

# Application of Paraffin Bait Technique to the Isolation of *Nocardia asteroides* from Clinical Specimens

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The principal findings of a study for further evaluating paraffin baiting as a routine laboratory diagnostic procedure in the isolation of *Nocardia asteroides*, the etiological agent of nocardiosis, are reported.

*Nocardia asteroides*, the etiological agent of nocardiosis, is a soil-inhabiting organism belonging to the actinomycetes. It has been repeatedly isolated (2, 4, 6) from soil with the help of a rather simple technique of paraffin baiting introduced by Gordon and Hagan in 1936 (2). The possibility that this technique may also be profitably applied to the isolation of *N. asteroides* from clinical specimens which frequently harbor a variety of contaminating microflora was suggested in an earlier study from this laboratory (P. V. Kurup, Ph.D. Thesis, Univ. of Delhi, 1967). In this communication, we report the principal findings of a study for further evaluating paraffin baiting as a routine laboratory diagnostic procedure in nocardiosis.

In all, 350 samples of sputum, 200 of bronchial aspirate, and 5 of gastric lavage, collected from 305 cases of bronchopulmonary diseases, were investigated. The procedures for processing the clinical specimens may be described briefly as follows. The sputa investigated were early morning specimens, collected in sterile wide-mouthed, glass-stoppered bottles after the patients had brushed their teeth and washed the mouth thoroughly with warm saline. The bronchoscopic aspirates and gastric washings were also collected under aseptic conditions. Each specimen was homogenized by vigorous shaking with sterile glass beads. It was then streaked on slants and plates of Sabouraud agar and glucose nutrient agar. For paraffin baiting, about 2 ml of each homogenized specimen was mixed thoroughly with approximately 5 ml of sterile carbon-free broth (6) of the following composition: NaNO<sub>3</sub>, 2 g; K<sub>2</sub>HPO<sub>4</sub>, 0.8 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g; FeCl<sub>3</sub>, 10 mg; MnCl<sub>2</sub>·4H<sub>2</sub>O, 8 mg; ZnSO<sub>4</sub>, 2 mg; distilled water, 1 liter; pH 7.2. The mixture was transferred to a sterile test tube. Into each of these test tubes was introduced a paraffin-coated

glass rod which had been sterilized by immersing overnight in 95% alcohol. Before use, the glass rod was drained of the excess alcohol by holding it against the wall of the container. Previous experience of investigators (5) in this laboratory had indicated that traces of alcohol which may possibly be carried into the broth with this method apparently had no adverse effect on the recovery of *N. asteroides*. Incubation was done at 37 C, and observations for nocardial growth were made after 1, 2, 4, and 6 weeks. White to cream or orange-colored growth appearing on paraffin-coated glass rods near the surface of the medium was scraped and further streaked on Sabouraud agar plates to pick up morphologically typical colonies of *Nocardia* species. These were further tested for morphological and biochemical characteristics to confirm the specific identity of *N. asteroides* (1, 3).

Of 555 clinical specimens investigated from 305 cases of bronchopulmonary diseases, 12 specimens originating from 6 patients yielded *N. asteroides* (Table 1). The positive specimens included 10 samples of sputum and one each of bronchial aspirate and gastric lavage. It is noteworthy that the paraffin bait technique was found to be effective in as many as 12 of 14 clinical specimens examined from 6 cases of nocardiosis. In strong contrast to this, with a routine culture technique, *N. asteroides* could be recovered from only a single clinical specimen, that is, a sample of bronchoscopic aspirate. However, even in this single instance of the success of routine culture technique, *N. asteroides* was also recovered by the paraffin bait technique. These findings are highly suggestive of the superiority of the paraffin bait technique over the routine culture technique.

With a view to further evaluating the usefulness of the paraffin bait technique, the following experimental study was carried out. A 0.1-ml

TABLE 1. Recovery of *N. asteroides* from six cases of nocardiosis; comparison of routine culture and paraffin bait techniques

Case no.	Specimen	No. examined	No. of <i>N. asteroides</i> isolates obtained with:	
			Routine culture technique <sup>a</sup>	Paraffin bait technique
1	Sputum	3	0	2
2	Sputum	2	0	2
3	Sputum	2	0	2
4	Sputum	3	0	3
5	Sputum	2	0	1
	Bronchial aspirate	1	1	1
6	Gastric lavage	1	0	1

<sup>a</sup> Refers to direct streaking of clinical specimens on Sabouraud agar and glucose nutrient agar media.

amount of a fine suspension of *N. asteroides* in normal saline was mixed with each of 50 samples of sputum collected from different patients with chronic chest diseases. These specimens were processed both by direct streaking on routine culture media and by paraffin baiting, as described earlier. Of the 50 sputum samples tested, *N. asteroides* was recovered from 48 by paraffin baiting, whereas only 24 of the specimens yielded the organism by direct streaking. These observations reinforce the conclusion that the paraffin bait technique is more efficacious than the routine culture technique in the recovery of *N. asteroides* from clinical specimens.

The successful application of paraffin baiting,

as apparent from the foregoing observations, promises to be a very useful laboratory procedure in the diagnosis of nocardiosis. According to the procedures currently used in various laboratories, the isolation of *N. asteroides* presents a difficult problem, particularly in the case of clinical specimens such as sputum, gastric lavage, etc., which commonly harbor a variety of contaminating microflora. These contaminants may overgrow *N. asteroides* and prevent its isolation. Selective culture media containing antibiotics are unsuitable for isolation of *N. asteroides*, owing to the sensitivity of this organism. The mechanism underlying the success of the paraffin bait technique appears to be a simple one, i.e., the ability of *N. asteroides* to utilize paraffin as sole source of carbon. This special technique overcomes the problem of many common contaminants because of their inability to grow on paraffin as a sole source of carbon.

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