JOURNAL OF BACTERIOLOGY, Apr., 1966 Vol. 91, No. 4
 Printed in U.S.A.

Effect of Normally Occurring Rabbit Antibodies on Fluorescent-Antibody Reactions

S. BERGMAN, A. FORSGREN, AND B. SWAHN

Department of Clinical Bacteriology, University of Umea, and Department of Medicine, University of Lund, Lund Sweden

Received for publication 4 January 1966

In methods in which fluorescent antibodies are used for identification of bacteria in different preparations, nonspecific fluorescent staining often constitutes an insurmountable problem. Various purification procedures and absorption methods have been tried in attempts to eliminate this difficulty (R. C. Nairn, *Fluorescent protein tracing*, E. S. Livingstone, Edinburgh, 1962; R. E. Dedmon, A. W. Holmes, and F. Deinhardt, J. Bacteriol. **89**:734, 1965).

Many nonspecific reactions can probably be attributed to the antibodies that occur normally in rabbit serum (M. D. Moody and W. L. Jones, J. Bacteriol. **86**:285, 1963). Since these are mainly directed against staphylococci and streptococci, these bacteria have been used to absorb sera. Some nonspecific reactions have been eliminated in this way, but the specific reactions have also been affected, resulting in lower titers. Blocking of the nonspecific staining with, for example, rhodamine-marked antisera against staphylococci and streptococci has been somewhat more satisfactory (W. B. Cherry and M. D. Moody, Bacteriol. Rev. **29**:222, 1965).

To estimate the titers of normally occurring antibodies, we tested pooled serum from nonimmunized rabbits against a number of strains of bacteria. Agglutination titers as well as fluorescent-antibody (FA) titers have been determined with a large number of strains of *Staphylococcus aureus*, *S. epidermidis*, streptococci of the pyogenic and Viridans groups, nonpathogenic types of *Neisseria*, and *Escherichia coli*. The agglutinin titer was determined with a test-tube agglutination technique and is expressed as the highest serum dilution that produced demonstrable agglutination of a bacterial suspension. The FA titer is the highest serum dilution that gave fluorescence at the bacterial periphery. The results are shown in Table 1.

TABLE 1. *Comparison of agglutination titers and fluorescent-antibody titers in pooled nonimmunized rabbit serum tested with different bacterial strains**

Strain	Titer																	
	Negative		1:10		1:20		1:40		1:80		1:160		1:320		1:640		1:1,280	
	AG†	FA†	AG	FA	AG	FA	AG	FA	AG	FA	AG	FA	AG	FA	AG	FA	AG	FA
Staphylococcus aureus	—	13	1	13	7	5	5	8	7	10	21	7	15	5	3	—	2	—
S. albus	5	18	6	1	6	2	3	—	1	—	—	—	—	—	—	—	—	—
α-Hemolytic streptococcus	—	3	—	—	—	—	—	—	2	—	—	—	—	—	1	2	—	—
β-Hemolytic streptococcus type A	3	6	—	2	—	—	—	—	1	—	2	—	1	—	1	—	—	—
β-Hemolytic streptococcus type C	2	1	2	2	1	2	—	—	—	—	—	—	—	—	—	—	—	—
β-Hemolytic streptococcus type G	—	3	1	—	—	—	—	—	2	1	—	—	—	—	—	—	—	—
Neisseria apatogen	3	2	1	1	1	2	—	—	—	—	—	—	—	—	—	—	—	—
Escherichia coli	17	41	15	2	4	6	4	—	3	—	3	—	1	—	2	—	—	—

* The number of strains against which the respective titer was observed is given.
† AG = agglutination; FA = fluorescent antibody.

The solutions of FA used were prepared according to Riggs et al. (Proc. Soc. Exptl. Biol. Med. **105**:655, 1960) with the following modification. The serum was dialyzed against 0.0175 M phosphate buffer (*p*H 6.5) and was then passed through a column of diethylaminoethyl-Sephadex A 50 equilibrated with the same buffer. The first fraction to emerge, consisting of α-globulin, was concentrated and conjugated with fluorescein isothiocyanate. The final solution was filtered through Sephadex G-25 medium.

The agglutinin titers ranged from zero to 1:1,280, with especially high titers against *S. aureus* and *E. coli*. The FA titers were consistently lower.

On the basis of the above, we have developed a technique of preinhibition. After fixing and rehydration, the preparation is exposed to a drop of pooled normal rabbit serum in a moist chamber for 10 min, after which a drop of specific fluorescein-marked antibody solution is applied and allowed to remain for 30 min. The preparation is then washed thoroughly and mounted.

Preinhibition with pooled normal rabbit serum appears to be an effective way to eliminate nonspecific staining, one of the most common sources of error in diagnostic FA technique. We have tested the method with specific sera against *Neisseria gonorrhoeae* and *Bacterium coli neapolitanum*. Preinhibition has not led to lowered titers or to loss of specificity in these systems. Preliminary results with other microorganisms are equally promising.

This investigation was supported by Försvarsmedicinska Forskningsdelegationen 7:025–2/65.