

## Survival of *Ureaplasma urealyticum* on Different Kinds of Swabs

SHARON A. POULIN,<sup>1</sup> RUTH B. KUNDSIN,<sup>1\*</sup> AND HERBERT W. HORNE, JR.<sup>2</sup>

*Department of Surgery, Peter Bent Brigham Hospital,<sup>1</sup> and Department of Obstetrics and Gynecology, Harvard Medical School,<sup>2</sup> Boston, Massachusetts 02115*

Received for publication 30 July 1979

The survival of *Ureaplasma urealyticum* on five different types of swabs routinely used for obtaining clinical specimens is limited to 1 h. Wooden applicator sticks inhibit the alkaline color change in broth and result in a significant loss of isolates if broth alone is used.

Many factors affect the isolation of *Ureaplasma urealyticum* from clinical specimens (2). This paper deals with the survival of ureaplasmas on different kinds of swabs.

Three different swab tips (cotton, rayon, and calcium alginate attached to wooden, plastic, or aluminum shafts) are routinely used to take cervical, vaginal, and urethral cultures and to transport these specimens to the laboratory. It became essential to evaluate these different swabs for their ability to support the survival of ureaplasmas and to determine whether any were actually inhibitory to ureaplasma survival.

Fresh urine positive for ureaplasmas was chosen as the inoculum so that all swabs to be tested could be immersed simultaneously and similarly contaminated. The presence of mucus or blood which might, in cervical, vaginal, and urethral samples, influence results was appreciated, but standardization of a technique was difficult.

Five basic types of swabs were evaluated in this study. The first was the Bardic Cul-Tube (C. R. Bard, Inc.). This individually wrapped sterile disposable culture unit consists of a cotton-tipped swab tightly spun around a wooden applicator stick. The wooden stick is fixed to the cap of the styrene plastic tube, and the swab tip can touch the sides of the plastic tube.

The second swab studied was the sterile disposable Swube (Falcon Plastics). This is also a cotton-tipped swab, but the cotton tip is held onto the wooden applicator stick by a small amount of glue instead of being spun onto the stick. There are two swabs in each individually wrapped polystyrene tube container. The swabs are not attached to the cap and can touch the sides and bottom of the tube. Only one of the two swabs was used in the experiments; the second swab was discarded.

A third type of swab tested was the Calgiswab, type 1 (Inolex Corp.). This is a calcium alginate

swab on a flexible aluminum shaft. The swab tip is attached onto the applicator shaft by glue. The swab is packaged in a sterile wax paper envelope. Because of the packaging, the Calgiswab was removed from the envelope and placed into a polystyrene plastic screw-capped tube (Falcon) to perform the test.

The fourth and fifth types of swab to be evaluated were the Cepti-Seal Culturette swab (Marion Scientific Co.) with and without, respectively, the carrying medium being released. This rayon-tipped swab is tightly wrapped around a polystyrene applicator, which is attached to the cap of the tube. The soft plastic tube is composed of a copolymer of propylene and polyethylene. The swab rests on a cotton pledget near the bottom of the Culturette. Under the cotton pledget is an ampoule containing 0.5 ml of modified Stuart transport medium. This ampoule can be crushed, releasing the carrying medium. When this is done, the sample will remain moist up to 72 h after the taking of the culture. The Culturette was studied in duplicate. One swab was studied with the ampoule intact, and the other was studied by using the carrying medium.

All swabs were treated identically. They were immersed in ureaplasma-positive urine for 30 s and allowed to drip dry for 2 min. All urines tested had a ureaplasma titer of  $\geq 10^4$  color changing units. The swabs were then returned to their original containers. All swabs were still wet when returned and came in contact with the sides and bottoms of the tubes. They were kept at room temperature and cultured at hourly intervals starting at time zero up to and including 3 h. A separate swab of each type was used for each time interval. The swabs were rolled on A7 solid agar (3) and then placed in Ford liquid medium (1). Ford medium was also added to the original swab containers to determine urea-

plasma survival in areas contacted by the swab. All cultures were incubated at 35°C and read daily up to day 7.

Four different urine samples were used to test the cotton-tipped Cul-Tube (Table 1). In all four cases, none of the cultured swabs gave a positive ureaplasma reaction in Ford medium. However, in three of the urine samples, all of the original Cul-Tube containers were positive. Colonies of *U. urealyticum* were visualized on A7 agar at time zero from all of the swabs. In only one sample were ureaplasmas cultured from the swab up to 3 h at room temperature. In two of the samples, ureaplasmas could be cultured on A7 at 1 h, but not thereafter.

Two of the urine samples were tested by using the cotton-tipped Swube. In both cases, results were similar to those obtained with the cotton-tipped Cul-Tube. There was no ureaplasma color change in any of the swabs in Ford broth. However, a positive color change was observed in all of the original Swube containers. With one urine sample, growth of *U. urealyticum* on A7 was observed from the cotton-tipped Swube that was kept at room temperature for up to 3 h. With a second urine sample, no growth on A7 occurred after 1 h at room temperature.

Although the Calgiswab was not inhibitory to ureaplasmas, poor ureaplasma recovery was observed in all three urine samples tested with it. This may be due to the fact that the swab was not as absorbent as the other swabs tested because of its small size.

