Increased Indole Detection for *Pasteurella multocida*

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A supplemented 2% peptone broth is described for the detection of indole production by *Pasteurella multocida*. The 96 isolates of *P. multocida* that were utilized in this evaluation were indole positive within 18 to 24 h.

*Pasteurella multocida* isolates from different hosts show a wide variability in physiological characteristics (1–5). In view of the fulminant course that *P. multocida* infections can take, rapid and accurate identification is imperative. However, positive identification can be difficult depending on the selection of biochemical media (5). Indole production by *P. multocida* is an important but variable characteristic of identification (2–5).

Heddleston (2) reported that 97 to 100% of 1,268 isolates of *P. multocida*, grouped by host origin, were indole positive when grown in 2% tryptone broth after a 5-day incubation. However, isolates from humans, rabbits, cattle, and sheep were inconsistent for indole production (2, 3). Lu et al. (4) reported that 15 of 32 and 8 of 10 rabbit isolates were indole positive. Oberhofer (5) observed that 90 to 100% of human isolates were indole positive after 5 days of incubation. His results varied with the different procedures used for detecting indole production (the conventional and indole spot test), selection of medium for the indole test, and the length of the incubation period. All of 62 *P. multocida* isolates of human, animal, and avian origin were indole positive after 4 days of incubation at 37°C in a 1% tryptone broth (J. Gadberry, unpublished data). This paper reports on a modified, supplemented peptone medium which provides rapid and reliable detection of indole production within 18 to 24 h with *P. multocida*.

Cultures were maintained by weekly transfer on 5% sheep blood agar plates. The medium used for the detection of indole production is a modification of a medium initially described by Rifkind and Pickett (7) and used as a basal culture medium in assaying for *Pasteurella* bacteriophage. They also used this medium for the detection of indole production. We have modified their method and report it in this paper.

The formulation and preparation of the medium (MRP broth) that we used included: 20 g of peptone (Difco Laboratories, Detroit, Mich.), 5.0 g of yeast autolysate (Sigma Chemical Co., St. Louis, Mo.), 5.0 g of NaCl, 990 ml of distilled water, and 10 ml of a 100× vitamin concentrate. The vitamin concentrate included: 500 mg of hemin, 100 mg of nicotinamide, 100 mg of calcium pantothenate, and 100 mg of thiamine hydrochloride (Sigma) in 1 liter of 0.01 N NaOH. The concentrated vitamin mixture was stored at 4°C and had a shelf life of at least 1 year. The final pH of the MRP broth was adjusted to 7.5 with 10 N NaOH, dispersed into 3-ml portions, and sterilized by autoclaving (15 min at 121°C).

Tubes of MRP broth were inoculated with isolates of *P. multocida* grown on 5% sheep blood agar at 37°C for 18 h. In preliminary studies, the inoculated MRP broth was incubated at 37°C as either aerated (shaker cultures, 150 rpm) or nonaerated cultures to determine which environment would provide adequate growth for the test. Samples of the MRP broth were then removed from each culture after 24 and 48 h and after 5 days of incubation. Kovac indole reagent (0.5 ml) was added to each tube which was then shaken gently. A deep-red color was read as a positive test for indole, and a negative test was recorded if this color did not develop after the addition of Kovac reagent (6). No difference in indole production was seen between the aerated and nonaerated cultures.

A total of 96 *P. multocida* isolates of human, animal, and avian origin (Table 1) were inoculated, in triplicate, in MRP broth. Isolates of *P. ureae* and *P. haemolytica* were used as indole-negative controls. All cultures were incubated at 37°C for no longer than 18 to 24 h. Kovac reagent was added to each tube as described above. Only the development of a deep-red color in the MRP broth was recorded as indole positive. Indole production was detected in all 96 isolates of *P. multocida* within 18 to 24 h after inoculation (Table 1), and as early as 12 h for some isolates. In comparison, Oberhofer (5) observed that 87 to 88% of his 48 strains of *P. multocida* were indole positive after 24 h of
incubation with the conventional test, and 100% of 31 strains were indole positive with the spot test.

Isolates from sources which previously were reported to give delayed or variable indole-positive reactions (2–5) with the conventional methods were included in this study. Detection of indole production was consistent and rapid for all isolates of *P. multocida* in the MRP broth. Indole production was not detected in the negative controls. This study demonstrates that MRP broth is highly superior to the conventional media and is as sensitive as the spot indole test, both of which detect indole production in *P. multocida*. The usual problems of poor growth and delayed reactions cited in the literature were not encountered with this method. The supplementation of the peptone broth was important for providing optimal growth conditions, which increased the rapidity and reliability of this test. Although it is somewhat complex to prepare, the MRP broth is a valuable tool for the detection of indole production in *P. multocida*. It should be considered for use along with the other media routinely used in the biochemical identification of *P. multocida*. Preliminary studies indicate that the MRP broth is reliable for the detection of indole in those species of the *Enterobacteriaceae* which are known to be indole positive. However, further evaluations are necessary.

### LITERATURE CITED