) until needed. If strips have already been removed from the base, place them into the base with the negative control well in row A and the Butyrate well (containing white paper disk) in row H.
B. Allow materials to come to room temperature.
C. Use a sterile swab to transfer a pure isolate (from Chocolate or Modified Thayer Martin agar) into a tube of Inoculation Buffer. Prepare a heavy suspension (equivalent to a 4.0 McFarland turbidity standard or higher). If isolate does not emulsify well, vortex and/or aspirate repeatedly until density of the solution is uniform in appearance. A low density may result in false-negative reactions. **CAUTION: Potential isolates of *Neisseria meningitidis* should be manipulated in a class II biological safety cabinet!**
D. Aseptically transfer 4 to 5 drops of the suspension into each well of the strip, except the second (B) and the seventh (G) wells (these wells are intentionally left empty).
E. Incubate strips uncovered at 35 °C aerobically. Do not incubate in a CO_2 atmosphere.
F. Read the butyrate well (row H) after 10 to 15 min. Read the carbohydrate wells (row A, C, D, E, and F) after 2 h and before 7 h.

V. Interpretation

A. The reaction in row H (butyrate) should be read after 10 to 15 min of incubation at 35 °C. A blue color is considered positive. Color changes that take place in the butyrate well after 25 min of incubation should be disregarded.
B. The other wells in the strip containing the carbohydrates (C through F) should be examined after 2 – 4 h. A change of color from red to orange or yellow is considered positive. Color reactions can be more easily read by placing the base with the reaction strips over the white reaction card found in the test kit. A carbohydrate reaction that is more orange/yellow than the control well (A) is considered positive. Color reactions are illustrated in Figure 1 below. **Note:** Color changes in the carbohydrate wells that take place after 7 h of incubation should be disregarded.

Figure 1



1 = *N. gonorrhoeae*
2 = *N. meningitidis*
3 = *N. lactamica*
4 = *N. sicca*
5 = *M. catarrhalis*

VI. Quality Control

Quality control is performed with each new lot or shipment using the organisms listed in the table below with the expected reactions.

Expected QC Results

Test Organism	ATCC	Control	Glucose	Maltose	Lactose	Sucrose	Butyrate
N. lactamica	23970	-	+	+	+	-	-
N. sicca	9913	-	+	+	-	+	-
M. catarrhalis	25238	-	-	-	-	-	+

VII. Limitations

A. This product is intended for pure cultures of oxidase-positive, gram-negative diplococci. Use of this test with other microorganisms should only be performed with evaluation of test results during Rounds.

B. Some strains may give a weak positive reaction, which is seen as an orange color in the wells. Carbohydrate reactions should be considered positive if and only if the well containing the carbohydrate is more orange/yellow than the negative control well (row A).

C. Butyrate reactions should be read within 25 min or erroneous results may occur.

D. False-negative may result from using too small of an inoculum or cultures greater than 48 h old.

VIII. Verification of Test Method

This kit was evaluated using both ATCC strains and clinical isolates that were identified using conventional CTA carbohydrate tubes. Eight

organisms were tested, including *M. catarrhalis* ATCC 25238, *M. catarrhalis* clinical isolate, *N. gonorrhoeae* ATCC 43069, *N. gonorrhoeae* clinical isolate, *N. meningitidis* ATCC 13090, *N. lactamica* ATCC 23970, and two *Neisseria* spp. clinical isolates. The results of the CarboFerm showed 100% agreement with the CTA carbohydrates.

This system was also evaluated with 5 fastidious gram-negative bacilli, including 2 *Haemophilus aphrophilus*, 1 *Eikenella corrodens*, 1 *Kingella kingae*, and 1 *Capnocytophaga* spp. All five isolates yielded expected results in the CarboFerm within 4 h. The CTA tubes with *Kingella* and *Capnocytophaga* required extended incubation beyond 1 wk to obtain expected results.

IX. References

A. Package insert: Hardy Diagnostics, CarboFerm Neisseria Kit, 2006.

B. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed., ASM Press, Washington, D.C.

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