

Screening Tests for the Presumptive Identification of *Corynebacterium diphtheriae* in a Diagnostic Laboratory

We were concerned to read the recent article by Pennie et al. (7) on the misidentification of toxigenic *Corynebacterium diphtheriae* as a *Corynebacterium* sp. with low virulence in a child with endocarditis. The paper raises several issues relating to current methods for the microbiological diagnosis of diphtheria. Firstly, specific guidelines on laboratory diagnosis have been issued by the World Health Organization (WHO) (5) and there have been several recent publications relating to this area in view of the resurgence of diphtheria in Eastern Europe and the emergence of other infections caused by non-toxin-producing strains (1, 3, 4).

Secondly, the fermentation of sucrose is not regarded as an essential test in the laboratory diagnosis of *C. diphtheriae* infection. Pennie et al. do not state the methodologies in use within the originating laboratory in Malaysia. It is stated by the WHO that screening tests for the presumptive identification of toxigenic *C. diphtheriae* are essential within the diagnostic laboratory. We refer in particular to tests for the enzymes pyrazinamidase and cystinase. It is likely that use of these simple screening tests would avoid misidentification of *C. diphtheriae*. *C. diphtheriae* (all biotypes), *C. ulcerans*, and *C. pseudotuberculosis* do not produce the enzyme pyrazinamidase but do, however, produce the enzyme cystinase. "*C. xerosis*" and other corynebacteria are usually pyrazinamidase positive and cystinase negative (5). The WHO manual makes recommendations for the use of positive and negative controls for these tests.

The definitive identification of *C. diphtheriae* to species and biotype levels relies upon biochemical tests, fermentation of sugars, hydrolysis of urea, and nitrate reduction, in addition to the detection of toxigenicity.

Lastly, a recent publication from Funke and colleagues states that the majority of "*C. xerosis*" strains reported in the literature may have been misidentified as *C. amycolatum*. They

further emphasize that from their data, "*C. xerosis*" is rarely encountered in clinical specimens (6). Data from Coyle and colleagues (2) clearly show the diversity among "*C. xerosis*" organisms: they appear to comprise six taxonomic groups, one of which is indistinguishable from *C. striatum*.

In view of the immense public health significance attached to the isolation of toxigenic *C. diphtheriae*, we fully support all attempts to ensure that accurate methodologies are in use within diagnostic and reference microbiology laboratories.

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Misidentification of *Corynebacterium diphtheriae*

Pennie et al. (6) described the misidentification of toxigenic *Corynebacterium diphtheriae* as *C. xerosis* in a child with endocarditis. According to the authors, this organism was misidentified because of an erroneous positive result of the sucrose fermentation test. Though sucrose-positive strains of *C. diphtheriae* are rare, their existence has long been appreciated (2). While some current standard references (4, 5) mention that such strains may be encountered, at least one other (1) does not. Though said to be "extremely rare" in the United States and Europe, sucrose-positive strains were described as being found to be "not uncommon" in one report of a study done in Brazil (3). This suggests the possibility of other geographic foci where they are not so rare. Our laboratory has received a toxigenic sucrose-positive strain isolated from the throat of a person who had been to Brazil. We have also received a toxigenic sucrose-positive strain isolated from the nose of a patient from San Diego, Calif. Travel history on this patient is unknown (unpublished data).

In the event that a laboratory isolates a gram-positive coryneform rod which resembles *C. diphtheriae* (catalase positive; positive fermentation tests in glucose, maltose, and, rarely, sucrose; urea, esculin, and gelatin hydrolysis negative), a few other tests should be performed. The Gram stain morphology on 18- to 24-h growth from Loeffler or Pai media is so unique to *C. diphtheriae* that experienced bacteriologists can give a presumptive positive report based on this morphology. Some laboratories routinely use 18- to 24-h growth of *C. diphtheriae* from Loeffler or Pai media as the quality control test for the Gram stain in order to keep the microbiologists familiar with this typical morphology.

Any organism which is suggestive of *C. diphtheriae* either by biochemical reactions or typical Gram stain morphology should also be cultured on Tinsdale medium. *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* will produce a typical brown halo around their colonies on this medium (not to be confused with black colonies alone, which are produced by

several *Corynebacterium* species and even some *Staphylococcus* species). *C. ulcerans* and *C. pseudotuberculosis* can be differentiated from *C. diphtheriae* by their production of urease (*C. diphtheriae* is urease negative) and by their cellular morphology.

As Pennie et al. (6) point out, accurate identification of *C. diphtheriae* is of concern in view of the dramatic increase in the number of cases in the New Independent States of the former Soviet Union. It is important for microbiologists to upgrade their skills in identifying this pathogen.

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Ed. Note: The article by Pennie et al. and the letters by Wong et al. and Efstratiou and George illustrate problems associated with the misidentification of isolates of toxigenic Corynebacterium diphtheriae. These articles stress the importance of maintaining appropriate experience with this group of organisms in order to avoid future problems and urge continued vigilance by clinical laboratorians and use of appropriate tests to ensure that these important pathogens are accurately identified.