JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1980, p. 620-623
0095-1137/80/10-0620/04$02.00/0

Vol. 12, No. 4

Use of the Rapid Fermentation Test in Determining Carbohydrate Reactions of Fastidious Bacteria in Clinical Laboratories

DANNIE G. HOLLIS,* FRANCES O. SOTTNEK, W. JERRY BROWN, AND ROBERT E. WEAVER
Center for Disease Control, Atlanta, Georgia 30333

The rapid fermentation test was used to determine the carbohydrate reactions of some of the fastidious bacteria encountered in clinical laboratories, such as: *Haemophilus* species, including *Haemophilus vaginalis*; *Actinobacillus actinomycetemcomitans*; *Cardiobacterium hominis*; *Kingella* species; *Corynebacterium* species; *Propionibacterium* species; and *Erysipelothrix rhusiopathiae*. Results were usually obtained within 4 h by using inocula from 24- or 48-h blood or chocolate agar media.

The demonstration of acid production from carbohydrates by fastidious bacteria is frequently delayed (2 to 7 days) when tested in broth media. In some instances, acid reactions are not detected unless serum or other enrichments are added to the carbohydrate media. Since the rapid fermentation test (RFT) has proven to be very reliable for detecting carbohydrate reactions of the fastidious gonococci (2-4, 7), it was decided to determine the suitability of this method for testing other fastidious organisms. The organisms studied were *Haemophilus* species, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Kingella* species, *Francisella tularensis*, groups DF-1, DF-2, HB-5, EF-4, J-K, and E, *Corynebacterium* species, *Propionibacterium* species, *Erysipelothrix rhusiopathiae*, and *Bacterionema matruchotii*.

All organisms tested were grown either on blood agar plates containing heart infusion agar and 5% rabbit blood or on chocolate agar (BBL Microbiology Systems) plates consisting of GC base, 1% hemoglobin, and 1% IsoVitaleX. We found that inocula from chocolate agar containing supplement B gave a false-positive sucrose reaction in the RFT, and therefore this agar should not be used when sucrose is included as one of the carbohydrates for characterization. The cultures were incubated in a candle jar atmosphere for 18 to 48 h at 35°C.

Brown's modification of the RFT for *Neisseria gonorrhoeae* (2) was used with a few changes: the desired carbohydrate solutions were made up to 20% concentrations in broth (peptone, 10 g; meat extract, 3 g; sodium chloride, 5 g; distilled water, 1 liter), and the pH was adjusted to 7.0. These solutions were filter-sterilized. With a sterile Pasteur pipette, 1 drop (0.04 ml) of the appropriate carbohydrate solution was placed in a disposable sterile culture tube (10 by 75 mm); 0.1 ml of buffer-salt solution (pH 7.0) was added to the tube, and then 1 drop of a very heavy cell suspension (2) from an 18- to 48-h culture (prepared in 0.35 ml of the buffer-salt solution) was added. A control tube containing a drop of the peptone-meat extract-sodium chloride broth instead of a drop of the carbohydrate solution was used with each culture. The tubes were covered with aluminum foil and incubated in a 35°C water bath. The reactions were read at 4 and 24 h. In some instances, reactions were positive within 30 min, and with most organisms reactions were positive in 4 h; however, reactions with a few organisms were weak or questionable at 4 h and stronger at 24 h. All of the carbohydrates used for the RFT were tested in this system with quality control cultures. It was necessary to test several brands of reagent-grade maltose to find one which was satisfactory (5). Difco maltose (lot 0168-15) was used in this study.

The carbohydrate reactions of the *Haemophilus* species tested by the RFT are listed in Table 1. These reactions agreed with those obtained in the standard carbohydrate bases used in our laboratory. These bases are peptone-meat extract-sodium chloride broth (5) and OF (oxidation-fermentation) medium (9). The carbohydrate bases were supplemented with peptic digest of blood (6) when determining the reactions of the factor-dependent *Haemophilus* strains. These strains were tested for glucose, xylose, and lactose reactions in the standard bases, but they were not tested for reactions in mannitol, sucrose, maltose, and fructose. Twenty-five *H. influenzae* strains, which included five strains of each of the five biotypes, 7 *H. haemolyticus* strains, 22 *H. parainfluenzae* strains (including

TABLE 1. *Carbohydrate reactions of Haemophilus species with the RFT*[a]

Strain	No. of strains	Reaction[b]						
		Glucose	Xylose	Mannitol	Lactose	Sucrose	Maltose	Fructose
H. influenzae	25							
Biotype 1	5	+	+	−	−	−	d	+
Biotype 2	5	+	d	−	−	−	d	+
Biotype 3	5	+	d	−	−	−	−	+
Biotype 4	5	+	+	−	−	−	d	+
Biotype 5	5	+	+	−	−	−	d	+
H. haemolyticus	7	+	−	−	−	−	−	+
H. parainfluenzae	22							
Biotype 1	6	+	−	−	−	+	+	+
Biotype 2	9	+	−	−	−	+	+	+
Biotype 3	7	+	−	−	−	+	+	+
H. segnis	5	+	−	−	−	+	+	+
H. paraphrophilus	8	+	−	−	+	+	+	+
H. aphrophilus	5	+	−	−	+	+	+	+
H. haemoglobinophilus	1	+	w	+	−	+	w	+
H. ducreyi	12	d w	−	−	−	−	−	−
H. vaginalis	7	+ or w	d	−	−	d	+ or w	+ or w
H. equigenitalis	1	−	−	−	−	−	−	−

[a] Reactions were read at 4 h.

