

## Evaluation of Copan Swabs with Liquid Transport Media for Use in the Gen-Probe Group A Strep Direct Test

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**The Gen-Probe Group A Streptococcus Direct Test (GASDT), which detects the presence or absence of group A streptococci directly from pharyngeal specimens, utilizes a specific relative light unit (RLU) cutoff of 4,500 to differentiate between positive and negative test results. In response to a report by a manufacturer that the background RLU values for the Copan rayon swabs with liquid media were higher than the RLU values typically observed with Culturette swabs, we tested multiple lots of Copan rayon swabs with liquid media and determined that the swabs are unacceptable for routine use in the GASDT. The high background RLU values for the Copan rayon swabs appear to be a direct result of the gamma irradiation used to sterilize the swabs. We also performed a comparative clinical evaluation of Copan Dacron swabs with liquid media and Culturette swabs for use in the GASDT. Overall, there was 97.5% agreement between the results obtained with the Copan Dacron swabs and those obtained with the Culturette swabs. Compared to Culturette swabs, the Copan Dacron swabs had a sensitivity and a specificity of 97 and 98%, respectively. Copan Dacron swabs with liquid media are an acceptable alternative to the swabs currently qualified for use with the GASDT, but Copan rayon swabs with liquid transport media should not be used in the GASDT.**

The Group A Streptococcus Direct Test (GASDT; Gen-Probe, Inc., San Diego, Calif.) is used to detect the presence or absence of group A streptococci directly from pharyngeal specimens. Because the GASDT utilizes chemiluminescence technology and a cutoff of 4,500 specific relative light units (RLU) to differentiate between positive and negative test results, it is important that individual swab types be qualified for use in the assay. We previously evaluated the use of the Culturette swab (Becton Dickinson Microbiology Systems, Cockeysville, Md.) when the assay was introduced in our laboratory (3). We subsequently performed an in-house comparison of the Culturette and Bacti-Swabs (Remel, Lenexa, Kans.) in the GASDT and detected no significant differences in the background RLU values of the two products. Both the Culturette and Bacti-Swabs utilize a rayon swab with liquid Stuart's medium.

In 2001, Becton Dickinson replaced the Culturette swab with the CultureSwab brand of swab transport devices. Copan (Brescia, Italy) manufactures the CultureSwab transport devices. Culturette swabs utilize a rayon swab in a single tube with Stuart's medium enclosed in a glass ampoule that is crushed after the inoculated specimen is placed into the tube. The product is sterilized with ethylene oxide. Copan utilizes a peel pouch containing a swab stick attached to a cap and a tube containing the transport medium in either a liquid form soaked into a polyurethane sponge or a gel. The Copan product that is considered comparable to the Culturette product is a rayon swab with liquid Stuart's transport medium that is sterilized with gamma irradiation.

As the Copan swab transport devices with liquid Stuart's medium were being introduced in the United States, we

learned from a manufacturer that Copan rayon swabs with liquid media had higher background RLU values in the GASDT than were typically observed with Culturette swabs, which resulted in apparent false-positive test results with the GASDT.

This study was initially designed to evaluate the *in vitro* use of the Copan rayon swabs with liquid media in the GASDT. When our evaluation of the Copan rayon swabs indicated that they were not acceptable for use in the GASDT, we then considered the use of Dacron swabs. In-house work at Copan had indicated that a Dacron swab with liquid Stuart's medium performed acceptably in the GASDT (N. Sharples, unpublished data). Using a Copan Dacron swab with liquid transport medium (Stuart's or Amies) and one of the then-qualified rayon swabs, the Culturette swab, we performed a comparative clinical evaluation of the two swabs for use with the GASDT.

Two swabs from each of 10 lots of Copan rayon swabs with liquid Amies transport medium were tested, uninoculated, with the GASDT. Each swab was placed into the transport tube containing the sponge and allowed to remain in contact with the sponge for approximately 15 min before the GASDT was performed. The GASDT was performed by following the instructions in the package insert as previously described (3).

The GASDT was performed on two swabs from each of five lots of the Copan rayon swabs with and without the polyurethane sponge present in the transport tube. Since the liquid transport medium (Amies or Stuart's) is in the sponge, testing without the sponge is equivalent to testing a dry rayon swab. For the swabs tested without the sponge, the sponge was carefully removed from the tube with sterile forceps before the swab was inserted into the tube.

Two swabs from each of two lots of Copan rayon swabs were also tested with and without the addition of the probe reagent for the GASDT. For those swabs tested without the probe

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TABLE 1. Effect of gamma irradiation on rayon swabs as measured with the Gen-Probe GASDT

Swab type	Mean RLU value <sup>a</sup> for indicated day of storage prior to testing		
	1	70	120
Irradiated	3,622	5,664	6,548
Nonirradiated		2,091	2,411

<sup>a</sup> Values are the mean results for 10 lots of swabs tested in duplicate.

reagent, the GASDT was performed by following the same standard protocol as that used for the paired control specimens (those with the probe reagent), with the exception that the 50  $\mu$ l of probe reagent was not added to the tubes.

