Effect of Delay in Processing on the Performance of Directigen for the Detection of Group A Streptococci in Throat Swabs

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We compared a latex agglutination method, Directigen (Hynson, Westcott & Dunning) with a culture-based system for detection of group A streptococci in 964 throat swabs. Discordants were resolved by Lancefield typing of beta-hemolytic colonies. After culture, swabs were randomly assigned to be tested by Directigen within 4 h of sampling or after overnight storage. For swabs that yielded more than 50 beta-hemolytic colonies from culture, there was a highly significant difference ($P < 0.01$) in sensitivity between the group tested early and the group tested after storage overnight. For swabs tested within 4 h of sampling, a trend ($P = 0.07$) existed between sensitivity and length of delay before testing.

Previous studies have shown that the Directigen (Hynson, Westcott & Dunning, Baltimore, Md.) latex agglutination test for detection of group A streptococci directly from throat swabs has a sensitivity between 71 and 91% and a specificity between 93 and 99% (3; G. R. Greene, N. Uchiyama, and E. Mendoza, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 24th, Washington, D.C., abstr. no. 18, 1984; R. E. Klicker, R. P. Gruninger, and S. M. Nelson, 24th ICAAC, abstr. no. 23; P. A. Kjos and J. P. Anhalt, 24th ICAAC, abstr. no. 13). Sensitivity has generally been less with swabs that yield fewer than 50 colonies on culture than with swabs that yield more colonies. Preliminary results from one study (P. A. Kjos and J. P. Anhalt, 24th ICAAC, abstr. no. 13) also showed that sensitivity might be affected by the length of time that specimens were stored before testing. We now report more extensive studies of the effects of processing delay on sensitivity and specificity of Directigen for detection of group A streptococci in throat swabs.

Study design. Oropharyngeal specimens from symptomatic patients seen at the Mayo Clinic and affiliated hospitals were collected and transported to the laboratory by use of the Culturette system (Marion Scientific, Kansas City, Mo.), which employs a rayon-tipped swab and modified Stuart transport medium. Specimens were entered into the study within 2 h of receipt by the laboratory, and no distinction was made on the basis of patient age. Specimens were excluded when the time after collection exceeded 4 h or when they were not received in the Culturette system. Between late August and early October, 964 swabs were accepted into the study.

Each swab was inoculated onto one plate of T-Soy agar containing 5% sheep blood (GIBCO Laboratories, Madison, Wis.) and was then assigned randomly to one of two groups for testing with Directigen either immediately (485 swabs) or after overnight storage at room temperature (479 swabs). Directigen testing was done exactly according to recommendations of the manufacturer. Particular care was taken to ensure that all reagents were at room temperature before use. Swabs were vortexed for 5 s with the extraction reagent, and glass plates used in the test were decontaminated with a phenolic solution (3% Amphyl, Sterling Drug, Montvale, N.J.) before rinsing. Incubation of swabs with the

Directigen extraction reagent was at 35°C in a conventional laboratory incubator. A full set of positive and negative controls was tested each day, and a negative control was tested with each batch of specimens. Occasional samples gave ambiguous agglutination results. These were retested by using the supernatant obtained after centrifugation of the extract at $1,400 \times g$ for 5 min.

Cultures were examined for beta-hemolytic colonies after overnight incubation in air (2, 5, 7). Negative cultures were incubated for an additional 24 h and examined before being discarded. Colonies showing beta-hemolysis were enumerated by using a grading scale of 1+ (<10 colonies), 2+ (10 to 50 colonies), 3+ (>50 colonies), and 4+ (colonies too numerous to count). Group A streptococci were identified by a conventional fluorescent-antibody (FA) technique (1, 4).