The last swabs to be tested were the rayon-tipped Culturette swabs. Four urines samples were set up, leaving the ampoule intact. A typical ureaplasma color change was observed at 1 h or more from the tested swabs and up to 3 h in the original Culturette containers. However, as with other types of swabs tested, 1 h at room temperature seems to be the limit *U. urealyticum* can survive on any swab for culture onto

A7. Three of the four urine samples were also evaluated by using the Culturette with the modified Stuart transport medium, that is, by crushing the ampoule. The carrying medium did not influence ureaplasma recovery, because the results were identical to those with the Culturette with carrying medium ampoule intact.

To determine the inhibitory characteristics of different parts of all of the swabs tested, the swab tip was peeled off and part of the applicator stick was cut and dropped into separate tubes of Ford broth. A 1-ml amount of *U. urealyticum*-positive urine was then added to each of the above tubes. As a control, Ford broth plus 1 ml of urine was set up at the same time. All cultures were incubated at 35°C and read daily for 4 days.

The control changed after 18 h of incubation, as did all of the parts of the swabs that showed no inhibition of ureaplasmas.

Neither cotton tip or pledget, nor rayon tip, nor calcium alginate tip, nor aluminum shaft, nor polystyrene applicator was, by itself, inhibitory to the ureaplasmas. What was inhibitory was the wooden applicator stick on which the cotton swab is glued or spun. This inhibitory effect of the wooden applicator stick was great enough to prevent color change in all ureaplasma-positive urine specimens.

All negative broths were subcultured onto A7 agar at 18 h. Colonies of *U. urealyticum* were visualized on all of the A7 plates. This indicates that certain parts of the swabs inhibit the typical color change in Ford medium that is associated with ureaplasmas, even though the organisms themselves are still viable in subculture.

To evaluate the inhibitory effect of the wooden applicator stick in a clinical situation, a study of the Cul-Tube and Culturette was set up in duplicate. Cervical specimens were taken by one of us (H.W.H.) by using the Cul-Tube first and then immediately the Culturette. A total of

TABLE 1. Comparison of swabs contaminated with ureaplasma-positive urine<sup>a</sup>

Type of swab	Urine samples tested	Swabs producing color change in Ford broth after:				Swab containers producing color change in Ford broth after:				Swabs producing growth on A7 agar after:			
		0 h	1 h	2 h	3 h	0 h	1 h	2 h	3 h	0 h	1 h	2 h	3 h
		Cul-Tube	4	0	0	0	0	4	3	3	3	4	3
Swube	2	0	0	0	0	2	2	2	2	2	2	1	1
Calgiswab	3	3	1	0	0	1	1	1	1	2	0	0	0
Culturette, without carrying medium	4	4	4	3	2	4	4	4	4	4	4	1	1
Culturette, with carrying medium	3	3	3	3	2	3	3	3	3	3	3	1	1

<sup>a</sup> Cul-Tube, Cotton-tipped swab tightly spun around a wooden applicator stick; Swube, cotton-tipped swab glued to a wooden applicator stick; Calgiswab, calcium alginate swab on an aluminum shaft; Culturette, rayon-tipped swab wrapped around a polystyrene applicator.

54 patients were cultured. All swabs were received in the laboratory for culture within 1 h after the specimens were taken.

Both the Cul-Tube swab and the Culturette swab were removed from their original containers and were rolled onto A7 agar. The swabs were then placed into separate tubes of Ford broth. Ford broth was also added to the original swab containers. All cultures were incubated at 35°C and held for 7 days.

The best agreement between solid medium and broth occurred when Culturettes were used (Table 2). Of the 54 cervical specimens, 25 were positive, and 29 were negative. Of the 25 A7 agar-positive samples, all of the Cul-Tube containers gave a positive ureaplasma color change, but only 11 of the cultured Cul-Tube swabs changed the Ford broth. Cul-Tube swabs gave false-negative reactions in 14 instances in broth. If broth cultures alone had been done, no color change would indicate a negative result for 14 patients, patients who were actually positive. In the 29 A7 agar-negative ureaplasma specimens, no color change was observed in any of the Ford broths with either the Culturette or the Cul-Tube.

In conclusion, swabs on wooden applicator sticks may yield confusing results when culturing for ureaplasmas. The wooden applicator stick prevents a color change in Ford medium when ureaplasmas may actually be present. The absence of a color change in liquid medium with

TABLE 2. Comparison of culture results with two swabs

Swab	Ford broth		A7 agar		Total done
	Color change	No color change	Positive	Negative	
Cul-Tube	11 <sup>a</sup>	43	25	29	54
Culturette	25 <sup>a</sup>	29	25	29	54

<sup>a</sup> Statistically significant difference  $P = 0.004$ .

the wooden applicator stick would yield a negative report when simultaneous agar cultures are not done for ureaplasmas. Because of these findings, we are now requesting that specimens be sent to us by using Culturettes which have a plastic-polystyrene applicator not found to be inhibitory to ureaplasmas in broth. No matter what type of swab is used, it is important that the laboratory receive a culture within 1 h of taking the specimen.

#### LITERATURE CITED

1. Ford, D. K., and J. MacDonald. 1967. Influence of urea on the growth of T-strain mycoplasmas. *J. Bacteriol.* **93**:1509-1512.
2. Kundsins, R. B., A. Parreno, and S. Poulin. 1978. Significance of appropriate techniques and media for isolation and identification of *Ureaplasma urealyticum* from clinical specimens. *J. Clin. Microbiol.* **8**:445-453.
3. Shepard, M. C., and C. D. Lunceford. 1976. Differential agar medium (A7) for identification of *Ureaplasma urealyticum* (human T mycoplasmas) in primary cultures of clinical material. *J. Clin. Microbiol.* **3**:613-625.