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Use of Monoclonal Antibodies To Serotype *Bordetella pertussis* Isolates: Comparison of Results Obtained by Indirect Whole-Cell Enzyme-Linked Immunosorbent Assay and Bacterial Microagglutination Methods

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Sixty-one *Bordetella pertussis* isolates were tested blindly in two laboratories to determine their serotype nature by monoclonal antibodies using two independent methods: the standard bacterial microagglutination assay and an indirect whole-cell enzyme-linked immunosorbent assay. Both methods gave concordant results in 60 of the 61 isolates.

Many methods have been described for distinguishing strains of *Bordetella pertussis*, and these range from the traditional method of serotyping (3) to the more sophisticated genetic methods such as pulsed-field gel electrophoresis (2, 5, 6), ribotyping (13), randomly amplified polymorphic DNA (8), and multilocus sequence typing (14). Undoubtedly the more sensitive DNA methods are highly discriminative in distinguishing many strains of *B. pertussis* into different fingerprints or sequence types. Serotyping results, on the other hand, may vary within the same profile. Moreover serotype seems to change with population immunity, and serotyping has provided data to suggest that immunity towards whooping cough depends on the serotype specificity of the pertussis bacteria (12). This was evident when vaccines lacking one serotype given to a population resulted in pertussis cases caused by strains of the serotype not present in the vaccine preparation (4, 11, 15). In 1979, the World Health Organization recommended that whole-cell pertussis vaccine should contain both serotype 2 and 3 antigens (16). Therefore, together with the more sensitive DNA methods, serotyping continues to be a useful laboratory surveillance tool for studying the epidemiology of pertussis.

Based on reactions with specific antisera, *B. pertussis* can be divided into three types: serotype 2, serotype 3, and serotype 2,3. The major serotyping antigens of *B. pertussis* have been determined to be associated with their fimbriae. Traditionally serotyping is done by the bacterial agglutination test using the slide agglutination method with bacteria mixed with specific serotyping antisera on glass slides. Hybridoma monoclonal antibodies to the serotype 2 and serotype 3 fimbria antigens have also been produced and characterized (7). Attempts to standardize the serotyping method by means of microagglutination have also been made and published (9). Nevertheless, the bacterial agglutination method is still subjective and depends on the ability of the bacteria to form a smooth suspension. Therefore, we have explored the possibility of using an objective method of indirect whole-cell ELISA for the serotyping of *B. pertussis* isolates. This assay development was evaluated independently at two laboratories with strains of *B. pertussis* that had been serotyped by the microagglutination method in one laboratory and then tested blindly in a second laboratory by the indirect whole-cell ELISA using different batches of the same serotyping monoclonal antibodies. In this communication, we report our findings and compare the two methods for the determination of serotypes of *B. pertussis* isolates.

B. pertussis isolates used in this study were mostly from the culture collection of the Swedish Institute for Infectious Disease Control (SIIDC) and were selected to represent isolates from different periods as well as expressing different serotyping antigens of Fim2, Fim3, and Fim2,3. All isolates were retyped before they were sent blindly to the National Microbiology Laboratory (NML) for testing by ELISA. A few Canadian patient isolates were typed by ELISA at NML and sent blindly to SIIDC for testing by the bacterial microagglutination assay.

Monoclonal antibodies that recognize serotype 2 and 3 fimbria antigens were made from hybridoma cell lines BPF2 (anti-Fim2) and BPC10 (anti-Fim3), which were originally developed by Brennan, Manclark, and Li (November 1992; U.S. patent 5,162,223) (7). Antibodies from the hybridoma cell lines were produced at the National Institute of Biological Standards and Control and made available to NML and SIIDC.

Serotyping of isolates by the bacterial microagglutination method was done as essentially described by Mooi et al. (9). Traditional slide agglutination was carried out according to the method described by Preston (10). Indirect whole-cell ELISA was done according to a procedure described for the serotyp-

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TABLE 1. Serotyping results for 54 *B. pertussis* strains from the Swedish Institute for Infectious Disease Control using bacterial agglutination and indirect whole-cell ELISA methods[a]

NML no.	Sweden no.	Serotype determined by bacterial agglutination	Mean ELISA OD reading with MAb[b]:	
			Fim2	Fim3
Test strains				
SS 020	P394/98	2	1.487	0.114
SS 055	T/68	2	1.867	0.069
SS 029	P694/99	2	1.901	0.066
SS 032	P238/00	2	2.859	0.078
SS 036	P618/00	2	2.933	0.081
SS 016	P164/97	2	2.938	0.064
SS 013	P87/97	2	2.938	0.078
SS 005	B63	2	2.944	0.062
SS 006	B75	2	2.953	0.117
SS 004	B22	2	2.994	0.056
SS 018	P169/98	2	3.000	0.075
SS 056	T87	2	3.001	0.070
SS 003	B37	2	3.010	0.079
SS 052	T10	2	3.021	0.072
SS 002	B16	2	3.022	0.117
SS 054	T25	2	3.031	0.126
SS 057	T43	2	3.036	0.073
SS 017	P24/98	2	3.057	0.063
SS 015	P97/97	2	3.061	0.084
SS 050	S9	2	3.096	0.086
SS 009	B44	2	3.117	0.060
SS 026	P341/99	2	3.151	0.083
SS 045	S2	2	3.227	0.094
SS 049	S8	3	0.057	1.543
SS 001	B43	3	0.042	1.665
SS 053	T18	3	0.064	1.665
SS 019	P337/98	3	0.047	1.675
SS 021	P456/98	3	0.037	1.680
SS 028	P656/99	3	0.046	1.687
SS 025	P307/99	3	0.039	1.693
SS 046	S3	3	0.049	1.703
SS 051	T9	3	0.052	1.709
SS 047	S5	3	0.044	1.717
SS 035	P563/00	3	0.054	1.717
SS 023	P611/98	3	0.052	1.727
SS 048	S6	3	0.047	1.742
SS 008	B28	3	0.058	1.750
SS 042	P123/03	3	0.060	1.766
SS 043	P47/04	3	0.045	1.779
SS 033	P257/00	3	0.051	1.779
SS 039	P89/03	3	0.052	1.785
SS 038	P915/00	3	0.049	1.790
SS 030	P871/99	3	0.039	1.798
SS 031	P1189/99	3	0.039	1.824
SS 037	P793/00	3	0.043	1.822
SS 041	P107/03	3	0.053	1.836
SS 027	P487/99	3	0.056	1.836
SS 040	P93/03	3	0.051	1.849
SS 034	P291/00	3	0.047	1.878
SS 011	B70	2,3	1.398	1.756
SS 010	B59	2,3	1.372	1.771
SS 007	B12	2,3	1.618	1.854
SS 012	FDA 460	2,3	2.821	0.820
SS 014	P96/97	3	1.027	1.708
Control strains[c]				
Fim2+ve	2558	2	3.038	0.064
Fim3+ve	Hav	3	0.023	1.393