Resolution of discrepancies. When Directigen gave a negative result and the FA result was positive for group A streptococci, a beta-hemolytic colony from the culture was tested with Directigen. If the Directigen test of the isolate was positive, the specimen was considered confirmed for containing group A streptococci, but the original Directigen result was counted as a false-negative test. If the Directigen test of the isolate was also negative, Lancefield grouping was done by the Rantz-Randall extraction method (6). When

TABLE 1. Performance of Directigen for detection of group A streptococci from throat swabs

Test group	Directigen result	Confirmed result		% Sensitivity ^a	% Specificity ^b
		Positive	Negative		
Immediate	Positive	57	3	61	99
	Negative	36	389		
Overnight	Positive	57	3	72	99
	Negative	22	397		
Combined	Positive	114	6 ^c	66	99
	Negative	58	786		

^a Percent sensitivity = (number of Directigen true positive results/number of confirmed positive specimens) $\times 100$.

^b Percent specificity = (number of Directigen true negative results/number of confirmed negative results) $\times 100$.

^c A group C *Streptococcus* was isolated from one specimen. The remaining specimens did not yield any beta-hemolytic colonies.

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Directigen was positive and FA testing was negative, the cotton plug in the Culturette was swirled in Todd-Hewitt broth, and a portion of the broth was inoculated onto sheep blood agar. After incubation for up to 48 h, any beta-hemolytic colonies present were identified by Lancefield grouping (6). No additional group A streptococci were recovered by this method.

Statistical analysis. Comparisons were evaluated for statistical significance by calculation of chi-square. A two-tailed distribution was assumed.

Results and discussion. Group A streptococci were confirmed in 172 of the 964 specimens (17.8%). Of these, Directigen detected 114, giving an overall sensitivity of 66%. Directigen correctly identified 786 of 792 specimens as negative, giving a specificity of 99% (Table 1). FA testing was positive for three specimens that were negative by both Directigen and Lancefield grouping. Specificity of Directigen was not affected by storage of swabs overnight; however, there was a trend ($P = 0.11$) toward increased sensitivity when stored swabs were tested (Table 1). This trend reached significance ($P < 0.01$) when only results from cultures with more than 50 beta-hemolytic colonies were considered (Table 2).

The exact time of specimen collection was determined for 75 swabs that yielded group A streptococci and were included in the group tested within 4 h of collection. The delay between collection and Directigen testing was classified into three ranges. Comparison of results for specimens tested within each time interval tended to show ($P = 0.07$) a progressive increase in sensitivity with delay before testing (Table 3).

Our results are in accord with those of McCusker et al. (3) that show Directigen to be highly specific for detection of group A streptococci in throat swabs. The sensitivity of Directigen for detection of group A streptococci was lower than that reported by McCusker et al. (3), but when results for swabs tested within 4 h of collection were excluded from analysis, the sensitivity we observed was within the range of sensitivities reported by other investigators. Several factors

TABLE 2. Effect of colony count and testing delay on Directigen sensitivity for detection of group A streptococci

Colony count ^a	Directigen result	Confirmed positive results			
		Immediate		Overnight	
		No.	% Sensitivity ^b	No.	% Sensitivity
>50	Positive	55	69	55	87
	Negative	25		8	
<50	Positive	2	15	2	13
	Negative	11		14	

^a Number of colonies per plate showing beta-hemolysis.

^b See Table 1, footnote a for definition.

TABLE 3. Effect of testing delay in the immediate test group on Directigen sensitivity

Delay (h) ^a	No. confirmed positive ^b	Directigen result		% Sensitivity
		Positive	Negative	
≤1	24	10	14	42
1-2	35	23	12	66
2-4	16	12	4	75

^a Measured from the time specimen was obtained from patient to initiation of Directigen test.

^b Delay could not be determined within the time limits shown for an additional 48 confirmed positive specimens.

probably affect sensitivity. McCusker et al. (3) used a heat block to incubate the extraction mixture, whereas we used a conventional bacteriological incubator. Whether this difference adversely affects sensitivity is currently under study. The effect of processing delay on sensitivity, as shown in the present study, would be expected to be most evident if some other factor, such as incubation conditions, combined to decrease sensitivity to a marginal level.

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