Paired Culturette and Copan Dacron swab specimens were simultaneously collected from 774 patients presenting to Geisinger Health System clinics. Specimens were collected from each patient with either a Culturette swab and a Copan Dacron swab with liquid Amies transport medium or a Culturette swab and a Copan Dacron swab with liquid Stuart's transport medium. Paired swab specimens were tested within 24 h of collection in the same run of the GASDT in the microbiology laboratory at Geisinger Medical Center. The GASDT was performed as previously described (3). For specimens with discrepant results, the pledgets were cultured and worked up as previously described (3).

Two swabs from each of 10 different lots of Copan rayon swabs with liquid Amies medium were tested with the GASDT with no added inocula. The mean RLU value for the 10 lots was 3,622. After initial testing of these 10 lots, we obtained nonirradiated swabs of the same lots from Copan. Approximately 14 and 17 weeks after the initial testing, we tested paired irradiated and nonirradiated swabs from the same lots at the same time by using the GASDT. For the testing at 14 and 17 weeks, the same protocol was employed as was previously utilized with the 10 lots of irradiated swabs; no inocula were added to any of the swabs. The results are summarized in Table 1. The RLU values were significantly higher ( $P < 0.001$  for both testing dates) for the irradiated swabs than for the nonirradiated swabs on both testing dates. On both testing dates, the swabs from the 10 lots that were irradiated had RLU values that would be interpreted as positive ( $\geq 4,500$ ), while all of the nonirradiated swabs had RLU values that would be interpreted as negative. For these 10 lots, the mean RLU values increased over the 14-week time period of the testing.

Five lots were also tested with the GASDT with and without the polyurethane sponge. The liquid transport medium is in the sponge. The mean RLU value for the five lots with the sponges was 4,173, while the mean RLU value for the five lots without the sponges was 4,095.

To better understand the contributions of different assay components to the total RLU values, we tested two lots of irradiated and nonirradiated swabs with and without the addition of the probe reagent to the assay. For the irradiated swabs, the mean RLU values were 4,764 with the probe and 2,399 without the probe. For the nonirradiated swabs, the mean RLU values were 2,004 with the probe and 1,202 without the probe.

TABLE 2. Comparison of results of GASDT performed with different swab types

Copan transport medium	No. of paired specimens with indicated GASDT result <sup>a</sup>			
	Culturette (+), Copan (+)	Culturette (-), Copan (-)	Culturette (+), Copan (-)	Culturette (-), Copan (+)
Liquid Amies	130	274	3	8
Liquid Stuart's	101	250	4	4
Total	231	524	7	12

<sup>a</sup> Results were positive (+) or negative (-).

A total of 774 paired clinical specimens were tested in this study, 415 that were collected with a Culturette swab and a Copan Dacron swab with Amies liquid transport medium and 359 that were collected with a Culturette swab and a Copan Dacron swab with liquid Stuart's transport medium. The results are summarized in Table 2. The combined overall agreement was 97.5% between the Culturette swabs and the Copan Dacron swabs with liquid medium (Stuart's or Amies), 97.4% between the Culturette swabs and the Copan Dacron swab with Amies medium, and 97.8% between the Culturette swabs and the Copan Dacron swab with Stuart's medium. By using the GASDT result obtained with the Culturette swab as the "gold standard," the sensitivity of the GASDT performed on the Copan Dacron swab with liquid Stuart's or Amies medium was 97% and the specificity was 98%.

Overall, there were 19 specimens with discrepant test results for the two comparative swab systems with the GASDT (Table 2). When the pledgets for these 19 specimens were cultured, group A streptococci grew from the pledgets for 15 of the specimens. Of the four specimens that failed to grow group A streptococci from the subculture of the pledgets, three had a positive GASDT result with the Copan swab only, and one had a positive GASDT result with the Culturette swab only.

We found the Copan swabs easier to use than the Culturette swabs because, unlike the Culturette swabs, the Copan swabs do not have an ampoule that has to be crushed. The Copan swabs were also easier to wring out in the Gen-Probe lysis buffer, apparently because the swabs' fibers are more tightly wound onto the sticks.

The GASDT utilizes a chemiluminescent probe to detect group A streptococci directly from pharyngeal specimens collected with swabs. As specified in the package insert, the determination of a positive test result is based upon an RLU value; a value greater than or equal to 4,500 RLU indicates a positive result, and a value less than 4,500 RLU indicates a negative result. Previous evaluations performed in our laboratory (3) and at the Mayo Clinic (6) have utilized Culturette swabs to validate the assay with the cutoff of 4,500 RLU.

In our experience, differences between commercially available swabs as well as between sterilization methods are not well understood by most clinical microbiologists. Both the Becton Dickinson Culturette swab and the Remel Bacti-Swab utilize swabs with rayon fibers and liquid Stuart's transport medium. Both are also sterilized by exposure to ethylene oxide gas. Rayon fiber is made from cellulose, a naturally occurring prod-

uct. Dacron fiber, on the other hand, is a synthetic polyester (5). Although both Dacron and rayon have historically been used for microbiological swabs, it is our impression that in the United States rayon is used more frequently. It is critical that when a new application of a swab is being considered, such as for the GASDT, careful evaluations be conducted to ensure that the swab materials perform comparably.

