Further Studies on the Cowan Strain of *Staphylococcus aureus* as an Aid for the Diagnosis of Influenza

**Brief Report**

By

E. ZALAN and C. WILSON

Virus Laboratory, Ontario Ministry of Health,
Toronto, Ontario, Canada

Accepted July 22, 1977

**Summary**

Standardization of procedures leading to the identification of influenza viruses with the aid of sensitized staphylococci and also comparative studies between coagglutination and HI during the 1976/77 influenza season are described.

In an earlier report (2) we described a new method for the rapid identification of influenza viral strains. The method was based on the fact that certain strains of *Staphylococcus aureus*, such as the Cowan type 1 strain, contain a protein in their cell wall which is capable of binding IgG serum globulins without affecting the antibody sites. The antibody bound to the bacterial cell wall can, therefore, react with its homologous antigen bringing about the agglutination of the IgG-coated (sensitized) bacteria.

This property of *Staphylococcus aureus* to bind serum globulins was found to be useful for the identification of influenza virus isolates, provided that the influenza specific antiserum used for the sensitization of bacteria was of high titre and free of non-viral agglutinins.

This report deals with the standardization of the technique and also presents data obtained by using the test during the 1976/77 influenza season.

The bacteria were grown in plastic plates in CCY medium (1) solidified by the addition of 1.5 per cent Difco agar. The cultures were incubated at 37°C for 18 hours, then washed off the agar in 5 ml of PBS, pH 7.2 containing 1 per cent glucose and 1 per cent 2, 3, 5-triphenyl-1H-tetrazolium chloride. After an additional 30 minutes of incubation at 37°C when the staphylococci developed a dark cherry-red color, the culture was harvested, washed several times in PBS, treated with 0.5 per cent formaldehyde in PBS for 3 hours and then exposed to heat at 80°C for 30 minutes under constant agitation in order to insure uniform heat-treatment of the individual bacterium. This step proved to be very important since
staphylococci which escaped heat-treatment reacted non-specifically with various substances such as normal allantoic or tissue culture fluid which are present in a viral sample to be tested. Finally the staphylococci were washed several times in PBS and resuspended in the same medium to a concentration of 10 per cent. The suspension was stored at 4°C for future use.

The immunizing antigen used for the production of antiserum was concentrated by ultracentrifugation and purified by sucrose density gradient sedimentation. It was found that antisera containing 640 or greater HI units/ml were suitable for the sensitization of staphylococci.

Small amounts of antichick antibodies which could interfere with the specificity of the test were removed by preparing a 10 per cent dilution of the hyperimmune serum in normal allantoic fluid then, after 30 minutes of incubation at room temperature, adding 1 part of a 25 per cent kaolin suspension to 10 parts of the serum-allantoic fluid mixture; after an additional 10 minutes of incubation the serum was clarified by centrifugation.

In order to achieve a test with high degree of specificity and sensitivity, the optimal serum dilution necessary for the sensitization of staphylococci must be determined. The procedure consists of the preparation of serial two-fold dilutions of serum in PBS in 1 ml volumes, then sensitizing identical volumes of a 0.5 per cent suspension of staphylococci with each serum dilution at room temperature for 10 minutes; the sensitized bacteria are then sedimented by light centrifugation, washed once in distilled water and resuspended in the same to twice of the original volume bringing the final concentration to 0.25 per cent. To determine the optimal sensitizing serum dilution, each preparation has to be tested against:

a) serial two-fold increasing dilutions of the homologous virus (sensitivity test),
and

b) a battery of type A and B influenza viruses (specificity test).

After determining the optimal serum concentration, a larger batch can be prepared which is then stabilized by the addition of 0.08 per cent sodium azide solution and 50 micrograms per ml gentamycin.

The preparation should be stored at -20°C. The sensitivity of working suspension, kept at refrigeration temperature may decrease somewhat, therefore, the replacement of unused bacterial suspension after two weeks is recommended.

The test can be carried out on either agglutination slides or in microplates.

Using silicone-treated agglutination slides, 50 microliter volumes of the viral fluid and the sensitized bacterial suspension are mixed and vigorously agitated manually or mechanically for 10 minutes. Weak antigens with HA titres of 1:16 or less may cause agglutination only after an additional 10 minutes of agitation. The fluid volume is sufficiently large to withstand drying due to evaporation.

Using microplates, preferably flat bottom plates, 25 microliter volumes of the viral suspension and the sensitized bacterial suspension are placed in the wells. The plate is then covered with a lid and agitated in a micromixer for a minimum of 20 minutes; however, weak reactions become visible only after additional agitation.

Although in most cases agglutination can be observed by the naked eye or with the help of a magnifying glass, it is preferable to use an inverted microscope for reading the test.