

False-Positive Cryptococcal Antigen Latex Agglutination Caused by Disinfectants and Soaps

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Five disinfectants or soaps were tested to determine if any could be responsible for false-positive results obtained with the Latex-Crypto Antigen Detection System kit (Immuno-Mycologics, Inc., Norman, Okla.). Three disinfectants or soaps (Derma soap, 7X, and Bacdown) produced false-positive agglutination after repeated washing of ring slides during testing of a known negative cerebrospinal fluid specimen.

The Latex-Crypto Antigen Detection System is a latex agglutination test that detects capsular polysaccharide antigen of *Cryptococcus neoformans* in cerebrospinal fluid (CSF) and serum (3). *C. neoformans* antigen can be detected in specimens from patients with central nervous system involvement (cryptococcal meningitis) and pulmonary cryptococcosis (3).

False-positive rates from latex cryptococcal antigen tests of as high as 15% have been reported (2). Explanations for such false-positive agglutination reactions have been addressed previously. For example, immunosuppressed patients with disseminated *Trichosporon beigelii* infection may generate false-positive agglutination results, because *C. neoformans* and *T. beigelii* share an antigen (4). Introduction of talc from latex gloves into CSF and surface condensation (syneresis fluid) from agar to the CSF, prior to latex testing, may also produce false-positive agglutination (1, 3). To our knowledge, this is the first report of false-positive latex agglutination caused by various disinfectants and soaps used to clean ring slides.

The Associated Regional and University Pathologists microbiology laboratory was obtaining intermittent false-positive agglutination results while using the Latex-Crypto Antigen Detection System kit. When all other published explanations for false-positive agglutinations were eliminated, we designed a two-part investigation to determine if disinfectants and soaps used to clean the ring slides could cause false-positive reactions when those slides were subsequently used in screening for cryptococcal antigens. The first experiment was designed to observe whether direct interaction of disinfectants and soaps with latex particles caused false-positive reactions. This evaluation was accomplished by screening individual disinfectant reagents with latex particles. The second observation was designed to simulate what occurs in the laboratory. This simulation was done by screening known negative specimens of serum and CSF on ring slides that were repeatedly washed with disinfectants or soaps. The test solutions used in this study were Derma soap (manufactured by Minnetonka Medical, Minnetonka, Minn.) (1:1 dilution), 7X (manufactured by ICN Biomedicals, Inc., Costa Mesa, Calif.) (1:1 dilution), bleach (manufactured by Clorox Company, Oakland, Calif.) (1:10 dilution), Amphyl (manufactured by Lehn and Fink Industrial Products divisions, Montvale, N.J.) (1:10 dilution), and Bac-

down (manufactured by Decon Laboratories, Inc., Bryn Mawr, Pa.) (2 oz. [~59 ml] quantum sufficit 1 gal [~4 liters]).

Positive (LCAC) and negative (NC) controls were supplied by the test kit manufacturer and were used in each trial.

Direct screening procedure. Aliquots of each disinfectant were placed in separately labeled nonsiliconized test tubes. Twenty-five microliters of latex reagent was dispensed on 7 different rings of an unused ring slide (12 rings per slide), direct from the manufacturer. To each of the seven rings was added one of the following substances: 25 μ l of LCAC, 25 μ l of NC, or 25 μ l of the various disinfectants and soaps. The suspensions were mixed with wooden applicators, and the ring slides were placed on a tabletop rotator (Wellcome Diagnostic) and rotated for 9½ min at 100 rpm. Three technologists were randomly selected to independently read each reaction of the positive and negative controls and the reaction of disinfectants and soaps in the three trials (nine readings). Also, an evaluation of the reliability of NC was added to this observation. Solutions (1:1) of the negative control, latex particles, and detergents and soaps were tested by the same method as described above to see whether false-positive agglutination occurred.

Positive results ranged from the formation of particles within a cloudy background (agglutination reaction of 1+) to large clumps of particles within a clear background (4+). Negative results were indicated by a cloudy suspension with no particles. For each of the trials, two of three technologists' readings had to agree before a reaction was called false positive. In each trial, all technologists agreed that all positive controls possessed reactions of 2+ to 3+ for agglutination and all negative controls were free from agglutination, including those for the testing with NC, latex particles, and detergents and soaps.

