Method for the Preservation of Diagnostic Sera for Field and Laboratory Work

HARRY L. SMITH, JR., AND ROBERT J. MANDLE

Department of Microbiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Received for publication 28 October 1970

Paper discs impregnated with antiserum, lyophilized, and stored at temperatures ranging from −21 to 33 C have yielded potent and specific reagents when rehydrated 370 days later. Applications are discussed.

When specific diagnostic sera are available, a clinical bacteriology laboratory can often identify isolates within minutes. These reagents are stable for years in the lyophilized or frozen states. When the sera are reconstituted or maintained in the fluid state, or both, preservation and stability may present problems. The volume of serum in a vial is frequently greater than is needed for immediate use. To conserve reagents, refrigeration aids in preserving the reconstituted sera for a few days, but this is not always available. Addition of chemical preservatives such as Merthiolate or phenol to the sera causes lysis of certain organisms such as Vibrio cholerae (unpublished data), preventing the use of the sera in agglutination tests.

This note describes a technique that reduces the waste of serum while allowing storage over a wide range of temperatures.

Stocks of V. cholerae diagnostic sera were prepared and lyophilized in portions which yielded 5 ml of reagent on rehydration. These include Group, Inaba, and Ogawa sera, the latter two being appropriately prepared. No preservatives were added. Samples of each serum were rehydrated with 1 ml of 1% saline, and 10 0.5-inch (ca. 1.27 cm) paper discs (Schleicher and Schuell no. 740E) were moistened with contents from each vial. The impregnated discs were placed at −70 C for 1 hr in 5-ml serum bottles with split-rubber stoppers. The vials were put into a VirTis vacuum stoppering and manifold chamber at room temperature. A vacuum of 5 μ of mercury and a condenser temperature of −45 C were employed for 18 hr. The vials were sealed under vacuum to prevent possible dispersal of the dried serum when the vacuum was released. After the chamber was opened, the rubber stoppers were raised briefly to allow equilibration of pressure. Batches of the three sera were prepared in this manner.

Discs were tested immediately after preparation. Different volumes of saline were added to discs and the supernatant fluids were tested by the slide agglutination method with live cholera vibrios as the antigens. It was found that 0.5 ml of 1% saline was the maximal amount of diluent which yielded serum for discs sufficient to give complete clumping of the organisms when the slide agglutination test was viewed with the naked eye. Since 10 discs contained the equivalent of 5 ml of the original serum, the recovery was quite good.

For tube agglutination tests, the sera were eluted by using 1.0 ml of saline per disc, a 1:2 dilution of the original sera. Serial twofold dilutions were made in saline. Suspensions of live cholera vibrios were used (4).

Vials containing the discs were placed at 33 C for 24 hr in 5-ml serum bottles with split-rubber stoppers. The vials were put into a VirTis vacuum stoppering and manifold chamber at room temperature. A vacuum of 5 μ of mercury and a condenser temperature of −45 C were employed for 18 hr. The vials were sealed under vacuum to prevent possible dispersal of the dried serum when the vacuum was released. After the chamber was opened, the rubber stoppers were raised briefly to allow equilibration of pressure. Batches of the three sera were prepared in this manner.

Discs were tested immediately after preparation. Different volumes of saline were added to discs and the supernatant fluids were tested by the slide agglutination method with live cholera vibrios as the antigens. It was found that 0.5 ml of 1% saline was the maximal amount of diluent which yielded serum for discs sufficient to give complete clumping of the organisms when the slide agglutination test was viewed with the naked eye. Since 10 discs contained the equivalent of 5 ml of the original serum, the recovery was quite good.

For tube agglutination tests, the sera were eluted by using 1.0 ml of saline per disc, a 1:2 dilution of the original sera. Serial twofold dilutions were made in saline. Suspensions of live cholera vibrios were used (4).

Vials containing the discs were placed at 33 C for 24 hr in 5-ml serum bottles with split-rubber stoppers. The vials were put into a VirTis vacuum stoppering and manifold chamber at room temperature. A vacuum of 5 μ of mercury and a condenser temperature of −45 C were employed for 18 hr. The vials were sealed under vacuum to prevent possible dispersal of the dried serum when the vacuum was released. After the chamber was opened, the rubber stoppers were raised briefly to allow equilibration of pressure. Batches of the three sera were prepared in this manner.

Discs were tested immediately after preparation. Different volumes of saline were added to discs and the supernatant fluids were tested by the slide agglutination method with live cholera vibrios as the antigens. It was found that 0.5 ml of 1% saline was the maximal amount of diluent which yielded serum for discs sufficient to give complete clumping of the organisms when the slide agglutination test was viewed with the naked eye. Since 10 discs contained the equivalent of 5 ml of the original serum, the recovery was quite good.
### Table 1. Results of slide agglutination tests with cholera diagnostic sera lyophilized on paper discs

| Antiserum | Storage | Ogawa serotype | Inaba serotype |
|-----------|---------|----------------|----------------|
|           |         | 34 38 628 1451 | 35 48 629 1449 |
| Group     | No storage | + b + + + + | + + + + + + |
|           | Stored for 370 days at 33 C | + + + + + + | + + + + + + |
|           | 20-25 C | + + + + + + | + + + + + + |
|           | 5 C | + + + + + + | + + + + + + |
|           | −21 C | + + + + + + | + + + + + + |
| Ogawa     | No storage | + + + + + + | − − − − − − |
|           | Stored for 370 days at 33 C | + + + + + + | − − − − − − |
|           | 20-25 C | + + + + + + | − − − − − − |
|           | 5 C | + + + + + + | − − − − − − |
|           | −21 C | + + + + + + | − − − − − − |
| Inaba     | No storage | − − − − − − | + + + + + + |
|           | Stored for 370 days at 33 C | − − − − − − | + + + + + + |
|           | 20-25 C | − − − − − − | + + + + + + |
|           | 5 C | − − − − − − | + + + + + + |
|           | −21 C | − − − − − − | + + + + + + |

