Comparison of Rapid Identification Method and Conventional Substrates for Identification of Corynebacterium Group JK Isolates

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In an effort to identify Corynebacterium group JK isolates rapidly, Rapid Identification Method (RIM series; Austin Biological Laboratories, Inc., Austin, Tex.) substrates were tested in parallel with conventional substrates. RIM reactions agreed with conventional substrate results, respectively, as follows: urea, 38 of 38; nitrate, 35 of 38; glucose, 35 of 38; maltose, 28 of 38; sucrose, 37 of 38; and o-nitrophenyl-β-D-galactopyranoside, 24 of 26. As a supplement to initial screening tests, the RIM tests offer a rapid method for identifying group JK isolates.

Corynebacterium group JK can be part of the normal skin flora. However, group JK isolates have been associated with sepsis, prosthetic heart valve infections, pyelonephritis, peritonitis, and meningitis (4, 7). The resistance antibiogram seen with group JK isolates can also complicate treatment. For these reasons the College of American Pathologists requires all extent-four laboratories to distinguish group JK isolates from other Corynebacterium spp. (2).

Conventional biochemical identification of group JK is cumbersome and time consuming, requiring an extended incubation period of at least 72 h. To identify the corynebacteria more rapidly, investigators have used rapid biochemical tests prepared in-house, i.e., the API 20S (Analytab Products, Plainview, N.Y.) and the MiniTest system (BBL Microbiology Systems, Cockeysville, Md.) (5, 6, 10, 11). Kelly et al. (6) found the API 20S reliable only for identification of groups JK and D2, whereas other investigators (5, 10, 11) found that rapid testing was reliable for carbohydrate reactions, nitrate reduction, and urease activity of group JK and other fastidious gram-positive organisms (9).

The Rapid Identification Method (RIM series; Austin Biological Laboratories, Inc., Austin, Tex.) offers tests for carbohydrate utilization, nitrate reduction, and urease activity that require less than 1 h of incubation. The RIM-N kit includes glucose, maltose, sucrose, and lactose carbohydrate substrates. Utilizing parameters for the conventional identification of group JK isolates as described by Coyle et al. (3) and Riley et al. (9), we devised a scheme to identify group JK isolates by incorporating overnight conventional tests with RIM tests. Our proposed identification scheme utilized the following parameters: Gram stain morphology; hemolysis on 5% sheep blood agar (SBAP); growth on triple sugar iron; catalase; motility; and RIM glucose, maltose, sucrose, nitrate, urea, and o-nitrophenyl-β-D-galactopyranoside (ONPG) reactions. To evaluate the reliability of the RIM method, RIM substrates were tested according to the instructions of the manufacturer and run in parallel with conventional metabolic substrates. The RIM method requires delivery of 3 substrate drops into each of the provided tubes. A loopful of fresh (18- to 24-h-old) culture is inoculated into the substrate. After 30 to 60 min of incubation, reagents can be added to the tube, if necessary, and the color reactions can be visually read.

The 38 organisms tested were isolated in pure culture from 21 patient blood cultures; 22 organisms represented multiple isolates from 5 patients. For inclusion in the study, isolates had to have a pleomorphic gram-positive bacillus (diphtheroid) Gram stain morphology. All isolates were collected over a period of several years and had been frozen in buffered glycerol broth at −70°C. Prior to testing, isolates were subcultured three times onto SBAP.

For conventional identification of group JK isolates, the following parameters (with required reactions in parentheses) were used as described by Coyle et al. (3) and Riley et al. (9): catalase (+); hemolysis on SBAP (−); nitrate reduction (−); urease (−); gelatin hydrolysis (−); motility (−); esculin hydrolysis (−); ONPG (v [variable]); growth on triple sugar iron (no growth); and carbohydrate utilization of glucose (+), maltose (v), sucrose (−), mannitol (−), and xylose (−). Andrade carbohydrate broths were supplemented with 3 drops of rabbit serum (GIBCO Laboratories, Grand Island, N.Y.) per tube and read daily for 7 days. Urease activity was detected on Christensen urea agar slants. Nitrate reduction was determined with in-house-prepared nitrate broth with a heart infusion base (1). Nutrient gelatin (1%) tubes (Regional Media Laboratories, Lenexa, Kans.) were read daily after 24 h of incubation at 35°C and subsequent incubation at room temperature for 8 days. Motility was determined from wet-mount preparations from growth on the 5% SBAP. We used the Centers for Disease Control method of distinguishing group JK isolates from Corynebacterium bovis, with ONPG tablets (Key Scientific Products Co. Inc., Los Angeles, Calif.) utilized according to the instructions of the manufacturer. Conventional media were obtained from BBL, incubated at 35°C in air, and read daily for 3 days unless otherwise indicated.

Conventional biochemical reactions identified 21 of 38 isolates as group JK. To identify group JK isolates, a positive glucose fermentation, which required a minimum of 72 h of incubation for the isolates, was needed. This lengthy incubation precludes the usefulness of conventional tests in providing timely results to clinicians.

RIM tests correlated well (≥92%) with conventional methods, with the exception of the maltose test (Table 1). Discrepant RIM glucose reactions were false-negative (Table 2), while 8 of 10 discrepant maltose reactions were

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false-positive. Excluding discrepant maltose and ONPG reactions which did not contribute to the misidentification of any of the group JK isolates, RIM tests would have identified 16 of 21 JK isolates upon initial testing. These five significant group JK identification discrepancies occurred with five patient isolates. None of the non-group JK isolates were misidentified as group JK.

Repeat testing corrected 12 of 19 RIM discrepancies (Table 1), increasing the corrected RIM identifications to 21 of 21 JK isolates. When negative glucose reactions were obtained by RIM, all other RIM tests were negative. These negative reactions should alert one to retest glucose fermentation capability and thus prevent JK misidentifications. The short incubation period for RIM allows the test to be repeated easily that same day.

Upon evaluating essential tests to identify group JK isolates, we found that maltose and ONPG could be eliminated from the battery of tests. The maltose reaction for group JK isolates is variable and therefore not a discriminating test. Group JK and C. bovis are biochemically similar; however, Riley et al. (9) and Coyle et al. (3) suggest the use of ONPG to differentiate between group JK (ONPG negative) and C. bovis (ONPG positive). Our group JK isolates were not consistently negative for ONPG; this finding supports the data of McGinley et al. (8). C. bovis is rarely isolated from human infections; consequently, other parameters, such as source of specimen, should be considered before reporting an ONPG-positive group JK isolate as C. bovis.

After malose and ONPG tests were eliminated, our JK identification scheme utilized the following parameters: Gram stain morphology, catalase, hemolysis on SBAp, motility, and growth on triple sugar iron in conjunction with RIM glucose, sucrose, nitrate, and urea. Negative glucose reactions by RIM should be evaluated with all other conventional parameters, including a resistance antibiogram, if present, to spot possible misidentifications. The RIM method offers rapid determination of biochemical properties for the pleomorphic gram-positive bacilli and helps identify group JK isolates days sooner than would a conventional identification.

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LITERATURE CITED