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Effect of Incubation Temperature on T-Agglutination Typing of *Streptococcus pyogenes*

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The temperature of incubation affected the typability of beta-hemolytic group A streptococci by T-agglutination tests. When strains could not be typed after routine incubation at 30 C, they were incubated at 22 to 25 C, and nearly a 10% increase in typability was achieved. The clinical source of the strains was related to their typability. Incubation at the lower temperature was required for successful typing of higher percentages of strains from the skin and other clinical sources than from the throat. Sixty per cent of the skin strains were represented by six serotypes. Of these, 53% of the strains required incubation at 22 to 25 C before they could be typed.

Successful typing of group A streptococci by T agglutination is affected by such factors as trypsin digestion, *p*H, and the temperature at which the culture is grown. Cultures incubated at 30 C are routinely used. If satisfactory suspensions are difficult to obtain, another culture is grown at 22 C, and testing is repeated (4).

This study demonstrates the effect of incubating cultures at different temperatures on the T typing of group A streptococci isolated from the throat, skin, and other clinical sources.

MATERIALS AND METHODS

Cultures. We used 3,127 strains of group A streptococci which had been received from state health departments and individual investigators throughout the United States. Only a few strains from any single streptococcal outbreak were included in the collection. All strains were grouped serologically by the method of Swift et al. (6) before typing was attempted.

T-agglutination typing procedure. Methods used for preparing antigens for typing by T-agglutination tests were described previously (2, 4). Beta-hemolytic group A streptococci were inoculated into 5 ml of modified Todd-Hewitt Broth (Difco) and incubated at 30 C for 16 to 20 hr. If cultures were rough or failed to react with any of the six antiserum pools shown in Table 1, another culture was incubated at 22 to 25 C for 16 to 20 hr. Occasionally, strains had to be incubated for an additional 24 to 48 hr because insufficient growth resulted.

Whenever agglutination occurred in more than one antiserum pool, the suspensions were placed in a water bath, held at 50 C for 15 min, and retested. If agglutination in more than one pool persisted, the warming period was repeated until a single pool reacted. When two or more pools remained reactive after four such treatments, the suspension was typed with individual sera of the reactive pools. The components of the agglutinating pools are the following: T pool, types 1, 3, 13, B3264; U pool, types 2, 4, 6, 28; W pool, types 5, 11, 12, 27, 44; X pool, types 8, 14, 25, Imp 19; Y pool, types 22, 23; Z pool, types 9, 18.

RESULTS

The results of the T-agglutination reactions of strains isolated from various sources are presented in Table 1. Seventy-five per cent were isolated from the throat, 15% from the skin, and 10% from all other sources. Nearly 92% of the strains reacted with a single agglutination pool, but 7% failed to react with any of the six pools. More than 94% of the strains from the throat, 90% from the skin, and 92% from all other sources were typable. Reactions (64%) of throat isolates occurred predominantly in the W or U pools, but reactions of skin isolates occurred predominantly (63%) in the T or X pools.

Table 2 shows the percentages of strains that required incubation at 22 C before T typing was achieved. Only 7% of the throat isolates required incubation at the lower temperature. Higher percentages (13 to 16%) of the throat isolates reacting with the T, X, or Y pools required incubation at the lower temperature than the percentages of those (4 to 6%) reacting with the U, W, or Z pools. Among the skin strains, 18% failed to react when grown at 30 C, but they did react when the cultures were grown at 22 C. At least 26% of the skin strains which reacted with the T or X pools required incubation at 22 C before typing was achieved. Fifteen per cent of the isolates from all other sources failed to react

TABLE 1. *Agglutination reactions of 3,127 strains of group A streptococci isolated from various sources*

Reacting pools	No. of reacting strains from various sources						Total no.	Per cent
	Throat	Skin	Nose	Blood	Wounds	Others		
T	348	136	22	11	7	36	560	18
U	567	30	1	12	5	22	637	20
W	842	74	11	17	18	50	1,012	32
X	210	164	4	4	10	14	406	13
Y	70	11	1	1	1	2	86	3
Z	166	8	0	3	12	8	197	6
Two pools	15	5	0	0	0	0	20	1
None	140	47	11	2	1	8	209	7
Total	2,358	475	50	50	54	140	3,127	100
Per cent	75	15	2	2	2	4	100	

TABLE 2. *Per cent of group A streptococci from throat, skin, and other sources requiring growth at 22 C for agglutination*

Reacting pool	Throat strains	Skin strains	Strains from other sources	Pool percentage
T	13	26	24	18
U	6	3	10	6
W	4	5	5	5
X	14	27	41	21
Y	16	18	60	19
Z	4	13	0	4
Two pools	13	20	0	15
Total	7	18	15	9

with any agglutination pool when cultures were incubated at 30 C, but they did agglutinate when grown at 22 C. Twenty-four to 41% of those cultures from other sources which reacted with the T or X pools required incubation at 22 C.

Table 2 also shows that an average of 4 to 6% of the strains reacting with the U or W pools required incubation at 22 C, regardless of the clinical source. However, an average of 18 to 21% of the strains that reacted with the T or X pools required incubation at the lower temperature. Among these, the percentage of strains from skin or other sources was approximately twice that of strains from throat cultures.

