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CarboFerm™ *Neisseria*: A New Test System for Identification of *Neisseria spp.* and *Moraxella catarrhalis*

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ABSTRACT

CarboFerm™ *Neisseria* (CF, Hardy Diagnostics, Santa Maria, CA) is a new rapid carbohydrate (CHO) utilization test for identification of *N. gonorrhoeae* (*GC*), *N. meningitidis* (*MC*), *N. lactamica* (*NL*), saprophytic *Neisseria* (*Nsp*), and *M. catarrhalis* (*Mcat*). The CF cuvette contains a control well, a butyrate esterase (BE) well, and 4 wells containing glucose (GLU), maltose (MAL), lactose (LAC), and sucrose (SUC), respectively. A suspension (greater than a No. 4 McFarland standard) of the organism is prepared in buffer, and 4-5 drops are placed in each well. After incubation for 15 min, a blue color in the BE well identifies the isolate as *Mcat*. Further incubation for 2 to 4 hours results in CHO patterns for *Neisseria* spp. CF was evaluated with 192 isolates of *Neisseria* spp. and 40 *Mcat* isolates and compared with conventional identification procedures. All *Mcat* were correctly identified by CF after 15 min. For *GC*, CF identified 97% and 100% of 64 isolates at 2 hr and 4 hr, respectively. For *MC*, CF identified 94% and 96% of 48 isolates after 2 hr and 4 hr, respectively. Two of 48 *MC* were MAL-negative and positive for GLU only. For *NL*, CF identified 79% and 91% of 33 isolates at 2 hr and 4 hr, respectively. Three *NL* were misidentified due to negative LAC reactions at 4 hrs. *N. cinerea* (*NC*, 15 isolates) were negative for all CHO's at 4 hr. Of 32 *Nsp*, 56% and 78% were identified at 2 hr and 4 hr, respectively. Misidentification of *Nsp* was due to negative GLU and/or SUC reactions after 2 and 4 hrs. CF performed well for identification of *GC*, *MC*, and *Mcat*, but additional testing may be warranted for *NL* and clinically significant *Nsp* isolates.

INTRODUCTION

Methods for identifying *Neisseria* species and *Moraxella catarrhalis* recovered from clinical specimens include conventional and modified carbohydrate (CHO) acidification procedures, commercial multi-test identification kits, and both immunologic (i.e., coagglutination, fluorescent antibody) and DNA probe tests for culture confirmation of *Neisseria gonorrhoeae* (*GC*). Traditional CHO acidification test procedures use cystine-tryptic digest agar with added CHO's and may require prolonged incubation. Modified CHO utilization techniques allow identification of *Neisseria* species in 1 to 4 hours (h); commercial adaptations of this method have compared favorably with conventional procedures.

Hardy Diagnostics (Santa Maria, CA) has recently marketed the CarboFerm™ *Neisseria* kit (CF), a new rapid CHO utilization test for identification of *Neisseria* species and *Mcat*. The kit is inoculated with a heavy suspension of gram-negative, oxidase-positive diplococcal isolates and provides an identification of these organisms after a 4 h incubation. In this study, the performance of CF was evaluated with 192 isolates of *Neisseria* species and 40 isolates of *Mcat* recovered from clinical specimens.

MATERIALS AND METHODS

Organisms

Organisms tested included fresh clinical isolates, stock strains, or clinical isolates that had been stored frozen at -70°C in serum:BHI broth (1:1). Clinical isolates were from cultures submitted to the Clinical Microbiology Laboratory of the University of Illinois Medical Center. Organisms were recoverd from a variety of body sites (Table 1). Frozen isolates were subcultured onto chocolate agar or modified Thayer-Martin Agar (Remel, Lenexa, KA) and were subcultured at least 3 times prior to testing on CF. Organisms producing discrepant results were re-tested with conventional procedures (below) and CF. Testing was done in a blinded fashion with 18-24 h subcultures.

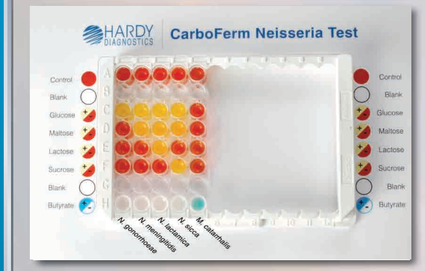

Table 1. Strains Tested in the Evaluation of CarboFerm™ *Neisseria* (CF)

Species	No. Strains Tested	Source	No. of Strains
N. gonorrhoeae (GC)	64	Endocervix	47
		Urethra	14
		Endometrium	1
		Blood	1
		Conjunctiva	1
N. meningitidis (MC)	48	Throat	18
		Respiratory	3
		CSF	6
		Blood	2
		Conjunctiva	1
		Urethra	4
		Rectal	14
N. lactamica (NL)	33	Throat	28
		CSF	2
		Urethra	2
		Rectal	1
N. cinerea (NC)	15	Respiratory	7
		Cervix	2
		Wound	2
		Blood	2
		Conjunctiva	1
		Urine	1
Neisseria spp. (Nsp)	32	Respiratory	13
		Throat	8
		Blood	7
		Abscess	2
		Conjunctiva	1
		Endocervix	1
M. catarrhalis (Mcat)	40	Respiratory	27
		Conjunctiva	8
		Blood	2
		Sinus aspirate	1
		Wound	1
		Urine	1

