A review of the correlation of T-agglutination patterns and M-protein typing and opacity factor production in the identification of group A streptococci

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Summary. The classical techniques of M protein and opacity factor (OF) typing and T agglutination typing remain the “gold standard” in identifying group A streptococci, although newer techniques have been proposed to assist laboratory scientists, microbiologists, epidemiologists and clinicians in the precise identification and characterisation of these organisms. Because of the current scarcity of M-typing sera and the increased use by many laboratories of T typing as the sole method of group A identification, a table is presented to indicate specific correlation between the T-agglutination pattern and the M serotype. The use of this table will enable not only more selective use of typing sera but also, perhaps, result in improved understanding and ultimately in correlating these defined patterns with newer and more sensitive techniques.

Introduction

Classically, the serological identification of group A streptococci has been based on cell-wall protein antigens and although new techniques have been reported recently to improve the means of strain identification,^{1–5} these classical techniques remain in use as the standard by which other methods are evaluated.

The most important of these classification methods relates to identification of the M antigen, the protein primarily responsible for conferring virulence on group A streptococci^{6,7}. At present, although there are approximately 74 recognised M serotypes,^{6,8} group A typing antisera required to identify these M types can be very difficult to prepare and are not available commercially.⁹ Extensive sets of typing sera are available in only a few specialised reference laboratories. Monoclonal antibodies for M typing are not available. A similar situation pertains to serum opacity factor (OF) typing sera. Although currently there are fewer than 30 identified and characterised OF-producing M serotypes,^{8,10,11} availability of monovalent OF-typing sera is even more of a problem. Unless a micro-technique that can be carried out with human sera is used,¹¹ it is difficult to perform these tests for identification.

Group A streptococci can also be characterised by

the T protein, another surface antigen.¹² These T proteins are identified by the use of one of several established serological assays utilising the approximately 20 accepted anti-T sera.^{6,9,13,14} T types correlate with, but are much less specific than, M types. It is also common for a single streptococcal strain to have more than one T-protein antigen, resulting in a pattern of T proteins rather than a single type. This occurrence of T patterns may be a source of confusion since “incomplete” patterns are commonly found. T typing sera are commercially available and are prepared by certain reference laboratories.

An understanding of the relationship between the T-agglutination pattern and M or OF serotype is necessary for those involved in the study of streptococcal epidemiology, for clinicians dealing with streptococcal infection and for scientists working on streptococcal strain identification. Also, because M-typing antisera are not commercially available and are difficult to obtain, laboratories using these, whether small laboratories or larger reference centres, frequently rely on the T type or pattern to indicate which M sera to use with a given strain.⁶ Even for those laboratories that may have only an incomplete set of M-typing sera, a table for ready reference would be advantageous to allow selective testing with M or OF sera and to conserve M-typing sera.

Methods

We have constructed a table which correlates T-agglutination patterns with M or OF serotype. This is based on the accumulated data and experience from

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Table. Association of T pattern and OF production with M serotype in group A streptococci

