Blood Coagulation Test for Citrate Utilization

JOSEPH T. CORDARO AND WALTER SELLERS

USAF School of Aerospace Medicine, Aerospace Medical Division (AFSC), Brooks Air Force Base, Texas 78235

Received for publication 28 August 1967

The rationale for this test is that, if an organism attacks the citrate in citrated blood, the blood will clot. If the organism (other than Staphylococcus aureus) is not capable of attacking citrate, no clot will form.

The idea for the test originated from an article by B. G. Bayliss and E. R. Hall (J. Bacteriol. 89:101, 1965), who demonstrated with paper chromatography that apparently positive coagulase tests with organisms other than S. aureus coincided with the disappearance of citrate from the citrated plasma used in the coagulase test.

No previous reports were found of a systematic effort to develop a rapid citrate test in which citrated blood or plasma served as the indicator for citrate utilization.

Seventy-one cultures, representing various genera and species of Enterobacteriaceae, were employed in developing the test. Tubes were heavily inoculated, since the reaction time was inoculum-dependent. One loopful of growth from an 18-hr Triple Sugar Iron Agar slant invariably produced a firm clot within 1 to 3.5 hr with each of the 30 citrate-utilizing bacterial species tested, except for Salmonella typhi. The delayed clotting time (6.5 hr) observed with two strains of S. typhi is in agreement with unpublished work of the Florida State Board of Health laboratories, whose investigators found that S. typhi required a longer incubation period than did other enteric organisms to produce a positive reaction on Christensen's Citrate Agar.

In corroboration of the work of Bayliss and Hall, a nutritive medium was found to be necessary for clotting to occur; saline was unsatisfactory. The addition of glucose delayed clotting time in proportion to the amount added to broth media. Clotting occurred more rapidly with Heart Infusion Broth (HIB) as a diluent than with other media tested. Assays of various concentrations of dehydrated HIB showed that use of half-strength (12.5 g/liter) HIB resulted in faster clotting times than did other concentrations. Blood was chosen rather than plasma because it clotted as fast and did not require centrifugation. The addition of calcium chloride to sequester excess citrate by forming inactive calcium citrate was markedly beneficial for rapid clotting. The composition of the final broth contained, per liter: 12.5 g of HIB, 100.0 mg of CaCl₂, and 2.5 g of NaCl (to prevent red blood cell lysis). The broth was distributed in 85-ml amounts in screw-capped bottles. These were autoclaved and stored until needed. When needed, 15 ml of outdated citrated bank blood was added to a bottle of the broth, and 1-ml amounts of this mixture were pipetted aseptically into small sterile tubes for testing citrate utilization. The 15% blood used for the test was the smallest.

Fig. 1. Difference in appearance between positive and negative tubes turned sideways with the blood coagulation test for citrate utilization. Each positive tube was inoculated with a different species of citrate-utilizing organism, including an Escherichia coli paracolon. The red blood cells had settled to the bottom of tube 3 before clotting took place owing to the longer clotting time required for this organism (Salmonella typhi). The negative reactions were obtained with various species and serotypes of Shigella and a culture of Proteus morganii. Tube C is an uninoculated control.
concentration of blood which would consistently produce a complete clot (Fig. 1). The remaining blood broth in the bottle was usable for 1 to 2 months when stored in the refrigerator.

We believe the greatest value of this test among the Enterobacteriaceae would be in the separation of Shigella species from Shigella-appearing paracolon organisms on TSI slants. Escherichia coli paracolons, as well as other cultures not eliminated as possible Shigella by use of the rapid urease test, completely clotted the medium within 1 to 3.5 hr. Shigella cultures did not clot the medium. This 3.5-hr test appears to be the fastest cultural procedure for the elimination of virtually all of these urease-negative Shigella-appearing paracolons from consideration as possible Shigella.