Conventional Methods

Conventional identification tests for *Neisseria* species and *Mcat* included Gram stain, oxidase/catalase tests, growth on modified Thayer-Martin agar (MTM, Remel, Lenexa, KA), acid production from GLU, MAL, FRU, SUC, and LAC by the rapid carbohydrate fermentation technique, ONPG hydrolysis, and DNase. Isolates were also characterized for presence of indoxyl butyrate esterase, prolyl-aminopeptidase, γ-glutamyl aminopeptidase, and β-galactosidase (BactiCard™ *Neisseria*, Remel). *MC* isolates with atypical CHO utilization patterns were serogrouped by slide agglutination using meningococcal antisera (Remel). Additional tests included nitrate and nitrite reduction, colistin susceptibility, and production of iodine-positive polysaccharide from sucrose.

CarboFerm™ *Neisseria* Test (CF)

The CF kit consists of an 8-well reagent cuvette packaged as 12 strips snapped into a plastic frame. Frame rows are labeled A through H. Cuvette wells are sealed with a plastic-cap strip. Wells in row A contain dehydrated peptone medium base with phenol red indicator and no CHO (control). Wells in rows C-F contain basal medium and pH indicator with CHO's: GLU (C), MAL (D), LAC (E) and SUC (F). Wells in row H contain bromo-chloro-indoxyl butyrate (BE, butyrate esterase substrate). Row B and G wells are empty. Tubes containing inoculation fluid are also included. CF is designed to identify the Gram-negative, oxidase-positive diplococci belonging to the genus *Neisseria* and *Moraxella catarrhalis*. To inoculate the CF, an organism suspension (greater than a No. 4 McFarland turbidity standard) is prepared in the buffer from an 18 to 24 hour culture on chocolate, blood, or selective medium. Four to five drops of inoculum are placed in each of the reagent-containing wells, and the cuvette is incubated in ambient air at 35°C.

After 15 min, well H (BE) is examined for presence of a blue color. If the well is teal-blue, the organism is identified as *Mcat*, and testing is complete. If the well is colorless, the BE reaction is scored negative, and the cuvette is re-incubated for up to 4 hours. The CHO control and the CHO wells are the inspected for color changes: red or red-orange is negative; orange, yellow-orange, and yellow are positive. In this study, the CHO control and CHO-containing wells for each isolate were read after 2 h and 4 h incubation at 35°C.

RESULTS

Table 2 Identification of *Neisseria* spp. and *Moraxella catarrhalis* by the CarboFerm™ *Neisseria* (CF)

Species	No. tested	No. identified at 2 h (%)	No. identified at 4 h (%)
N. gonorrhoeae (GC)	64	62 (97)	64 (100)
N. meningitidis (MC)	48	45 (94)	46 (96)
N. lactamica (NL)	33	26 (79)	30 (91)
N. cinerea (NC)	15	15 (100)	15 (100)
Neisseria spp. (Nsp)	32	18 (56)	25 (78)
		No. identified at 15 min (%)	Not Applicable
M. catarrhalis (Mcat)	40	40 (100)	

All 40 isolates of *Mcat* were correctly identified by the CF after 15 min incubation.

All 64 isolates of *GC* were correctly identified after 4 hours incubation. After two hours, 2 of the 64 isolates had GLU reactions that were interpreted as negative (i.e., red orange).

Of 48 *MC* tested, 45 (94%) were correctly identified after 4 h. One isolate had a negative GLU (with a positive MAL reaction) after 2 h that was clearly positive after 4 h. Two isolates of *MC* not identified after 4 h were actually MAL-negative strains (a group C throat isolate and a group Y blood isolate). Without additional testing, these two isolates would have been misidentified as *GC* on CF.

Of 33 *NL* strains tested, 79% were identified after 2 h, and 91% were identified after 4 h. incubation. Three strains not correctly identified by CF produced negative (red-orange) reactions for LAC after 4 h; the same results were obtained on repeat testing.

The 15 *NC* strains tested produced negative BE reactions at 15 min and negative CHO reactions after 2 and 4 h. The CHO control and CHO wells were similar in color after incubation, so no false-positive CHO reactions were noted with the *NC* isolates for any CHO's on CF.

Among 32 *Nsp*, (56%) were identified after 2 h, and 78% were identified after 4 h incubation. Seven isolates were not identified after 4 h.; 2 were misidentified as *Mcat* (BE-positive) after 15 min, 2 were GLU-negative after 4 h, and 3 were GLU- and MAL-positive but SUC- and LAC-negative after 4 h. These latter isolates would have been identified as *MC* by CF. Conventional testing indicated these latter isolates were GLU-, MAL-, and FRU-positive, SUC- and LAC-negative, did not reduce nitrate, reduced nitrite to N_2 and did not produce iodine-positive polysaccharide from sucrose, confirming them as isolates of *N. subflava* bv *flava*. Because FRU is not present on CF, these SUC-negative isolates would be identified as *MC* by CF testing.

In conclusion, the CF performed well for identification of *GC*, *MC*, and *Mcat*. Additional testing may be warranted for identification of *NL* and clinically significant non-gonococcal, non-meningococcal *Neisseria* species isolates.