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Coagglutination of *Vibrio cholerae*, *Vibrio mimicus*, and *Vibrio vulnificus* with Anti-Flagellar Monoclonal Antibody

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Monoclonal antibodies (MAbs) with serological activity for purified flagellar (H) core protein prepared from *Vibrio cholerae* were identified by enzyme-linked immunosorbent assay. Four of these MAbs reacted with the flagella of *V. cholerae* and *V. mimicus* exclusively, while eight MAbs reacted with at least 1 of 30 heterologous *Vibrio* species tested by enzyme-linked immunosorbent assay or coagglutination. It appears that *V. cholerae* and *V. mimicus* express similar, if not identical, H determinants unique to these two *Vibrio* species. *Staphylococcus aureus* cells or latex beads armed with the four species-specific MAbs coagglutinated each of 47 isolates identified bacteriologically as *V. cholerae* or *V. mimicus* from among 103 *Vibrio* isolates tested. One coagglutination reagent armed with anti-*V. vulnificus* H MAb exhibited species specificity in that only *V. vulnificus* cells were coagglutinated from among the 31 *Vibrio* species examined. This reagent coagglutinated 20 isolates identified bacteriologically as *V. vulnificus* in a serological survey. MAb coagglutination reagents offer a rapid, specific, and economical alternative to the classical bacteriological approach to identify the human pathogens *V. cholerae*, *V. mimicus*, and *V. vulnificus*.

Coagglutination reagents armed with species-specific anti-flagellar (H) antibody offer a sensitive, specific, and economical approach to the rapid identification of *Vibrio* pathogens. The specificity of this serological test is predicated on the knowledge that each *Vibrio* pathogen examined to date expresses H determinants unique to that species (1, 2, 7, 9, 13, 14, 19, 21–23). We previously reported (19) that coagglutination reagents bearing anti-*V. vulnificus* H antibody, produced in rabbits immunized with flagellar core (FC) protein, agglutinated 99% of those bacterial isolates identified bacteriologically as *V. vulnificus*. These reagents did not agglutinate any of 723 environmental isolates identified as *V. fluvialis*, *V. cholerae*, *V. mimicus*, or *V. parahaemolyticus*.

Vibrio cholerae, however, has serologically detectable H determinants in common with *V. metschnikovii*, *V. anguillarum*, *V. fluvialis*, *V. mimicus*, and *V. ordalii* (1, 19, 21, 22), a situation which precludes the use of rabbit anti-H serum to identify *V. cholerae* serologically. Castellani-Pastoris et al. (4, 18) described a direct-immune slide agglutination test in which rabbit anti-H serum agglutinated 98.9% of 1,081 *V. cholerae* isolates examined. It was their objective to facilitate rapid identification of O1 and non-O1 *V. cholerae* serovars recovered from clinical specimens. They did not conduct an extensive serological survey to assess the species specificity but noted that *V. anguillarum* cells were agglutinated with anti-*V. cholerae* H serum. If polyclonal anti-*V. cholerae* H serum were used to screen, serologically, sucrose-positive isolates recovered from seafood or environmental specimens in an attempt to identify the source of an O1 or non-O1 human infection, the findings might be fraught with nonspecific agglutination reactions (19). Polyclonal anti-*V. cholerae* H serum must be absorbed sequentially with motile-cell preparations of *V. anguillarum*, *V. metschnikovii*, and *V. fluvialis* to impart species specificity to the antiserum (21), a time-consuming task which would have to be repeated with each new lot of antiserum.

Alternatively, coagglutination reagents coated with anti-H monoclonal antibody (MAb) should exhibit the desired species specificity. This report describes a coagglutination reagent bearing anti-H MAb which possesses serological activity for O1 and non-O1 *V. cholerae* serovars exclusively. The H coagglutination reagent provides a rapid, sensitive, and economical test by which to identify these vibrios from among sucrose-positive isolates recovered from clinical, seafood, and environmental specimens. The findings also support previous reports which suggested that the flagella of *V. cholerae* and *V. mimicus* express similar, if not identical, H determinants (19, 21, 22).

MATERIALS AND METHODS

Flagellar core purification. Flagellar cores were purified from motile strains of each of the following *Vibrio* species by methods described previously (19, 24): *V. cholerae* Ogawa ATCC 14035, *V. vulnificus* ATCC 27562, *V. anguillarum* ATCC 19264, *V. diazotrophicus* ATCC 33466, *V. fluvialis* ATCC 33810, *V. nigrapulchritudo* ATCC 27043, *V. metschnikovii* ATCC 7708, *V. ordalii* ATCC 33509, *V. mimicus* ATCC 33653, and *V. proteolyticus* NCMB 1326. Each flagellar core preparation was examined by electron microscopy to determine purity. Total protein determinations were made on each flagellar core preparation by the method of Lowry et al. (8).