[b] Symbols: +, positive reaction; −, negative reaction; d, some reactions positive, some negative; w, weak positive reaction; + or w, some reactions positive, some weak.

five or more strains of each of the three biotypes), five strains with characteristics of *H. segnis*, a new species described by Kilian (8), 8 *H. paraphrophilus* strains, 5 *H. aphrophilus* strains, and 1 *H. haemoglobinophilus* strain had reactions similar to those reported by Kilian (8) and Back and Oberhofer (1). Twelve strains of *H. ducreyi* were tested. Five had questionable reactions in glucose at 4 h, and the reactions of four of these were stronger at 18 to 24 h. Inocula from 48-h plates were used when testing *H. vaginalis*. One strain was weakly positive in glucose, maltose, and fructose; one strain was sucrose negative at 4 h and positive at 18 to 24 h. *H. equigenitalis* did not react in any of the carbohydrates tested by using the RFT or the standard carbohydrate bases.

A representative number of strains of other fastidious gram-negative rods also were tested by using the RFT (Table 2). All *A. actinomycetemcomitans* strains tested were positive in glucose and maltose. Some strains were weak in xylose and mannitol in the RFT; however, these same strains had weak or delayed reactions in xylose and mannitol when first studied in our laboratory in the standard broth base. The five strains of *C. hominis* were positive within 4 h in glucose, mannitol, sucrose, and maltose. Positive reactions with our standard fermentation broth base were obtained in 2 to 7 days. For one strain it also was necessary to add serum to the basal media. *Kingella kingae* strains were positive in maltose and weak in glucose. This was also true when this organism was tested in our standard carbohydrate basal media. *Kingella denitrificans* strains were positive in glucose within 4 h in the RFT. When the fermentation broth base was used, these same organisms were positive in glucose only after 3 to 7 days or longer. With two strains serum had to be added to the media. In the standard carbohydrate test, if the organism did not grow or only grew poorly by 48 h, the tube was reinoculated and approximately 3% serum was added. In the RFT the carbohydrate reactions are not dependent upon growth of the organism, and no serum was used. The glucose reaction of *F. tularensis* was weakly acid. We were unable to demonstrate acid reactions with

TABLE 2. *Carbohydrate reactions of some other fastidious gram-negative rods with the RFT*[a]

Strain	No. of strains	Reaction[b]					
		Glucose	Xylose	Mannitol	Lactose	Sucrose	Maltose
Actinobacillus actinomycetemcomitans	6	+	d	d	−	−	d
Cardiobacterium hominis	5	+	−	+	−	+	+
Kingella kingae	6	w	−	−	−	−	+
Kingella denitrificans	8	+	−	−	−	−	−
Francisella tularensis	6	w	−	−	−	−	−
DF-1	5	+	−	−	+	+	+
DF-2	16	+	−	−	+	−	+
HB-5	5	+	−	−	−	−	−
EF-4	6	+	−	−	−	−	−

[a] Reactions were read at 4 h.
[b] Symbols used are the same as for Table 1.

strains of this organism in our standard basal media; however, acid reactions can be obtained in 2 to 7 days from glucose in a cysteine agar medium containing phenol red. DF-1, DF-2, HB-5, and EF-4 groups reacted well in the RFT. In contrast, several of these groups had weak or delayed (3 to 7 days) carbohydrate reactions in the fermentation broth base. One group in particular, DF-2, which is usually isolated from blood and associated with dog bites, produced weak and often delayed acid reactions in the fermentation basal media, but in the RFT the reactions were positive within 30 min to 4 h. The RFT has been very helpful in an early identification of this organism. With this system this organism can be identified in 1 to 2 days; previously, 3 to 7 days or longer was required for identification.

In Table 3 are the results of the carbohydrate reactions in the RFT with some fastidious gram-positive organisms: *E. rhusiopathiae*, *Corynebacterium haemolyticum*, *C. pyogenes*, *C. bovis*, *C. pseudotuberculosis*, group J-K, *B. matruchotii*, *Propionibacterium* sp., and group E. The reactions of the strains tested in each of these groups were positive at 4 h. Most of these same strains were not positive until 2 to 7 days in the fermentation broth media, and frequently it was necessary to add serum and reinoculate or repeat the carbohydrate tests. Using the RFT to determine the carbohydrate reactions of these organisms can help to identify them sooner.

The results of this limited study suggest that the RFT is useful for the carbohydrate characterization of many of the fastidious organisms encountered in clinical laboratories. It appears that by using the RFT for carbohydrate reactions and a few additional rapid tests, i.e., spot

TABLE 3. *Carbohydrate reactions of some fastidious gram-positive rods with the RFT*[a]

Strain	No. of strains	Reaction[b]					
		Glucose	Xylose	Mannitol	Lactose	Sucrose	Maltose
Erysipelothrix rhusiopathiae	5	+	−	−	+	−	+
Corynebacterium haemolyticum	5	+	−	−	+	d	+
Corynebacterium pyogenes	5	+	+	−	+	+	+
Corynebacterium bovis	3	+	−	−	d	−	+
Corynebacterium pseudotuberculosis	5	+	−	−	−	−	+
Group J-K	12	+	−	−	−	−	d
Bacterionema matruchotii	5	+	−	d	−	+	+
Propionibacterium sp.	5	+	−	−	−	+	+
Group E	4	+	+	−	−	+	+

[a] Reactions were read at 4 h.
[b] Symbols used are the same as for Table 1.

indole, oxidase, urease, catalase, and ornithine decarboxylase, many fastidious bacteria can be quickly identified in a clinical laboratory.

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