3-1986

Comparison of a New Commercially Prepared Porphyrin Test and the Conventional Satellite Test for the Identification of Haemophilus Species That Require the X Factor

Joseph L. Gadberry

Mary Ann Amos

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1986, p. 637–639
0095-1137/86/030637-03$02.00/0

Vol. 23, No. 3

Comparison of a New Commercially Prepared Porphyrin Test and the Conventional Satellite Test for the Identification of *Haemophilus* Species That Require the X Factor

JOSEPH L. GADBERRY[1]* AND MARY ANN AMOS[2]

Department of Microbiology, The University of Health Sciences College of Osteopathic Medicine, Kansas City, Missouri 64124,[1] and Grebe and Amos, Inc., Leawood, Kansas 66202[2]

Received 24 July 1985/Accepted 23 November 1985

A test with a commercially developed porphyrin test agar was examined for the identification of *Haemophilus* spp. The porphyrin test agar method was compared with the conventional paper strip satellite method in tests with 187 isolates and was found to be easier to perform and interpret, giving a sensitivity of 98.7% and specificity of 94.7%.

The genus *Haemophilus* contains a number of species which are important human and animal pathogens, as well as several species which reside in humans as endogenous flora. Speciation of *Haemophilus* isolates in the clinical laboratory has been primarily based on the requirement for hemin (X factor) or NAD (also known as V factor or coenzyme I) or both. *Haemophilus influenzae* is a frequently isolated bacterial pathogen causing pyogenic meningitis, arthritis, epiglottitis, and facial and orbital cellulitis in the pediatric population (8, 15, 20). It is also a frequent cause of pneumonia and other infections in adults (4, 15, 16). This bacterium requires both the X and V factors. The endogenous respiratory species, *Haemophilus parainfluenzae,* although rarely pathogenic, has recently become a more frequently isolated pathogen in clinical specimens (1–3, 5–7, 12–14, 16, 22, 24, 25, 27). It requires only the V factor. The methods currently used for the determination of these growth factor requirements rely on the supplements being added to defined agar media or being impregnated in disks or strips placed on agar plates. These methods are time consuming to perform, and interpretation of the results is often difficult and can lack reproducibility (9–11, 21). Another method for differentiation is based on the ability of non-hemin-requiring species to use δ-aminolevulinic acid (ALA) as a substrate for porphyrin synthesis (17). Until recently the only methods for porphyrin testing relied on a tube test (17, 21) or a reagent disk (23). We evaluated a new commercially available porphyrin test agar (PTA) (Remel, Lenexa, Kans.) to determine its practicality and reliability when compared with a conventional satellite method (which is frequently used in clinical laboratories) in which impregnated paper strips are used. Since PTA is a proprietary product, the composition of the agar is not available, but it does contain chocolate agar as an ingredient of the medium.

Laboratory strains and isolates obtained from clinical specimens were grown for 18 to 24 h on chocolate agar. In the conventional method, a saline suspension of six or more colonies was made and used to inoculate a Mueller-Hinton (MH) agar plate onto which X and V factor strips (BBL Microbiology Systems, Cockeysville, Md.) were placed as recommended by the manufacturer. The PTA was inoculated with a single colony from the same chocolate agar plates. The inoculation was carried out by making a pencil line streak and stab. Both the MH and the PTA plates were incubated for 18 to 24 h at 35°C under 5 to 10% CO_2.

The MH plates were examined for growth around the paper strips. Growth around both the X and V strips indicated dependence on hemin and NAD, whereas growth around the V strip only indicated hemin independence. The PTA plates were examined under UV illumination (wavelength, 350 nm). Bacteria capable of utilizing ALA in the biosynthesis of porphyrins produced orange fluorescence along the streak or the stab or both. Hemin-dependent organisms, which lack the enzymes necessary for heme synthesis and do not synthesize porphyrins, produced no fluorescence. Each method was interpreted independently without knowledge of the results of the other method.

We compared the results obtained from the PTA test with the results from the conventional satellite method, a test which is routinely used in clinical microbiology laboratories for the identification of *H. influenzae* and *H. parainfluenzae* (Table 1). Of the 187 isolates which we tested, 38 were found to be non-hemin requiring by the conventional method. We also found 38 isolates to be porphyrin positive by the PTA method. Among the porphyrin-positive isolates, two isolates gave false-positive results (i.e., fluorescence when both the X and V factors were required) and two isolates gave false-negative results (i.e., no fluorescence and no X factor requirement), as was confirmed by the conventional satellite test. We further observed four isolates which were weakly positive or fluoresced in the area of the stab only.

There was a 98% agreement between the PTA method and conventional satellite methods when the two procedures were compared for determining X factor requirements of *Haemophilus* isolates. We found that the sensitivity of the PTA test was 98.7% and the specificity was 94.7% compared with the conventional method.

The bacterial strains giving inconsistent results were further tested by using quad plates (Remel) and the biochemical tests, ornithine, urea, and indole. One of the PTA-false-positive isolates was found to be porphyrin negative upon retesting and was identified as *H. influenzae* by the biochemical testing coupled with the hemolysis results on horse blood. Four isolates which were weakly positive on PTA were identified as *H. parainfluenzae*. Three of these isolates were biotype II and one fit the classification of the unnamed taxon B described by Kilian (18). Of the two false-negative

* Corresponding author.