Immunization. BALB/c mice were immunized at 2-week intervals for 4 to 8 weeks by intraperitoneal injection of 100 μg of purified flagellar core protein obtained from *V. cholerae* or *V. vulnificus*. Prior to fusion, the serum anti-H flocculation titer was determined (22). Those mice which exhibited elevated H titers were boosted with 500 μg of flagellin, and their spleen cells were collected 4 days later.

Hybridoma production. The protocol used to promote B-cell–myeloma cell fusion was modified from that described by Oi and Herzenberg (10). Briefly, spleen cells were fused at a 4:1 ratio with log-phase Sp2/0-Ag 14 nonsecreting myeloma cells. Following fusion, the cells were sedimented and suspended in culture medium to give 7.5×10^6 spleen cells per ml. From this suspension, 0.1-ml samples were distributed to each well of a 96-well flat-bottom tissue culture plate which contained a BALB/c thymocyte feeder layer.

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Hybridomas were fed medium which contained 100 μM hypoxanthine, 4×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine on each of the first 5 days following fusion. Hybridoma cell cultures were maintained at 37°C in a humidified atmosphere of 5 to 7% CO_2 in air in RPMI 1640 which contained 15% fetal bovine serum, 2 mM glutamine, 1% nonessential amino acids, and 100 U of penicillin-streptomycin per ml.

Anti-H ELISA. Supernatant fluid from each well which contained hybridomas was screened by enzyme-linked immunosorbent assay (ELISA) for anti-*V. cholerae* H or anti-*V. vulnificus* H antibody activity. A 100-μl sample of the appropriate flagellar core preparation (4 μg of protein per 100 μl of coupling buffer [5]) was dispensed into each well of an Immulon II (Dynatech Laboratories, Inc., Alexandria, Va.) microtiter plate and incubated at 4°C for at least 18 h. Following removal of coupling buffer, 200 μl of blocking buffer (1% bovine serum albumin and 0.02% NaN_3 in phosphate-buffered saline) was delivered to each well. The blocking buffer was removed after 1 h, 50 μl of cell culture supernatant fluid was dispensed to each well, and the plates were incubated at 25°C for 1 h. Each well was washed four times with a solution which contained 1.5 mM $MgCl_2$, 2 mM 2-mercaptoethanol, 0.1% bovine serum albumin, and 0.02% NaN_3 in phosphate-buffered saline, and then 50 μl of alkaline phosphatase-labeled goat anti-mouse immunoglobulin (Kirkegard and Perry Laboratories, Gaithersburg, Md.) diluted 1:250 in washing solution was added to each well. The plates were incubated at 25°C for 1 h, and the wells were washed four times. A 200-μl sample of *p*-nitrophenyl phosphate at a concentration of 1 mg/ml in diethanolamine buffer (pH 9.8) was added to each well and incubated for 30 min at 37°C. The reaction was stopped by the addition of 30 μl of 3 N NaOH per well, and the A_{405} was determined. Anti-H-secreting hybridomas were cloned three times by limiting dilution (10).

MAb purification and characterization. Four-liter roller bottles which contained 500 ml of culture medium were seeded with 50 ml of log-phase cells and incubated for 2 weeks. Ascites tumors were induced in BALB/c mice primed with 2,6,10,14-tetramethylpentadecane 10 and 3 days before the intraperitoneal injection of 10^7 hybridoma cells. Ascites fluid was collected from each mouse 1 to 2 weeks following hybridoma cell injection. Ascites and cell culture supernatant fluids were clarified by centrifugation, and the immunoglobulin fraction was precipitated by the addition of an equal volume of saturated $(NH_4)_2SO_4$ solution (pH 7.0). Following dialysis, immunoglobulin M (IgM) MAbs were purified by Sephadex G-200 filtration in 0.5 M NaCl and 0.05 M Tris hydrochloride (pH 7.8). MAbs of the IgG class were purified by affinity chromatography on protein A-Sepharose (Sigma Chemical Co., St. Louis, Mo.). MAbs were isotyped by ELISA with a double-antibody detection system (Hyclone Laboratories, Logan, Utah). Twofold dilutions of each of the 13 MAbs starting at 100 μg/ml were tested against homologous and heterologous flagellar core proteins by ELISA. MAb dilutions which exhibited an A_{405} of 0.2 or less were considered negative.