[a] Eight of the strains listed here were also studied at the NIBSC by whole-cell ELISA using the same set of monoclonal antibodies, but antigens, buffers, enzyme conjugate, and substrate were their own in-house reagents. Basically the same ELISA results were obtained at NIBSC, which were further confirmed by their traditional slide agglutination test.

[b] Although a cutoff value in the optical density (OD) of the ELISA method was not set before this study, all ELISA-positive isolates showed ODs of ≥0.8, while ELISA-negative isolates had ODs of <0.2. MAb, monoclonal antibody.

[c] Control strains expressing only Fim2 antigen (strain 2558) or Fim3 antigen (strain Hav) were included in each assay, and the optical densities given in this table for these two strains were representative data. Although day-to-day variations occurred, the margin of difference between the positive and negative strains was wide enough that no indeterminate values were encountered during the numerous assays performed for the method development evaluation stage. +ve, positive.

ing of meningococci (1). Briefly, a smooth suspension of a loopful of bacteria grown for 48 h on a Bordet-Gengou agar plate was prepared in pH 7.4 sterile phosphate-buffered saline and heat inactivated at 56°C for 1 hour. The inactivated bacterial suspension was cooled and stored at 4°C until ready for testing. Such inactivated cell suspensions for the ELISA were found to be stable at 4°C for months and can be reused as antigens (e.g., as controls) in multiple assays. Antigen coating was done by adding 100 μl per well of the inactivated *B. pertussis* bacterial antigen, diluted in phosphate-buffered saline to give an optical density of about 0.1 at 620 nm, to a Nunc Maxisorp 96-well flat-bottomed Immuno microtiter plate (Nalge Nunc International, Rochester, NY). Detection of binding of the serotyping monoclonal antibodies to the bacterial cells was done by addition of a 1:5,000 dilution of horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G $F(ab')_2$ fragment-specific antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.). Each assay was done in the presence of both a Fim2-positive control strain and a Fim3-positive control strain. Details of the different serological methods are available from the authors.

Optimal dilutions of the anti-Fim2 and anti-Fim3 monoclonal antibodies for use in the indirect whole-cell ELISA were determined by titration of each antibody against Fim2-positive and Fim3-positive reference strains. A dilution of 1:1,000 was chosen for both antibodies in subsequent ELISAs for the serotyping of *B. pertussis* isolates.

Out of the 54 Swedish isolates tested blindly by the indirect whole-cell ELISA method, 53 (98%) gave concordant results with the bacterial microagglutination method (Table 1). Of these 53 Swedish isolates that gave concordant results with both methods, 23 were serotype 2, 26 were serotype 3, and 4 were serotype 2,3. The only isolate that gave a discordant result was an isolate that was found to be serotype 3 by bacterial microagglutination but that was identified as serotype 2,3 by indirect whole-cell ELISA. This strain was retyped by both laboratories, and the results did not change. Potential reasons for this discrepant result may include the following. The microagglutination test was done on bacteria grown on charcoal agar plates for 72 h while the indirect ELISA was done on antigens prepared from cells grown for 48 h on Bordet-Gengou agar plates. Cells grown under different conditions may have different expression of their fimbria antigens (e.g., cells grown on charcoal agar may express only Fim3 antigen, or the Fim2 antigens may be expressed at low quantities or different qualities or at a subsurface location and therefore not be accessible to antibodies).

To further evaluate the ELISA method, eight Canadian isolates expressing the Fim3 antigens as determined by ELISA along with the single Swedish isolate that did not provide a matching result were sent blindly from NML to the SIIDC for testing by bacterial microagglutination. All eight Canadian isolates gave identical results regardless of the method of testing used, but the single Swedish isolate still tested as serotype 3 by the bacterial agglutination method but was typed as serotype 2,3 by ELISA.

Our data presented here show that the indirect whole-cell ELISA method for serotyping of *B. pertussis* is at least as good as the bacterial microagglutination assay (9). Several advantages of the indirect whole-cell ELISA method may make it a potentially attractive alternative method for serotyping pertussis strains. These advantages may include the method's objectivity and reproducibility and use of very small amounts of antibodies as well as its suitability for screening large numbers of strains. Therefore, based on this preliminary result, we propose to further evaluate and validate this method for the routine serotyping of *B. pertussis* in an interlaboratory trial involving several laboratories. Such studies may also involve the exchange of antigen preparations by different participating laboratories to compare antigenic presentation in cells prepared under different laboratory conditions, including the type of medium used to grow the bacteria, as well as to allow different laboratories to test the same batch of cells or antigens.

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