Evaluation of the RapID NH System for Identification of *Haemophilus somnus*, *Pasteurella multocida*, *Pasteurella haemolytica*, and *Actinobacillus pleuropneumoniae* Isolated from Cattle and Pigs with Respiratory Disease

SARAH A. SALMON,* JEFFREY L. WATTS, AND ROBERT J. YANCEY, JR.

The Upjohn Company, Animal Health Therapeutics Research, Kalamazoo, Michigan 49001

Received 21 December 1992/Accepted 19 February 1993

*Haemophilus somnus*, *Pasteurella haemolytica*, *Pasteurella multocida*, and *Actinobacillus pleuropneumoniae* from cattle and pigs with respiratory disease were used to evaluate the RapID NH system (Innovative Diagnostics, Atlanta, Ga.). Minor modifications of the RapID NH system to include animal source and growth requirements would permit the identification of all isolates tested.

*Haemophilus somnus*, *Pasteurella haemolytica*, *Pasteurella multocida*, and *Actinobacillus pleuropneumoniae* are gram-negative bacilli commonly isolated from cattle, swine, poultry, sheep, and goats (2, 5-8). Under conditions of stress and/or in association with viral respiratory agents, *H. somnus*, *P. haemolytica*, and *P. multocida* may produce an acute bronchopneumonia known as bovine respiratory disease (BRD) or shipping fever (3). These organisms have also been associated with respiratory disease processes in cattle and other animals (1, 2, 4, 6, 7). *A. pleuropneumoniae* is a causative agent of pleuropneumonia in pigs, a swine respiratory disease (SRD), and has also been implicated in porcine arthritis and meningitis (8, 9). Current methods for identification of these BRD and SRD pathogens are based upon conventional macrotube methods and rely heavily on the experience of the veterinary microobiologist for accurate interpretation (1, 2, 4). Many macrotube methods are tedious and time-consuming, and clinical microbiology laboratories performing veterinary bacteriology procedures and small veterinary diagnostic laboratories lack the expertise to readily identify BRD and SRD pathogens. Thus, accurate, convenient methods for the identification of these organisms are needed. The RapID NH system (Innovative Diagnostics, Atlanta, Ga.) is designed for the rapid identification of species of *Neisseria* and *Haemophilus* isolated from humans. This system does not currently include veterinary pathogens in its data base. The purpose of this study was to determine whether the RapID NH system could be easily adapted to accurately identify these BRD and SRD pathogens.

Isolates used in this study had been previously identified by veterinary reference laboratories in the United States and Canada and submitted to The Upjohn Animal Health Therapeutics Culture Collection from 1988 to 1992. Confirmation of identification was performed upon receipt by using previously described conventional methods (2, 6-8). The following type strains of each organism were also used: *H. somnus* ATCC 43625, *P. haemolytica* ATCC 33396, *P. multocida* ATCC 43137, and *A. pleuropneumoniae* type 1 ATCC 27088. Isolates were maintained at −70°C in 1.0-ml vials on sterile 3-mm glass beads in 0.5 ml of tryptic soy broth (Difco, Detroit, Mich.) with 10% glycerol until used. Cultures were subcultured twice onto freshly prepared blood agar base (Difco) plates supplemented with 5% sheep blood (*H. somnus*, *P. haemolytica*, and *P. multocida*) or chocolate agar plates with 2% supplement C (Difco) (*A. pleuropneumoniae*) and incubated at 37°C in 5% CO2. These 18- to 24-h isolates were suspended with sterile cotton swabs in RapID inoculation fluid (purchased separately) to a turbidity equivalent to or greater than that of a no. 3 McFarland standard. Suspensions were hand mixed, and the entire suspension was used to inoculate the test panel. Inoculated panels were incubated aerobically at 37°C for 4 h. After incubation, reactions were interpreted according to the manufacturer's instructions and four-digit profile numbers (biocodes) for all isolates were compared with those listed in the RapID NH code compendium.

All 101 *H. somnus* isolates tested yielded a unique biocode (0146) which was not listed in the RapID NH code compendium. The addition of this organism to the RapID NH data base would permit the system to identify this bovine pathogen. *P. haemolytica* could also be easily identified by this system. Fifty-one of 53 (92.5%) *P. haemolytica* isolates were identified by the RapID NH as *Haemophilus segnis*. *H. segnis* has been isolated most commonly from the dental plaque of humans and requires NAD for growth (6), unlike *P. haemolytica*, which grows well on blood agar and is commonly associated with disease in cattle, sheep, or goats (2, 7). Eighty-three percent of the *P. multocida* isolates were correctly identified by the system. The remaining 9 isolates yielded identifications of *Haemophilus influenzae* or inadequate identifications with probability overlap between *H. influenzae* and *P. multocida*. However, *H. influenzae* requires both X and V factors for growth and has not been detected in cattle or pigs (6), while *P. multocida* requires no factors for growth and is one of the most common pathogens isolated from respiratory diseases of animals (2, 7). All 51 isolates of *A. pleuropneumoniae* tested with the system were identified as *Haemophilus parainfluenzae*. *A. pleuropneumoniae* was first classified as *H. parainfluenzae* (8), an organism commonly isolated from humans (5, 6). Later studies reclassified the organism as a separate species, *Haemophilus pleuropneumoniae* (5). More recent studies transferred this organism to the genus *Actinobacillus* on the basis of phenotypic and DNA relatedness to this genus (9). Since the reclassification to *A. pleuropneumoniae*, *H. parainfluenzae* has not been reported from domestic animal...
sources (5, 6). Inclusion of *A. pleuropneumoniae* in the data base would prevent this organism from being incorrectly identified as *H. parainfluenzae* by clinical laboratories which may be unfamiliar with this veterinary pathogen.

The results of this study indicate that the RapID NH system could be easily modified to permit the accurate and rapid identification of BRD and SRD pathogens. With an increasing number of clinical microbiology and small veterinary laboratories performing veterinary bacteriological procedures, a convenient system such as the RapID NH would be of great utility. Furthermore, many clinical microbiology laboratories are familiar with this system, and expansion of the RapID NH data base to include these organisms would permit clinical laboratories to use the same system to identify both human and veterinary isolates.

In conclusion, minor modifications of the RapID NH data base to include current taxonomy, animal host species, and growth requirements would permit accurate, convenient identification of *H. somnus, P. haemolytica, P. multocida,* and *A. pleuropneumoniae*. Further studies are necessary to determine whether this system would also be useful in the identification of other members of the family *Pasteurellaceae* of veterinary significance.

REFERENCES


