

Reuse of *Salmonella* and *Shigella* Absorbing Cells for Preparing Monospecific *Salmonella* O and *Shigella* Antisera

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Salmonella and *Shigella* organisms used as absorbing cells for preparing group- or type-specific *Salmonella* O or *Shigella* antisera may be reused four or more times without qualitatively reducing their capacity to remove undesired antibodies. The cells may be reclaimed by heating in flowing steam or by treatment with N/5 HCl. The former method is preferred.

Serotype identification of organisms in the family *Enterobacteriaceae* requires monospecific antisera. One of the major problems in the production of these antisera is the preparation of a sufficient volume of absorbing cells, particularly when a large volume of antiserum is to be absorbed. Edwards and Ewing (1) recommend using the growth from 10 heavily inoculated 90-mm petri plates for each milliliter of *Salmonella* O or *Shigella* antiserum to be absorbed. By this method a packed cell-to-serum ratio of approximately 1 is obtained. Thus, the preparation of absorbing cells becomes an expensive and time-consuming operation in the preparation of these reagents. We investigated methods of reclaiming cells used for absorption of *Salmonella* O and *Shigella* antisera and reusing them for additional absorption. Updyke and Conroy (4) reported reusing streptococcus cells four or more times for absorption of group A streptococci antisera after treating them with N/5 HCl in saline. Also, absorbing suspensions used for preparing T-agglutination antisera for typing group A streptococcus were used up to three times by Moody et al. (3) by autoclaving them at 20 lb of pressure for 20 min between uses.

The cultures used for absorption and for evaluation of the antisera and the method of growing the cells were those recommended by Edwards and Ewing (1). After the cells were harvested they were washed two times with 0.85% NaCl containing 0.5% formalin (formalinized saline), resuspended to a 10% (wt/vol) concentration in formalinized saline, and stored at 4 C. For absorption, the desired volume of cell suspension was washed one time, and the antiserum was added to the packed cells at a

ratio of 0.5 g (wet weight) of cells per ml of undiluted serum. The absorbing cells were completely resuspended in the antiserum, and the mixture was placed in a 48 C water bath for 1 h for 50 ml or less of serum and for 2 h for larger volumes. After absorption, the cells were separated from the serum by centrifugation, resuspended in formalinized saline, and, depending on the volume of cells, heated in an Arnold steam sterilizer at 100 C for 1 or 2 h. The cells were then washed two times and resuspended to a 10% (wt/vol) concentration with formalinized saline. The specificity of the antiserum was determined by slide agglutination tests with homologous and heterologous alcohol-treated antigens (1). When necessary, the antiserum was reabsorbed with cells of the same or a different serotype.

To absorb *Salmonella* O and *Shigella* antisera, Edwards and Ewing (1) recommend dividing the desired quantity of absorbing cells into two portions and doing two separate absorptions on the same sample of serum. We have found that frequently the first absorption removes the detectable agglutinins for the absorbing cells, and, therefore, the second absorption is unnecessary. The use of one absorption has the advantage of not only requiring a smaller volume of absorbing cells but also of not subjecting the antiserum to an additional absorption.

Absorbing cells may be reused by either treating them overnight with N/5 HCl in saline or heating at 100 C for 1 h or longer. The latter method is easier to perform and is preferred in our laboratory. To date, absorbing cells of nine different *Shigella flexneri* and seven different *Salmonella* serotypes have been reused one or

more times without qualitatively reducing their absorbing capacity. Neither of the procedures is applicable to absorbing cells used to prepare monospecific *Salmonella* H antiserum because they destroy the flagella antigens.

Table 1 shows the results of absorbing three portions of *S. flexneri* 2b antiserum with the same *S. flexneri* 1b absorbing cells. The cells which were heated and washed after each absorption were just as effective in removing the cross-reactions the third time they were used as they were the first time. In another example, four different *Salmonella* O antisera that cross-reacted with *Salmonella* 9,12 antigen were absorbed with the same 9,12 cells that were heated and washed after each absorption (Table 2). The cross-reacting of the O (3), (15), 34 antiserum with the 9,12 antigen was probably due to the known antigenic relationship between O-antigen 12₂ and O-antigen 34 (2). Although not indicated in Table 2, the *Salmonella* 9,12 cells used the fourth time for absorption had been stored at 4 C in formalinized saline for 12 months, which indicates the stability of the absorbing capacity of these cells.

Absorbing cells of the other serotypes of *Salmonella* and *Shigella* have also retained their absorbing capacity during storage at 4 C.

Preliminary studies of this nature have also been done on other organisms used for absorption of antisera. To date, we have been able to reuse three species of *Candida* and one species of *Rhodotorular* cells for absorption of a *Blas-tomyces dermatitidis-Histoplasma capsulatum* FA conjugate. However, these cells could only be reused by suspending them overnight in N/5 HCl in saline, and washing three to four times in saline. When they were heated to 100 C, their capacity to absorb the cross-reacting antibodies was destroyed. On the other hand, attempts to reuse *Neisseria meningitidis* groups B or C cells after treatment by either method were unsuccessful, indicating that the absorbing antigen was either removed from the cells and lost during washing or was destroyed by these manipulations. Thus, the procedures described for reusing *Salmonella* and *Shigella*-absorbing cells cannot be used indiscriminately, but where applicable they may be very useful for preparing absorbed antisera.

TABLE 1. Reuse of *S. flexneri* 1b cells for absorption of *S. flexneri* 2b antiserum

Antiserum	Slide agglutination reaction with <i>S. flexneri</i> antigens										
	1a	1b	2a	2b	3a	3b	3c	4a	4b	5	6
Unabsorbed	3 ^a	2	4	4	4	4	4	2	4	4	—
Absorbed with: ^b											
Untreated cells	—	—	4	4	—	—	—	±	±	—	—
1 × Treated cells ^c	—	—	4	4	—	—	—	—	—	—	—
2 × Treated cells	—	—	4	4	—	—	—	—	±	—	—

^a Agglutinin reaction of undiluted antiserum (— = no agglutination).

^b Absorbed with 0.5 g (wet weight) of packed cells per ml of undiluted antiserum.

^c After each absorption cells were heated at 100 C for 1 h in flowing steam and washed two times with 0.5% formalinized saline.

TABLE 2. Reuse of the same *Salmonella* 9,12 cells for absorption of different *Salmonella* O antisera cross-reacting with 9,12 antigens

Antisera crossing with 9,12 antigen	Slide agglutination reaction with <i>Salmonella</i> 9,12 antigen	
	Prior to absorption	After absorption ^a
<i>Salmonella</i> O 1, 2, 12 antiserum absorbed with untreated 9,12 cells	3+	Negative
<i>Salmonella</i> O 4, 5, 12 antiserum absorbed with 9,12 cells treated 1 ×	3+	Negative
<i>Salmonella</i> O (9), 46 antiserum absorbed with 9,12 cells treated 2 ×	3+	Negative
<i>Salmonella</i> O (3), (15), 34 antiserum absorbed with 9,12 cells treated 3 ×	2+	Negative

^a Conditions were the same as in Table 1.

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