M type	OF	Pattern(s)*		
		Common patterns	Rarer patterns	Patterns from literature†
1	N	1	3/13/B3264	1/B3264 , 1/28 , 2 , 22
2	P	2, 2/28, 8/25/Imp. 19	5/27/44, 6, 2/25, 28, 2/8/25/Imp. 19	
3	N	3, 3/13/B3264		25/B3264
4	P	4	8/25/Imp. 19, 4/28	2/4 , 11
5	N	5, 5/27/44	11	12/44 , 25 , 5/12/44‡
6	N	6	1, 2, 9, Imp. 19, B3264	2/6 , 6/25 , 6/B3264 , 6/14/25
8	P	8, 8/25/Imp. 19		
9	P	9, 9/18	5/9, 3/B3264/9	
11	P	11	5/27/44, 5	5/12/27‡
12	N	12	5/27/44, 11/12	12/44 , 12/25 , 12/27/44 , 11/12/44 , 4 , 2 , 5/12/27‡
13	P	13, 3/13/B3264, 4/28		
14	N	14, 14/49		
15	N	18, 23		15/23/47‡
17	N	23		15/23/47‡
18	N	18, 9, 9/18	23, 27, 8/27/Imp. 19	
19	N	18, 9, 23		15/23/47‡
22	P	22, 12, 12/B3264, 12/3/13	11/12, 12/3/B3264, 3	
23	N	23, 18/23, 9, 18	1	15/23/47‡
24	N	4, 4/28		
25	P	25, 8/25/Imp. 19		12/27/44
26	N	4/28		
27	P	27, 5/27/44		5/12/27‡
28	P	28, 4/28	11/28, 4	
29	N	4/28, 4, 28	23	
30	N	23		15/23/47¶
31	N	8/25/Imp. 19		
32	N	9/18, 9/27		
33	N	3/13/B3264		3
34	N	28		
36	N	9/18		
38	N	9, 18		
39	N	9/B3264		3/13/B3264‡
40	N	4	4/6	
41	N	3/13	6/23, 18/23, 9/23	3 , B3264 , 9
42	N	3/B3264	14	NT
43	N	3/13	4/3/13	
44	P	44, 5/27/44		27/44 , 5/12/27‡
46	N	4, 4/28		
47	N	18/23, 9/23		15/23/47¶
48	P	28, 4/28		
49	P	14, 14/49	11/12	
50	N	NT		
51	N	14, 14/49		
52	N	3/13/B3264	8/25/Imp. 19, 11/27	
53	N	3/13/B3264	14, 9/3/13/B3264	
54	N	23, 3/13/B3264, 28, 28/3/13	15/17/19/23/47	

Table. (cont.)

M type	OF	Pattern(s)*		
		Common patterns	Rarer patterns	Patterns from literature†
55	N	8/25, 25/Imp. 19		
56	N	25/Imp. 19, 28/3/13	4/28	3/13/B3264‡, 3/13/B3264/28§
57	N	8/25, 8, 25/Imp. 19		
58	P	25, 8/25/Imp. 19, 28, 2/28		
59	P	11, 11/12, 14	9, 25/Imp. 19	8/25/Imp. 19¶, 14/25/Imp. 19§
60	P	4		4/28‡
61	P	11, 9/11/12		5/12/27‡, 9/11§
62	P	12		5/12/27¶
63	P	4		4/28¶
64**	P	11		NT§
65	N	NT, 2/8/25		8/25/Imp. 19¶
66	P	12		
67	N	9/28		
68	P	1		
69	N	3		
70	N	28		
71	N	9, 9/18		
72	N	12/3/13, 3/13/B3264		
73	P	3/13/B3264	NT	
74	N	9		
75	P	25, 8/25/Imp. 19		
76	P	12, 11/12	3/12/B3264	
77	P	13, 13/B3264, 9/13, 9, 28	B3264	
78	P	11	11/27	
79	P	25		
80	N	14		
81	P	NT, 3/13		

P, serotype produces OF.

N, serotype does not produce OF

* Selection of combinations of T patterns. Typing patterns in laboratory may include only part of these.

† T patterns described in the literature but not observed in our laboratory.

‡ Reference 6.

§ Reference 8.

|| Reference 9.

¶ Reference 21.

** M-64 has been described as "nearly identical with" M-52⁸ but a final determination of the status of this M-type has not been made.

studies carried out in this laboratory since 1953 in which T typing and M typing of group A streptococci have been used. During this period, group A streptococcal strains have been obtained from all regions of the USA as well as from many other areas of the world, e.g., Europe, Latin America, Oceania, Asia and the Middle East. M-serotypes reference strains from established and recognised streptococcal reference laboratories have also been studied. Group A strains

were isolated from many different clinical and epidemiological circumstances, ranging from uncomplicated pharyngitis and impetigo to fulminating systemic infections, rheumatic fever and glomerulonephritis. More than 25000 serotyped group A strains form this data base.

