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Brief Communication

IDENTIFICATION OF HAEMOPHILUS EQUIGENITALIS BY MEANS OF CO-AGGLUTINATION

Contagious equine metritis (CEM) was first reported by *Crowhurst* (1977), and the causal organism, a Gram-negative, fastidious and microaerophilic coccobacillus, was first isolated by *Platt et al.* (1977). The organism was described in detail by *Taylor et al.* (1978) who proposed the name Haemophilus equigenitalis.

Several workers (*Fleming & Tribe* 1977, *Swaney & Sahu* 1978, *Platt et al.* 1978, *Swerczek* 1978) observed the best growth of H. equigenitalis on chocolate agar prepared from Eugon agar (BBL*). However, as indicated by *Swerczek*, on this medium the growth acquires a wax-like consistency which is disadvantageous to emulsification for the performance of Gram-staining and slide agglutination. Moreover, *Platt et al.* (1978) observed that H. equigenitalis auto-agglutinated when cultured on Eugon agar. These disadvantages can be overcome by culturing the organism on Columbia agar (Oxoid). Faced with growing of the organism on Eugon agar while waiting for the delivery of Columbia agar and having previously achieved successful results with grouping of streptococci by means of co-agglutination (*Saxegaard* 1977), an attempt was made to see if the co-agglutination test could be applied to the identification of H. equigenitalis.

Four days old cultures of H. equigenitalis strain 61717 on chocolated Eugon agar were harvested in saline with 0.3 % formalin, washed twice, resuspended in saline with formalin and adapted to McFarland tube No. 3 or 9×10^8 cells per ml. One rabbit was immunized by six intravenous injections, starting with 1 ml and increasing to 2 ml. Two injections were given per week and the rabbit was bled one week after the last injection. A 10 % suspension, in phosphate-buffered saline, of formaldehyde and heat-treated Staphylococcus aureus containing protein A (NCTC** 8530) was prepared according to the method of *Kronvall* (1973) at the National Institute of Public Health, Oslo.

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* Baltimore Biological Laboratories.

** National Collection of Type Cultures, London.

The coating procedure was performed as described by *Christensen et al.* (1973). Prior to co-agglutination, four days old cultures of H. equigenitalis in Eugon broth with 10 % horse serum were centrifuged and the cells resuspended in 0.5 ml trisbuffer*, 0.2 M, pH 8.0. Three drops of a 5 % solution of trypsin were added and the suspension left in a water bath at 37 °C for 1 hr. To test for cross-reactivity, other strains of bacteria were cultured for 48 hrs. in Eugon broth with serum before trypsinization. The co-agglutination test was performed by mixing one drop of trypsinized culture and one drop of coated staphylococcal reagent on a glass slide. The slide was tilted and observed for co-agglutination for 1 min. Later trypsinization of H. equigenitalis was omitted and the co-agglutination test performed directly with cultures on chocolated Columbia agar.

H. equigenitalis showed distinct reaction in the co-agglutination test and no auto-agglutination when cultered in Eugon broth. No cross-reactivity was observed between the coated staphylococcal reagent and trypsinized cultures** of Actinobacillus equuli, A. lignieresii, Brucella abortus, B. canis, Bordetella bronchiseptica, Haemophilus gallinarum, Pasteurella multocida, Yersinia enterocolitica and Y. pseudotuberculosis. Nor was any cross-reactivity observed between the staphylococcal reagent and cultures of these organisms taken direct from blood agar plates.

The co-agglutination test appears to be a useful test in the serological identification of H. equigenitalis. The test is simple, rapid and easy to perform, especially when cultures can be taken direct from Columbia agar plates and mixed with the staphylococcal reagent. As mentioned by *Taylor et al.*, H. equigenitalis is biochemically very inactive. Conclusively, it is all the more important to verify the diagnosis by means of serological methods, and for this purpose it is convenient to have two direct tests available. Of these the co-agglutination test, because of the larger aggregates due to the presence of staphylococci containing protein A, gives more distinct reaction than the slide agglutination test.

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* Tris(hydroxymethyl) aminomethane, Sigma.

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