Preparation of anti-H MAb coagglutination reagents. IgG MAbs were fixed to Formalin-killed *Staphylococcus aureus* Cowan 1 ATCC 12598 cells by mixing 0.5 ml of $(NH_4)_2SO_4$-fractionated cell culture fluid with 0.5 ml of a 10% suspension (vol/vol) of prepared *Staphylococcus* cells (6, 19). IgM MAbs were fixed to 0.8-μm unmodified latex beads (Sigma). The latex particles were washed three times in saline which contained 0.1 M glycine and 0.1% NaN_3 (glycine-buffered saline, pH 8.2). Following the last wash step, a 5% latex suspension in glycine-buffered saline was prepared. A 100- to 150-μg sample of IgM MAb diluted in glycine-buffered saline mixed with an equal volume of latex beads was incubated at 37°C for 2 h, sedimented at 1,500 × *g* for 15 min, and suspended in 1 volume of 0.1% bovine serum albumin in glycine-buffered saline.

Coagglutination. Each *Vibrio* isolate to be tested by coagglutination was propagated on modified alkaline-peptone agar slants (1% peptone, 2% NaCl, 0.4% KCl, 0.4% $MgCl_2 \cdot 6H_2O$, 0.2% yeast extract, 1.5% agar [pH 7.4 to 7.8]) for 24 to 48 h at 30°C. The cells were harvested in 1 to 2 ml of 0.1 M Tris buffer (pH 7.8) containing 0.1 mM EDTA, 1% Triton X-100, and 0.001% thimerosal (TET buffer) (19). Each anti-H MAb coagglutination reagent was tested by slide agglutination against TET buffer suspensions of 31 *Vibrio* species. In addition to the *Vibrio* species used for flagellar core purification, the following *Vibrio* species were tested: *V. aestuarianus* ATCC 35048, *V. alginolyticus* ATCC 33787, *V. campbellii* ATCC 25920, *V. carchariae* ATCC 35084, *V. cincinnatiensis* ATCC 35912, *V. costicola* NCMB 701, *V. damsela* ATCC 35083, *V. fischeri* NCMB 1281, *V. furnissii* ATCC 35016, *V. gazogenes* ATCC 29980, *V. harveyi* NCMB 1280, *V. hollisae* ATCC 33564, *V. logei* ATCC 29985, *V. mediterranei* ATCC 43341, *V. natriegens* ATCC 14048, *V. nereis* ATCC 25917, *V. orientalis* ATCC 33934, *V. pelagius* I ATCC 25916, *V. parahaemolyticus* ATCC 10136, *V. splendidus* I ATCC 33125, *V. splendidus* II ATCC 25914, and *V. tubiashii* ATCC 19106.

Both clinical and environmental strains of *V. cholerae* O1 and non-O1 serovars, *V. mimicus*, *V. parahaemolyticus*, and *V. vulnificus* were provided by Lynelle Ford (Department of Health and Human Resources, State of Louisiana). Cultures of *V. anguillarum* and *V. ordalii* were sent to us by G. L. Bulloch (National Fisheries Research Laboratory, Kearneysville, W.Va.), and the *V. fluvialis* isolates used were part of a collection maintained in the Department of Microbiology, Louisiana State University.

RESULTS AND DISCUSSION

Serological characterization of MAbs. Thirteen hybridomas secreting antibodies specific for FC protein were selected and established. Twelve hybridomas obtained from one fusion experiment secreted MAbs which reacted, by ELISA, with *V. cholerae* core protein. Two of these clones, 1F4 and 3F4, secreted IgM MAb, as did clone G7 obtained from a second fusion experiment in which spleen-cell-donor mice were immunized with *V. vulnificus* FC. The remaining 10 MAbs were isotyped as either IgG2 or IgG3.