M-typing sera used, in addition to those produced in our own laboratory, were obtained from several sources including the Central Public Health Labora-

tory, Colindale, London, the laboratory of Dr Rebecca Lancefield at the Rockefeller University, the United States Centers for Disease Control (CDC), Atlanta, GA, and the World Health Organization Collaborating Center for Reference and Research on Streptococci, Prague, Czechoslovakia. M antigen for serotyping was extracted from group A streptococci by the hot acid method of Lancefield.^{9, 15} During the first two decades of the study period, M typing was performed by the capillary precipitin method,¹⁶ thereafter all M typing in our laboratory has been by the Ouchterlony gel diffusion method described by Rotta.¹⁷

T-typing sera were mainly obtained from the Central Public Health Laboratories, Colindale, London, from the Centers for Disease Control, and from the Institute of Sera and Vaccines (Sevac), Prague, Czechoslovakia. T typing was performed by established methods.^{9, 13}

OF typing has been performed in this laboratory since the late 1960s by various methods utilising improvements in techniques. The OF-typing method developed in this laboratory by Top and Wannamaker¹⁸ was used initially and then was replaced in the early 1970s by the agar method described by Maxted.¹⁰ Since 1987 the microwell OF-typing method has been used.¹¹ Antisera used for OF typing were produced in our laboratory either by immunising guinea-pigs,¹⁹ by screening M antisera raised against OF-positive serotypes for anti-OF activity,¹⁹ or by the use of our battery of human sera containing type-specific OF antibodies.¹¹

Discussion

The use of a table, such as the one presented here, to determine the M types most likely to be associated with streptococcal strains of a given T pattern would seem simple. However, although T typing is, generally, a reliable and reproducible method for characterising group A streptococci, the idiosyncratic nature of T typing may cause the T pattern obtained with some strains to vary from laboratory to laboratory and even within the same laboratory.

This variation in T type or pattern is most strongly influenced by variation in the quality of typing antisera and in the method used to prepare (trypsinise) the streptococci for T typing.¹⁴ Although efforts are made to standardise T typing antisera during production, differences in the antibody response of the immunised animal and absorption techniques used in individual laboratories mean that sera prepared at different times or in different laboratories against the same serotype, but perhaps a different reference strain, may vary both in titre and in specificity.

Similarly, trypsinisation conditions may dramatically affect T-typing results.¹⁴ For example, the occurrence of non-typable strains may be due to excess trypsinisation of the streptococcal cells. Also, strains

carrying more than one T antigen, e.g., the very common T patterns 3/13/B3264 and 8/25/Imp 19, may give very different results depending on the intensity of trypsin treatment. For example, a strain that gives a 3/13/B3264 pattern with one combination of antisera and trypsinisation conditions may type as only a 3/B3264 with a second combination and react only with type 3 T serum with a third. This variation in T pattern is minimised when strains that are compared are tested simultaneously by the same person with standardised reagents and techniques.

Therefore, it is important to be aware that the inter-related effects of variations in reagents and in techniques will strongly influence the serotype(s) in a pattern of reactions that will dominate and determine the final T pattern of a given strain. Thus, the interpretation of the T pattern and the determination of the correlation of M type with T pattern can be much more complicated than is at first apparent.

The world-wide scarcity of M- and OF-typing sera has resulted in many laboratories using the less precise procedure of attempting to characterise group A isolates by T antigen alone. This often results in inconclusive data because not only can T patterns change due to subtle variations in technique, but multiple isolates, and even serial isolates from the same patient, may have identical T-agglutination patterns although carrying different M proteins. For example, a single T type may be found in association with several different M types, e.g., T 12 may occur with M types 12, 22, 62 among others.²⁰ Conversely, different strains of the same M type may carry different T proteins, e.g., M 2 may occur with T types 2 or 25/Imp-19).⁹ Over the years certain M types that were described were later found to be wrongly designated. These were M types 7, 10, 16, 20, 21, 35 and 45.⁶

The table presented here has been synthesised from experience in our laboratory of more than four decades, as well as from selected published sources.^{6, 8, 9, 21} An initial attempt was made to include one reference or all references for each of the described type associations, but this proved to be impractical. We hope that this table can serve as the basis for update at regular intervals in the future. No such comprehensive table relating T-agglutination pattern with all currently recognised M types has been published, as far as we are aware.

In the examination of alternative methods for the identification of group A strains, it is advantageous to have information about the relationship between T and M type for streptococcal characterisation. This will facilitate further studies on this relationship by newer techniques.

Many colleagues from laboratories around the world have contributed to the accumulated experience presented in the table. It is impossible to acknowledge each and every one and their specific contributions. However, special mention is given to the late L. W. Wannamaker, and to Margaret Ragan, whose contributions have been essential to this compilation.

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