TABLE 1. Comparison of the PTA test with the conventional satellite test for hemin-independent porphyrin synthesis

Test	No. of isolates giving the following result:			
	True-positive	True-negative	False-positive	False-negative
PTA	36	147	2	2
Satellite	38	149	0	0

isolates, one was identified as *H. parainfluenzae* biotype II and the other was lost to further identification.

Isolates of *Haemophilus* spp. which gave different results on the PTA versus the conventional satellite test were further evaluated by using the porphyrin tube test (PTT) (Remel). The PTT was done as recommended by the manufacturer. Twenty Isolates, including American Type Culture Collection cultures of *H. influenzae* and *H. parainfluenzae*, were tested simultaneously to determine the correlation between the two porphyrin tests. There was good correlation between these two methods. Two isolates gave weak positive reactions with the PTT, whereas only one isolate was weakly positive with the PTA test. There was only one false-negative isolate with the PTA test and the PTT gave only a weakly positive reaction for the same isolate. There was a 94% sensitivity with the PTA test when compared with the PTT.

The PTA method of determining porphyrin production was also compared with the commercially available ALA reagent disk (Remel) (25). For the 53 isolates tested (Table 2), the PTA method was more sensitive than the ALA disk method, identifying six porphyrin-producing isolates. The disk method identified only four isolates. Results of both methods were verified by the conventional satellite strips.

The serious nature of many *Haemophilus* infections dictates that proper identification of species be made. The porphyrin test has been recommended as the method of choice in identifying *Haemophilus* spp. (19). The results in this study indicate that the PTA test is more sensitive than the ALA-containing disks in this determination.

The PTA test was considerably easier and faster to interpret than the growth around the X and V strips. Oftentimes the growth on the MH plates was very faint, making it difficult to interpret the results. The porphyrin media also eliminated the interpretation problem which can arise if the basal media, i.e., MH, tryptic soy agar, or brain heart infusion, used in the satellite test contains trace amounts of hemin. Erroneous results have been reported due to inconsistencies in the X factor content in these complex media (9–11). Carry-over of X factor with the inoculum has also been reported in a significant number of cases when testing for X factor requirement in the conventional manner, and *H. influenzae* may store hemin when grown on primary isolation medium (26). This problem also is avoided with porphyrin testing.

Another advantage of the system described here was the method of inoculation, which required only one colony, as opposed to the suspension of six or more colonies that was required for the satellite plates. Therefore, the time necessary for inoculation of plates was less. The PTA test was more economical than the satellite test since multiple bacterial isolates could be inoculated onto a single PTA plate. The satellite test required saline, basal agar plates, and X and V strips.

TABLE 2. Comparison of the PTA test and the ALA disk test for hemin independent porphyrin synthesis

Test	No. of isolates giving the following result:			
	True-positive	True-negative	False-positive	False-negative
PTA	6	47	0	0
ALA disk	4	47	0	2

Although the cost of each porphyrin test is about the same, there are several advantages to using the PTA test rather than the PTT. The PTA plates are much easier to inoculate (requiring only a pencil line streak from a single colony). At least five separate isolates can be grown on a single plate. The orange fluorescence produced by bacteria capable of utilizing ALA in the biosynthesis of porphyrins on PTA is not inoculum dependent. Therefore, there is no need for subculturing from primary isolation media. The PTT requires that a heavy inoculum from growth on an appropriate medium be suspended in the ALA substrate (19). This often requires a subculture from the primary isolation plate onto a chocolate agar plate for overnignt growth before inoculation of the PTT.

The stability of the ALA is also a factor. Stability of the substrate in the PTA plates is maintained at 4°C for 8 weeks from the date of manufacture. Although not an insurmountable problem, the ALA substrate for the PTT does necessitate special storage requirements (−20°C) and additional handling.

This study suggests that the PTA test, coupled with horse blood agar hemolysis, is as effective as the PTT for the identification of *Haemophilus* spp. requiring the X factor. However, because oxidase-positive and catalase-positive bacteria commonly found in the oropharynx can make heme and heme precursors from the ALA in the porphyrin agar, assurances must be made that the morphology of the bacteria being tested is consistent with *Haemophilus* spp.

(A preliminary report of this work has been presented [J. L. Gadberry and M. A. Amos, Abstr Annu. Meet. Am. Soc. Microbiol. 1984, C269, p. 281].)

We thank Delight Cain for secretarial assistance and typing the manuscript.