Each of the 13 MAbs was tested by ELISA against FC prepared from six heterologous *Vibrio* species reported to share H determinants with *V. cholerae* (1, 19, 21, 22) and from two species, *V. diazotrophicus* and *V. nigrapulchritudo*, which agglutinated with anti-*V. cholerae* polyclonal H coagglutination reagents in preliminary experiments. Six anti-*V. cholerae* H MAbs reacted with the FC of *V. cholerae* and *V. mimicus* exclusively (Table 1), which supports evidence that these two vibrios express similar, if not identical, H determinants (19, 22). Two of these MAbs, 1A10 and 2B1, produced identical ELISA titers with FC protein prepared from both *V. cholerae* and *V. mimicus*, while four other MAbs reacted at higher titers with *V. cholerae* flagellar protein (Table 1). The disparity in titers displayed by the latter four MAbs suggests that they may recognize a second epitope present in lower concentration or are not serologically accessible on *V. mimicus* FC. Competition experi-

TABLE 1. H-antigen relationships among flagellar core proteins prepared from *Vibrio* species examined by ELISA with anti-*V. cholerae* H and anti-*V. vulnificus* H MAbs

Species[a]	Titer for anti-H MAb[b]:												
	1A10	2B1	4C11	1F2	1D6	1C5	3G9	3G4	1F6	3F4	3D6	1F4	G7
V. cholerae	400	2,560	1,600	1,600	800	800	400	1,600	1,600	160	400	40	0
V. mimicus	400	2,560	1,280	1,280	320	160	400	1,600	1,600	320	400	40	0
V. proteolyticus	0	0	0	0	0	0	10	1,280	0	160	320	40	0
V. ordalii	0	0	0	0	0	0	0	0	160	160	640	0	0
V. diazotrophicus	0	0	0	0	0	0	0	0	160	160	640	40	0
V. anguillarum	0	0	0	0	0	0	0	0	0	0	320	80	0
V. fluvialis	0	0	0	0	0	0	0	0	0	0	160	0	0
V. nigrapulchritudo	0	0	0	0	0	0	0	0	0	0	0	40	0
V. metschnikovii	0	0	0	0	0	0	0	0	0	0	0	0	0
V. vulnificus	0	0	0	0	0	0	0	0	0	0	0	0	320

[a] 4 μg of flagellar core protein from each species was added to wells of each ELISA plate.
[b] Greatest dilution of MAb, starting with 100 μg of MAb in well 1, which gave A_{405} reading of 0.2 or greater in three separate experiments. All MAbs were anti-*V. cholerae* H except for G7, which was anti-*V. vulnificus* H. Clone G7 was generated and established from a fusion experiment from mice immunized with flagellar core proteins prepared from *V. vulnificus*.

ments would reveal the minimum number of species-specific H determinants expressed on *V. cholerae* and *V. mimicus* flagella.

Six anti-*V. cholerae* H MAbs reacted with FC of either one, two, three, or five heterologous *Vibrio* species in addition to FC of the homologous vaccine strain (Table 1). Predicated on the serological findings of previous investigations, it was anticipated that MAbs which reacted with the flagella of *V. anguillarum*, *V. diazotrophicus*, *V. fluvialis*, *V. metschnikovii*, *V. ordalii*, and *V. proteolyticus* would be identified. With the exception of *V. metschnikovii* MAb, the six MAbs reacted in different combinations with the FC prepared from these vibrios. Clone G7 (Table 1) produced MAb specific for an H antigen exhibited on the flagella of *V. vulnificus*.

Coagglutination reactions. The primary objective of this investigation was to prepare H coagglutination reagents which would agglutinate and identify *V. cholerae* from among sucrose-positive colonies selected from thiosulfate-citrate-bile salts-sucrose (TCBS) agar. Although *V. mimicus* appears to express the H determinants in common with *V. cholerae*, it is sucrose negative on TCBS. Coagglutination reagents prepared with each of the six *V. cholerae*- and *V. mimicus*-specific anti-H MAbs were tested against TET suspensions of 31 *Vibrio* species (Table 2). MAb 1F2 agglutinated *V. cholerae* and *V. mimicus* cells within 30 s, while three other reagents agglutinated TET suspensions of the two vibrios in 1 to 3 min. Two reagents, MAbs 4C11 and 1C5, also agglutinated *V. ordalii* cells, a result not predicted by FC ELISA findings.

With a few exceptions, each of the six MAbs which displayed heterologous activity in the FC ELISA coagglutinated whole-cell TET suspensions of each of the six vibrios described above (Tables 1 and 2). MAbs 3G9 and 3D6 each failed to coagglutinate one heterologous *Vibrio* sp. that FC ELISA findings predicted should be coagglutinated, while MAbs 1F6 and 3F4 each agglutinated one *Vibrio* sp. that was negative in the FC ELISA. MAb 1F4, which reacted with the FC of six *Vibrio* spp. in ELISA (Table 1), failed to coagglutinate whole-cell suspensions of these vibrios (Table 2).