Repeated cleansing procedure. The investigation of disinfectant buildup on ring slides was observed by cleaning labeled ring slides, each with a designated disinfectant or soap, for 10 cleaning cycles. After each trial, the slides were washed with the assigned disinfectant or soap and then were rinsed with distilled water and air dried. A known negative serum specimen and a known negative CSF specimen were treated with detach enzyme and placed in a 56°C heating block for 30 min. Each specimen was treated with enzyme inhibitor to discontinue the reaction between the specimen and the detach enzyme. On a ring slide that had been cleaned with one of the aforementioned disinfectants, 25 μ l of latex that was later mixed with 25 μ l of LCAC, 25 μ l of NC, or 25 μ l of known negative patient specimens was added to four different wells.

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TABLE 1. Direct screening of disinfectants and soaps with cryptococcal latex particles performed by three technologists

Trial and technologist	Agglutination reaction ^a with cleansing agent				
	Derma soap	7X	Bleach	Amphyl	Bacdown
Trial 1					
1	-	1+	-	1+	1+
2	-	1+	-	1+	1+
3	-	1+	-	-	1+
Trial 2					
1	-	1+	-	-	1+
2	-	1+	-	-	1+
3	-	1+	-	1+	1+
Trial 3					
1	-	1+	-	1+	1+
2	-	1+	-	1+	1+
3	-	1+	-	1+	1+

^a Procedure was performed as described in the section on direct screening procedures. Results are for CSF only.

The suspensions were mixed with wooden applicators, and the slide was placed on a tabletop rotator and rotated for 9½ min at 100 rpm. Again, three random technologists, not involved in the study design, independently read each of the quality controls and the reaction of each disinfectant or soap in the 10 trials, and the results were recorded. The positive and negative results were interpreted as previously mentioned.

In the course of the direct screening investigation, three test solutions produced false-positive agglutination. Test solutions 7X, Amphyl, and Bacdown produced false-positive agglutination reactions (Table 1). The 7X and Bacdown products generated 1+ false-positive results in all three trials, while Amphyl was considered to be positive in two of three replications. The NC did not agglutinate with any of the detergents.

Table 2 shows the results of disinfectant or soap buildup on ring slides. False-positive agglutination occurred with Derma soap, 7X, and Bacdown. Both Derma soap and 7X produced a 1+ false-positive agglutination in 1 of 10 trials. Bacdown produced 1+ to 4+ false-positive agglutination in 5 of 10 trials. The more often this reagent was used to cleanse ring slides the more intense the false-positive agglutination. All false-positive agglutinations occurred with the CSF specimen and not with the serum.

This study provides evidence that disinfectants and soaps used to clean ring slides can affect cryptococcal antigen latex agglutination results. All disinfectants and soaps except Amphyl were used in dilutions suggested by the manufacturer. It is unclear why the disinfectants and soaps cause false-positive

TABLE 2. Results of repeated cleansing of ring slides with disinfectants and soaps during CSF screening for cryptococcal antigen, performed by three technologists

Soap	Technologist	Agglutination reaction ^a for trial									
		1	2	3	4	5	6	7	8	9	10
Derma soap	1	-	-	-	-	-	1+	-	-	-	-
	2	-	-	-	-	-	1+	-	-	-	-
	3	-	-	-	-	-	1+	-	-	-	-
7X	1	-	1+	-	-	1+	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	1+	-	-	-	-	-	-	-	1+
Bacdown	1	-	1+	1+	-	3+	-	-	-	4+	3+
	2	-	1+	1+	-	3+	-	-	-	3+	2+
	3	1+	1+	1+	-	1+	-	-	1+	4+	3+
Amphyl	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-
Bleach	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-

^a Procedure was performed as described in the section on direct screening procedures.

agglutination and why CSF but not serum specimens are affected.

On the basis of these observations, clinical microbiology laboratories should be aware that disinfectants and soaps can produce false-positive agglutination with a latex reagent. False-positive agglutination may be eliminated by rinsing ring slides with bleach (10%) and distilled water after cleaning with a disinfectant or soap. However, soaking repeatedly used ring slides in bleach may present problems such as etching of the slides and destruction of the materials used to make the ring. This surface deterioration may provide a reservoir for protein buildup and may cause false-positive agglutination. One may consider the use of disposable ring slides to avoid false-positive agglutination produced by disinfectants and soaps.

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