We learned from a manufacturer about a potential problem with the use of the Copan rayon swabs with liquid media for the GASDT. This prompted us to test multiple lots of Copan rayon swabs in the GASDT. After initially testing irradiated swabs from 10 lots, we performed testing with paired irradiated and nonirradiated swabs from the same lots. When tested in parallel on two occasions, all the irradiated swabs yielded RLU values that were interpreted as positive with the GASDT, while all the nonirradiated swabs yielded RLU values that were interpreted as negative. In comparison to the results shown in Table 1, the mean RLU value for 759 negative specimens collected on Bacti-Swabs from a previous study was 1,854 (P. Bourbeau and B. Heiter, *Abstr. 102nd Annu. Meet. Am. Soc. for Microbiol.*, abstr. C-245, 2002). Additional studies demonstrated that the elevated RLU values were not due to the medium or the sponge, as results without the sponge were comparable to results with the sponge present. Even when the acridinium ester-labeled probe, which generates the chemiluminescent product for the assay, was removed, relatively higher RLU values were observed for the irradiated swabs than for the nonirradiated swabs. We conclude that the effect of gamma irradiation on rayon swabs makes them unacceptable for use with the GASDT.

We are aware of only one other reported problem in performing the GASDT with the Copan rayon swabs with liquid media. Chapin and colleagues reported that the Copan rayon swab gave irreproducible results with the GASDT, but they provided no specific data (2). Although we have tested only Copan swabs, we believe that our conclusions regarding the appropriateness of gamma-irradiated rayon swabs for the GASDT should be applicable to comparable swabs made by other manufacturers as well.

The effects of gamma irradiation on polymers are well described in the industrial literature (7, 9). Gamma rays are high-energy photons which lead to the production of free electrons and ultimately to ionization and free-radical production. When oxygen is present, superoxide molecules can be formed and the oxidation of polymers occurs. Polymer deterioration and discoloration (yellowing) have been reported. Minimizing exposure to oxygen lessens but does not eliminate the effects of gamma irradiation on polymers. Furthermore, spontaneous chemiluminescence can result from exposure to gamma irradiation (7, 9). While we have no way of specifically determining exactly what occurs when rayon fibers are exposed to gamma irradiation, our results clearly demonstrate the deleterious effect of gamma irradiation on the utility of rayon swabs in the GASDT.

All Copan swabs with media are sterilized by gamma irradiation (N. Sharples, unpublished data). Ethylene oxide cannot be utilized because sterilization with ethylene oxide produces residual ethylene oxide in the media that would be toxic for microorganisms. Ethylene oxide is appropriate for the Cul-

turette and Bacti-Swab swabs, where the medium is in an ampoule and thus not directly exposed to the ethylene oxide.

Our results in a clinical evaluation indicated that a Copan Dacron swab, utilizing liquid media and sterilized with gamma irradiation, gives acceptable performance in the GASDT. Particularly germane to this evaluation was whether a cutoff of 4,500 RLU is acceptable for discriminating between positive and negative test results when the gamma-irradiated Copan Dacron swab is used. We noted no significant differences in performance between the Culturette and Copan Dacron swabs. Overall agreement between the test results for the GASDT was greater than 97%. For 15 of the 19 specimens with discrepant test results, the presence of group A streptococci was confirmed by the subculture of a pledget. Our experience with evaluations of the GASDT suggests that many of the specimens that yield discrepant results contain small numbers of group A streptococci. Unequal distribution of organisms between two swabs has been clearly demonstrated in other studies. Kellogg has reported that sampling variations may lead to a false-negative result rate of about 10% (4).

The only other study of which we are aware that has utilized Copan Dacron swabs with liquid media in the GASDT was performed by Chapin and colleagues (2). They compared the GASDT with a rapid antigen test and with culture. The sensitivity of the GASDT in their study was 94.8% compared to a sensitivity of 99.4% for culture.

Our purpose in testing Copan Dacron swabs with two different liquid transport media, Amies and Stuart's, was to determine whether there were any performance differences in the GASDT between the two media in the Copan Dacron swabs in comparison to a Culturette swab. Amies transport medium was developed in an attempt to improve on the performance of Stuart's medium for culture (1, 8). We are aware of no previous evaluations of the GASDT which have tested swabs with different liquid transport media. In this study, we noted no significant difference in the performance of the two transport media in the Copan Dacron swabs in comparison to that of the Culturette swabs with liquid Stuart's medium. Nonetheless, in our opinion, further comparisons of Amies and Stuart's liquid transport media for use in the GASDT may be merited.

In conclusion, Copan rayon swabs with liquid media are not acceptable for use in the GASDT. However, Copan Dacron swabs with liquid media are acceptable for use in the GASDT and, indeed, yield results that are comparable to the results obtained with Culturette swabs. This is the only study of which we are aware that has specifically done a performance comparison in the GASDT of Culturette and gamma-irradiated Dacron swabs with liquid media.

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