TABLE 2. H-antigen relationships among *Vibrio* species examined serologically by coagglutination with *S. aureus* cells or latex beads armed with anti-*V. cholerae* H and anti-*V. vulnificus* H MAbs

Species[a]	Agglutination reaction for anti-H MAb[b]:												
	1A10	2B1	4C11	1F2	1D6	1C5	3G9	3G4	1F6	3F4	3D6	1F4	G7
V. cholerae	++	++	+++	+++	++	+++	++	++	+++	+++	+++	0	0
V. mimicus	++	++	+++	+++	++	+	++	++	+++	+++	+++	0	0
V. proteolyticus	0	0	0	0	0	0	0	+++	+++	+	+++	0	0
V. ordalii	0	0	+	0	0	+	0	0	++	++	++	0	0
V. diazotrophicus	0	0	+	0	0	0	0	0	++	+++	++	0	0
V. anguillarum	0	0	0	0	0	0	0	0	0	0	0	0	0
V. fluvialis	0	0	0	0	0	0	0	0	0	0	++	0	0
V. nigrapulchritudo	0	0	0	0	0	0	0	0	0	++	0	0	0
V. metschnikovii	0	0	0	0	0	0	0	0	0	0	0	0	0
V. furnissii	0	0	0	0	0	0	0	0	+++	0	+++	0	0
V. orientalis	0	0	0	0	0	0	0	0	+++	+	+++	0	0
V. tubiashii	0	0	0	0	0	0	0	+	0	+++	+++	0	0
V. vulnificus	0	0	0	0	0	0	0	0	0	0	0	0	+++

[a] Also tested were *V. aestuarianus*, *V. alginolyticus*, *V. campbellii*, *V. carchariae*, *V. cincinnatiensis*, *V. costacola*, *V. damsela*, *V. fischeri*, *V. gasogenes*, *V. harveyi*, *V. hollisae*, *V. logei*, *V. natriegens*, *V. nereus*, *V. pelagius* I and II, *V. parahaemolyticus*, *V. splendidus* I and II, and *V. mediterranei*. *Vibrio* cells were suspended in TET buffer.
[b] G7 MAb was generated from a fusion experiment in which mice were immunized with flagellar core protein prepared from *V. vulnificus*. All other MAbs were anti-*V. cholerae*. Agglutination reactions were examined and scored to indicate agglutination at 30 s or less (+++), 30 to 60 s (++), or 1 to 3 min (+).

TABLE 3. Serological survey of clinical and environmental *Vibrio* isolates by coagglutination with reagents armed with anti-*V. cholerae* H and anti-*V. vulnificus* H MAbs

Species[a] (no. of isolates tested)	No. of isolates coagglutinated by anti-H MAb[b]:				
	1A10	2B1	1F2	1D6	G7
V. cholerae					
O1 (10)	10	10	10	10	0
Non-O1 (27)	27[c]	27	27	27	0
V. mimicus (10)	10	10	10	10	0
V. fluvialis (10)	0	0	0	0	0
V. anguillarum (12)	0	0	0	0	0
V. ordalii (4)	0	0	0	0	0
V. vulnificus					
Clinical (10)	0	0	0	0	10
Environmental (10)	0	0	0	0	10
V. parahaemolyticus (5)	0	0	0	0	0
Vibrio species (unknown) (5)	0	0	0	0	0

[a] Isolates identified bacteriologically as these species. Each isolate was suspended in TET buffer for 1 h before testing.

[b] All MAbs were anti-*V. cholerae* H except for clone G7, which was anti-*V. vulnificus* H.

[c] Three isolates identified bacteriologically as *V. cholerae* failed to agglutinate upon first testing but did when they were reisolated on TCBS agar or passed through motility medium.

Since FC ELISA titers were low, the inability of the 1F4 anti-H coagglutination reagent to agglutinate the relevant vibrios could be attributed to low affinity for the H determinants. Alternatively, 1F4 MAb activity may be directed to an internal flagellar determinant, exposed on denatured flagellin utilized in the FC ELISA, which is not accessible on native flagella tested in the coagglutination assay. A common internal H antigen was identified by Shinoda et al. (15–17) which was serologically detectable on flagellin monomers of each *Vibrio* species they examined. In our study, mice were immunized with the same preparation of FC used in the ELISA. It is possible that a hybridoma secreting MAb specific for an internal antigen was selected. In addition, the concentration of each reagent was defined and controlled in the FC ELISA, while in the slide coagglutination test the concentration of flagellar antigen exposed following TET treatment and the quantity of MAb fixed to the surface of the *S. aureus* cell or latex bead were, to some extent, beyond the control of the investigator. The sensitivity of the coagglutination reaction may also be influenced by the release of outer membrane proteins, some of which exhibit serological identity with H core protein (12). If such proteins are exposed and released by TET treatment, they may serve to supplement the available H antigen.