|                | Antiserum | Storage | Ogawa serotype | Inaba serotype |
|----------------|-----------|---------|----------------|----------------|
|                | Group     |         | 38 1451        | 48 1449        |
|                | No storage | 64 a 64 | 64 128 64 32   |                |
|                | Stored for 370 days at 33 C | 64 64 64 64 64 64 64 64 |                |
|                | 20-25 C | 64 64 64 64 64 64 64 64 |                |
|                | 5 C | 64 64 64 64 64 64 64 64 |                |
|                | −21 C | 64 64 64 64 64 64 64 64 |                |
| Ogawa          | No storage | 64 64 | <4 <4 <4 <4 |                |
|                | Stored for 370 days at 33 C | 32 64 <4 <4 <4 |                |
|                | 20-25 C | 64 32 <4 <4 <4 |                |
|                | 5 C | 64 32 <4 <4 <4 |                |
|                | −21 C | 64 64 <4 <4 <4 |                |
| Inaba          | No storage | <4 <4 | 32 16 32 16   |                |
|                | Stored for 370 days at 33 C | <4 <4 <4 <4 <4 |                |
|                | 20-25 C | <4 <4 <4 <4 <4 |                |
|                | 5 C | <4 <4 <4 <4 <4 |                |
|                | −21 C | <4 <4 <4 <4 <4 |                |

a Vibrio Reference Laboratory Number: 34, NIH 41, U.S. vaccine strain, Ogawa; 35, NIH 35A3, U.S. vaccine strain, Inaba; 38, isolated Thailand, 1958, Ogawa; 48, isolated Thailand, 1958, Inaba; 628, isolated Hong Kong, 1961, Ogawa;629, isolated Hong Kong, 1961, Inaba; 1449, isolated E. Pakistan, 1962, Inaba; 1451, isolated E. Pakistan, 1962, Ogawa.
b +, Agglutination; −, no agglutination.

### Table 2. Results of tube agglutination tests with cholera diagnostic sera lyophilized on paper discs

| Antiserum | Storage | Ogawa serotype | Inaba serotype |
|-----------|---------|----------------|----------------|
|           |         | 38 1451        | 48 1449        |
| Group     | No storage | 64 a 64 | 64 128 32 64   |                |
|           | Stored for 370 days at 33 C | 64 64 64 64 64 64 64 64 |                |
|           | 20-25 C | 64 64 64 64 64 64 64 64 |                |
|           | 5 C | 64 64 64 64 64 64 64 64 |                |
|           | −21 C | 64 64 64 64 64 64 64 64 |                |
| Ogawa     | No storage | 64 64 | <4 <4 <4 <4 |                |
|           | Stored for 370 days at 33 C | 32 64 <4 <4 <4 |                |
|           | 20-25 C | 64 32 <4 <4 <4 |                |
|           | 5 C | 64 32 <4 <4 <4 |                |
|           | −21 C | 64 64 <4 <4 <4 |                |
| Inaba     | No storage | <4 <4 | 32 16 32 16   |                |
|           | Stored for 370 days at 33 C | <4 <4 <4 <4 <4 |                |
|           | 20-25 C | <4 <4 <4 <4 <4 |                |
|           | 5 C | <4 <4 <4 <4 <4 |                |
|           | −21 C | <4 <4 <4 <4 <4 |                |

a Numbers represent reciprocal of the final serum dilution.
whole sera to discs followed by freeze-drying has yielded satisfactory preparations on rehydration. We did not investigate the effect of extreme changes in humidity on the discs. This potential hazard could probably be avoided by packaging the discs in tightly sealed vials.

It is obvious that this technique could be applied to a variety of sera. Preliminary studies using agglutinating sera against other organisms have given promising results. The potential use of this procedure in serological surveys should not be overlooked. From a practical standpoint, the use of lyophilized serum-impregnated discs should reduce the cost of preparation and shipment while increasing the effective utilization of diagnostic sera.

This investigation was supported by the United States-Japan Cooperative Medical Science Program and Public Health Service grant AI-08403, National Institute of Allergy and Infectious Diseases, Public Health Service contract NIH-70-2188, and by the Office of Naval Research grant N00014-68-A-0516.

LITERATURE CITED
1. Adams, E., and R. P. Hanson. 1956. A procedure for absorbing virus neutralizing antibodies on paper disks. J. Bacteriol. 72: 572.
2. Brody, J. A., R. McAlister, R. Haseley, and P. Lee. 1964. Use of dried whole blood collected on filter paper discs in adenovirus complement fixation and measles hemagglutination inhibition test. J. Immunol. 92:854-857.
3. Crawford, Y. E. 1966. A laboratory guide to the mycoplasmas of human origin, 4th ed. Naval Med. Research Unit, Great Lakes, Ill.
4. Goodner, K., H. L. Smith, Jr., and H. Stempen. 1960. Serologic diagnosis of cholera. J. Albert Einstein Med. Center 8:143-147.
5. Stainbridge, E., and L. Hayflick. 1967 Growth inhibition test for identification of Mycoplasma species utilizing dried anti-serum-impregnated paper discs. J. Bacteriol. 93:1392-1396.