Letters to the Editor

Choice of Buffer and Use of Surface Blocking in an Enzyme-Linked Immunosorbent Assay for Antibodies to *Ureaplasma urealyticum* Serotypes

In an article by Wiley and Quinn (5), the search for and establishment of a good assay are described. There are, however, some points of discussion as to the use of plain buffer as the serum control and the composition of dilution buffers. Furthermore, a surface blocking was not used after the primary coating of the reaction surface.

A problem with the nonspecific binding of reagents may result from the way in which the serum control and the blocking of possible free binding spaces on the solid phase after coating were undertaken. As an "indirect" technique for determination of antibodies in rabbit antiserum, the coating was with the respective antigen (purified fractions of different serotypes of *Ureaplasma urealyticum*), followed by antiserum and terminated with anti-antibodies conjugated to an enzyme.

This sequence is used in many types of assays (4). From these studies, it is also known that the concentrations of the reagents must be optimized and that it is necessary to block possible remaining free binding spaces on the plastic after coating with the antigen (1, 2, 4). This blocking is often done with bovine serum albumin (BSA). Detergent alone is a weak blocking molecule because of the few physical bonds that can be established between detergent and the solid phase.

The binding strength in all cases is the combined strength of many nonspecific physical bonds to single molecules. Therefore, big molecules with many bonds will have a high binding strength to plastic surfaces, whereas small molecules such as detergents will bind weakly.

In the work by Wiley and Quinn (5), a binding of 0.40 to 1.60 μg/ml in 0.1 ml per well was used, giving 67 to 267 ng/cm^2, but the Nunc-Immuno plates bind up to 400 ng/cm^2 (3). Thus, only a part of the surface was used, and the rest was left unused and able to bind another molecule in one of the later incubations. There was no blocking of unused plastic surface parts but merely a wash with "PBS-T" (I presume that "T" means Tween or Triton, i.e., detergent), which is suboptimal as the detergent can detach from the surface, leaving it free to bind, (e.g., enzyme-conjugated antibodies). Finally, in the "serum control," there were no adsorbing molecules at all, as only 0.05 M carbonate buffer was used for incubations. A serum control ought to be a serum without the antigen (1–4).

All of this combined will, in the high-binding capacity Nunc plate (that has preference for binding proteins compared to detergent), result in a high readout value in control and reference wells. This is, as I have tried to explain, a result that necessarily will be found due to the nonoptimal procedure, and the solution of the problem is to block the surface with, e.g., BSA after the coating with the antigen, and to include BSA in the dilution buffers hereafter.

Further, I should like to mention that the signal-to-noise ratios as cited in the article were low, and a better ratio might have been observed if the solid phase had been blocked. Another way to design the assay would be to choose a solid phase with a lower binding capacity (e.g., Nunc catalog no. 269620). This will only bind approximately 100 ng/cm^2 and therefore give only few or no unused surface parts that need to be blocked after the primary coating. The readout values in the positive reaction wells will be approximately the same, whereas the controls/negative wells will be much lower, giving a better signal-to-noise ratio.

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5. **Wiley, C. A., and P. A. Quinn.** 1984. Enzyme-linked immunosorbent assay for detection of specific antibodies to *Ureaplasma urealyticum* serotypes. J. Clin. Microbiol. **19:** 421–426.

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Author's Reply

In response to Dr. Løvborg's letter, the statement in paragraph 1 that ". . . a surface blocking was not used after the primary coating of the reaction surface" is not true. According to the "ELISA method" described in our article (4) (page 422, paragraph 6), the antisera and conjugates were diluted in "PBS containing 1% bovine serum albumin and 0.05% Tween 20 (PBS-T). . . ." Thus, surface blocking agents bovine serum albumin (BSA) and Tween were used with antisera and conjugate to surface block any free sites, according to the technique of Voller et al. (3).

The use of plain carbonate buffer in the serum control is adequate. The carbonate buffer used to dilute the antigen was added in the first step to the control well. The serum was then added diluted in PBS-T, i.e., PBS plus BSA plus Tween 20, and not "T means Tween or Triton," as he stated. Thus, the BSA in excess would competitively bind to the plates, whereas antisera should not bind.

With respect to paragraph 3 of Dr. Løvborg's letter, we did optimize the concentrations of antigen required for each specific antigen, as stated in the results. Any remaining sites were blocked with BSA in the serum diluent buffer. The optimum antigen concentration was that determined to give an optical density of 1.0, as shown in Fig. 2 of our article.

With respect to the information on binding capacities of Nunc plates (2), we cannot be held responsible for data published in 1984 when our paper was submitted for publication in 1983.

With respect to paragraph 6 of Dr. Løvborg's letter, the solid phase was blocked with BSA and Tween as described. The assessment of the plates did not occur in only a few experiments. We worked with the Nunc plates using the complete ELISA procedure as described for many months before evaluating other types of plates.

Finally, the view held by Dr. Løvborg in paragraph 3 that detergent is a weak blocking molecule is open to question. Kenny and colleagues (1) have shown that the detergents Tween and Triton are stronger competitive blockers than BSA.

We feel that Dr. Løvborg's comments are unfounded in light of the misunderstanding of the use of the term "PBS-T."

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Sodium Polyanetholesulfonate in the Identification of *Garnerella vaginalis*

I read with interest the article by Reimer and Reller (2) describing the use of sodium polyanetholesulfonate (SPS) disks in the identification of *Gardnerella vaginalis* organisms. I have also tested this compound (Liquoid; Roche Ltd.) against organisms isolated from vaginal specimens, in conjunction with my own methods of identification (1).

Lawns of the organisms were spread over the surface of Columbia agar plates containing 10% horse serum. A disk was applied and the cultures incubated overnight. The results were as shown in Table 1.

I concluded that, although the SPS test helped to exclude catalase-positive vaginal coryneforms and lactobacilli, it did nothing to differentiate true *G. vaginalis* from the atypical, catalase-negative "G. vaginalis-like" organisms from the vagina, which still remains the major problem in vaginal bacteriology.

I also compared the easier, direct application of a loopful of 5% SPS onto seeded lawns and found it to be as effective as prepared disks in producing zones of growth inhibition. The 5% solution of SPS proved stable for up to 2 months in a refrigerator and eventually obviated the chore of manufacturing disks for this test.

TABLE 1. Identification of *G. vaginalis*

Organism (no.)	No. with typical *G. vaginalis* morphology	No. with positive catalase test	No. inhibited by:	
			H_2O_2	SPS
G. vaginalis (41)	41	0	41	31
G. vaginalis-like (19)[a]	0	0	19	8
Vaginal "diphtheroids" (16)	0	16	16	0
Vaginal lactobacilli (7)	0	0	7	0

[a] These organisms were of atypical Gram stain morphology.

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Author's Reply

The following is in reply to B. M. Jones concerning the sodium polyanetholesulfonate (SPS) disk test for identification of *Gardnerella vaginalis*.

The test conditions for the performing the SPS disk test were critical in our evaluation. Both the inoculum size and agar medium were very important. We found the test to work well only by using a suspension containing 10^8 CFU/ml for streaking the agar surface and by using brucella agar supplemented with 5% sheep blood. We tried Columbia agar with and without human or sheep blood and found that, although good growth of the organism was produced, small or absent zone sizes were seen. In addition, a positive test in our study required a zone size of >12 mm. A few other organisms we tested were inhibited but had smaller zone sizes. Because of these critical steps in the test, we are not surprised that the results obtained by Jones are different from our own.

We did not try to identify or separate *G. vaginalis*-like (GVL) organisms, as described by Bailey et al. (1), from true