The four species-specific coagglutination reagents identified in Table 2 were tested to assess their capacities to agglutinate the relevant *Vibrio* species from among clinical and environmental isolates. One hundred three cultures of the *Vibrio* species identified in Table 3 were propagated on alkaline-peptone agar for 24 h, and the growth from each slant was suspended in 1 to 2 ml of TET buffer and tested. The anti-*V. cholerae* H MAb reagents agglutinated each of the 47 organisms identified bacteriologically as *V. cholerae* or *V. mimicus* and did not agglutinate the 56 heterologous vibrios examined (Table 3). Three *V. cholerae* non-O1 isolates failed to agglutinate in the first round of testing. One isolate agglutinated following passage through motility-enhancement medium, and the other two agglutinated following reisolation on TCBS agar, suggesting that the original cultures may have been contaminated. *S. aureus* cells armed with MAbs 1F2 and 1A10 consistently agglutinated *V. cholerae* and *V. mimicus* cell suspensions within 30 s. In concert with this survey, latex beads coated with anti-*V. vulnificus* H MAb (clone G7, Table 2) correctly identified 20 isolates identified bacteriologically as *V. vulnificus*.

Elevated FC ELISA titers did not necessarily imply that an MAb would be the best candidate for construction of a coagglutination reagent. MAb 2B1 consistently produced marginal coagglutination reactions compared with those of MAb 1A10, yet MAb 2B1 produced an ELISA titer of 2,560, compared with 400 for MAb 1A10.

It is desirable to construct coagglutination reagents which discriminate between *V. cholerae* and *V. mimicus*, a goal that may not be achievable if MAb activity is directed toward recognition of H determinants. Previously we reported that polyclonal anti-*V. cholerae* H serum produced in rabbits immunized with Formalin-killed whole-cell vaccines (21, 22) and FC preparations (19) produced anti-H flocculation titers with *V. mimicus* equal to those obtained with *V. cholerae*. Rabbit anti-*V. cholerae* H serum absorbed with motile *V. mimicus* cells no longer possessed serologically detectable anti-*V. cholerae* H activity. The existence of 80 or more O-antigen serovars (3, 20) within the species *V. cholerae*, many of which are shared with *V. mimicus*, precludes the use of anti-O MAb reagents.

Bhattacharyya (1) proposed a serological nomenclature to identify the serologically detectable H antigens and their distribution among the three *Vibrio* species examined. The species-specific H antigens expressed by *V. cholerae*, *V. anguillarum*, and *V. metschnikovii* were designated A, B, and C, respectively, and the H antigens shared by *V. cholerae* and *V. anguillarum* and by *V. cholerae* and *V. metschnikovii* were designated D and F, respectively. Tassin et al. (21, 22) reported that *V. fluvialis* expresses H determinants in common with these three vibrios in addition to a species-specific antigen. The collective serological activity of the six anti-*V. cholerae* H MAbs which reacted with heterologous vibrio H antigens does not reflect all possible H-antigen relationships among these *Vibrio* species but supports in part the serological findings for polyclonal anti-H serum (1, 2, 19, 21, 22). Before expanding the H-antigen scheme, it might be advisable to select an alternative serological nomenclature by which to identify H antigens of *Vibrio* species, since A, B, and C are the accepted designations for the lipopolysaccharide-associated serovar-specific cell wall antigens for *V. cholerae* O1.

The H-coagglutination test provides several advantages and applications in the laboratory setting as follows. (i) The reagent can rapidly identify *V. cholerae*, *V. mimicus*, and *V. vulnificus* from among sucrose-negative and -positive colonies on TCBS agar. (ii) Since the H reagents maintain optimal serological activity for 1 year or longer, clinical laboratories need not stock the specialized media required to identify these three vibrio pathogens, which are encountered infrequently. The O1 serovars of *V. cholerae* can be identified serologically by direct slide agglutination with the appropriate anti-O serum or by anti-O coagglutination (11), following agglutination with H reagents. (iii) Public health and regulatory laboratory personnel must frequently process seafood and environmental specimens when tracking the

source of vibrio-associated human infections. H reagents inserted into the identification protocols would save time and resources when large numbers of specimens are examined. (iv) Finally, laboratory personnel need no formal training in serology to perform tests or interpret test results.

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