LITERATURE CITED

1. **Back, E., B. Carlson, and B. Hylander.** 1981. Urinary tract infection from *Haemophilus parainfluenzae*. Nephron **29:** 117–118.
2. **Blair, D. C., and W. Walker.** 1977. Bacterial endocarditis due to *Haemophilus parainfluenzae*. Chest **71:**146–149.
3. **Blaylock, B. L., and S. Baker.** 1980. Urinary tract infection caused by *Haemophilus parainfluenzae*. Am. J. Clin. Pathol. **74:**285–287.
4. **Brabender, W., G. R. Hodges, and W. G. Barnes.** 1984. Clinical significance of serotype, biotype, and β-lactamase production of respiratory isolates of *Haemophilus influenzae*. Am. J. Clin. Pathol. **81:**85–88.
5. **Chattopadhyay, B., P. H. Silverstone, and R. S. Winwood.** 1983. Liver abscess caused by *Haemophilus parainfluenzae*. Postgrad. Med. J. **59:**788–789.
6. **Chunn, C. J., S. R. Jones, J. A. McCutchan, E. J. Young, and D. N. Gilbert.** 1977. *Haemophilus parainfluenzae* infective endocarditis. Medicine **56:**99–113.
7. **Cooney, T. G., B. R. Harwood, and D. J. Meisner.** 1981. *Haemophilus parainfluenzae* thoracic empyema. Arch Intern. Med. **141:**940–941.
8. **Dajani, A. S., B. I. Asmar, and M. C. Thirumoorthi.** 1979. Systemic *Haemophilus influenzae* disease: an overview. J. Pediatr. **94:**335–363.

9. **Doern, G. V., and K. C. Chapin.** 1984. Laboratory identification of *Haemophilus influenzae*: effects of basal media on the results of the satellitism test and evaluation of the rapID NH system. J. Clin. Microbiol. **20**:599–601.
10. **Evans, N. M., S. M. Bell, and D. D. Smith.** 1975. New satellitism test for isolation and identification of *Haemophilus influenzae* and *Haemophilus parainfluenzae* in sputum. J. Clin. Microbiol. **1**:89–95.
11. **Evans, N. M., and D. D. Smith.** 1972. The effect of the medium and source of growth factors on the satellitism test for *Haemophilus* species. J. Med. Microbiol. **5**:509–514.
12. **Gallant, T. E., L. R. Malinak, D. W. Gump, and P. B. Mead.** 1977. *Hemophilus parainfluenzae* peritonitis associated with an intrauterine contraceptive device. Am. J. Obstet. Gynecol. **129**: 702–703.
13. **Geraci, J. E., C. J. Wilkowske, W. R. Wilson, and J. A. Washington II.** 1977. *Haemophilus* endocarditis. Report of 14 patients. Mayo Clin. Proc. **52**:209–215.
14. **Goldberg, R., and J. A. Washington II.** 1978. The taxonomy and antimicrobial susceptibility of *Haemophilus* species in clinical specimens. Am. J. Clin. Pathol. **70**:899–904.
15. **Hirschmann, J. V., and E. D. Everett.** 1979. *Haemophilus influenzae* infections in adults and a review of the literature. Medicine **58**:80–94.
16. **Jemsek, J. G., S. B. Greenberg, L. O. Gentry, D. E. Welton, and K. L. Mattox.** 1979. *Haemophilus parainfluenzae* endocarditis. Two cases and review of the literature in the past decade. Am. J. Med. **66**:51–57.
17. **Kilian, M.** 1974. A rapid method for the differentiation of *Haemophilus* strains. Acta Pathol. Microbiol. Scand. Sect. B **82**:835–842.
18. **Kilian, M.** 1976. A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. J. Gen. Microbiol. **93**:9–62.
19. **Kilian, M.** 1980. *Haemophilus*, p. 330-336. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of Clinical Microbiology, 3rd ed. American Society for Microbiology. Washington, D.C.
20. **Long, S. S., Teter, M. J., and P. H. Gilligan.** 1983. Biotype of *Haemophilus influenzae:* correlation with virulence and ampicillin resistance. J. Infect Dis. **147**:800–806.
21. **Lund, M. E., and D. J. Blazevic.** 1977. Rapid speciation of *Haemophilus* with the porphyrin production test versus the satellite test for X. J. Clin. microbiol. **5**:142–144.
22. **Maller, R., S. Ansehn, and A. Fryden.** 1977. *Haemophilus parainfluenzae* infection of the central nervous system. Scand. J. Infect. Dis. **9**:241–242.
23. **Matthews, J. S., J. A. Reynolds, D. E. Weesner, J. L. Perry, and A. L. Jenkins.** 1983. Rapid species identification and biotyping of respiratory isolates of *Haemophilus* spp. J. Clin. Microbiol. **18**:472–475.
24. **Nakamura, K. T., D. W. Beal, F. P. Koontz, and E. F. Bell.** 1984. Fulminant neonatal septicemia due to *Haemophilus parainfluenzae*. Am. J. Clin. Pathol. **81**:388–389.
25. **Oill, P. A., A. W. Chow, and L. B. Guze.** 1979. Adult bacteremic *Haemophilus parainfluenzae* infections. Arch. Intern. Med. **139**:985–988.
26. **Santanam, P.** 1984. A modified method for differentiation of *Haemophilus influenzae* from *Haemophilus parainfluenzae*. Eur. J. Clin. Microbiol. **3**:150–151.
27. **Warren, S. T., E. Reinitz, and R. S. Klein.** 1981. *Haemophilus parainfluenzae* septic arthritis in an adult J. Am. Med. Assoc. **246**:868–869.