Table 3 shows the agglutination reactions of strains which reacted with the T pool. Strains of T type 1 were more commonly isolated from the throat than from other sources and were easier to type with suspensions grown at 30 C than were other types in the pool. Types 3/13 and 3/13/B3264 were frequently isolated from both the throat and skin. Of these types, fewer throat (11 to 18%) than skin (22 to 31%) isolants required incubation at 22 C. These two types represented 89% of the skin cultures which agglutinated the T pool.

Table 4 shows the agglutination reactions of the strains which reacted with the X pool. Four types which occurred more frequently than others are 14, 8/14/25/Imp 19, 8/25/Imp 19, and 25/Imp 19. The skin strains were more difficult to type than the throat strains. In one case, 8/25/Imp 19, the difference was quite pronounced. Only 4% of the throat isolates required growth at 22 C before reactions were observed, but 41% of the skin isolants required such growth. One type, 8/14/25/Imp 19, was isolated from the skin more commonly than from the throat. This type was easier to type than the other skin strains reacting

TABLE 3. *Types of group A streptococci from throat, skin, and other sources reacting with T pool and percentages requiring incubation at 22 C*

Types	Throat		Skin		Other sources		Total	
	No. of strains	Per cent at 22 C	No. of strains	Per cent at 22 C	No. of strains	Per cent at 22 C	No. of strains	Per cent at 22 C
1	60	3	4	25	7	14	71	6
3	20	0	9	22	7	14	36	8
B3264	0	0	1	0	0	0	1	0
3/13	200	11	61	31	31	32	292	17
3/B3264	2	0	0	0	0	0	2	0
1/3/B3264	8	0	0	0	1	0	9	0
3/13/B3264	49	18	59	22	30	23	130	22

TABLE 4. *Types of group A streptococci from throat, skin, and other sources reacting with X pool and percentages requiring incubation at 22 C*

Types	Throat		Skin		Other sources		Total	
	No. of strains	Per cent at 22 C	No. of strains	Per cent at 22 C	No. of strains	Per cent at 22 C	No. of strains	Per cent at 22 C
14	76	16	42	33	8	13	126	21
25	1	100	2	0	0	0	3	33
Imp 19	11	18	11	36	8	13	30	23
8/25	7	0	10	40	0	0	17	24
8/25/Imp 19	27	4	32	41	0	0	59	24
8/25/Imp 19/14	2	0	30	15	0	0	32	13
14/25	0	0	1	0	0	0	1	0
14/25/Imp 19	1	0	4	75	0	0	5	60
25/Imp 19	84	14	41	29	18	11	143	18

with this pool. The above four types represented 84% of the skin cultures agglutinating the X pool.

DISCUSSION

Many investigators have found typing by T-agglutination tests a valuable tool for characterizing group A streptococci (1, 3–5, 7; W. Maxted and M. T. Parker, *personal communication*; M. T. Parker, presented at Symposium of Streptococcal Infections, Jena, Germany). Experience with the test indicated that the source of the strain was frequently associated with its typability. The percentage of nontypable strains was higher among strains from the skin than from the throat. In this respect, the T-typing system was similar to the M-typing system.

It was demonstrated that typability of strains by T typing could be increased nearly 10% by testing cultures incubated at 22 C. This increase was higher for strains isolated from the skin (18%) and other sources (14.6%) than from the throat (6.8%).

There was an appreciable difference in the difficulty involved in typing the throat isolants, depending upon the pool that the strain agglutinated. For example, among the throat strains requiring incubation at the lower temperature, the percentage of strains that reacted with pools T, X, and Y was twice that of those which reacted with pools U, W, and Z. This phenomenon was more pronounced among the skin isolants.

A relationship existed between the tendency of a strain to require a lower incubation temperature to attain typability and the serotypes of the strains. The types which were most commonly associated with the skin reacted with the T pool and were types 3/13 and 3/13/B3264. The skin strains of the X pool which presented the greatest problem were 14, 8/25/Imp 19, 25/Imp 19, and 8/14/25/Imp 19. These types represented 60% of the total skin strains. Of these strains, 53% required incubation at room temperature before typing was achieved.

The special difficulty of typing strains reacting with the T and X pools did not seem to be correlated with a lower titer of the T and X pools of antisera. All monospecific sera were adjusted so that a 1:160 dilution reacted satisfactorily with the testing strains after pools were made. Increasing strain typability probably resulted from increased T-antigen production in the strains at the lower temperature and not from the antisera.

Although it has been demonstrated that T-agglutination typing for certain strains can be achieved more readily at the lower temperature, evidence indicating that all strains should be grown at 22 to 25 C rather than at 30 C is not available. Also, no data are available which would indicate that strains should first be grown at 22 to 25 C and then at 30 C. Maintaining standard procedures and incubating all strains at 30 C for preparation of suspensions seem advisable. Then, if typing is not achieved on the first attempt, the lower incubation temperature can be used as an alternative.

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