

Comparison of Paraffin Baiting and Conventional Culture Techniques for Isolation of *Nocardia asteroides* from Sputum

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Of 1,510 sputum samples examined from 1,016 patients with bronchopulmonary disorders, *Nocardia asteroides* was isolated from 67 samples by paraffin baiting, as compared with only 30 isolations by the conventional technique of culturing on Sabouraud dextrose agar. This higher efficacy of the paraffin bait technique was found to be statistically significant ($P < 0.001$).

It is well known that *Nocardia asteroides*, the principal etiologic agent of nocardiosis, can be readily isolated from soil by a simple technique of paraffin baiting introduced in 1936 by Gordon and Hagan and further developed in 1960 by McClung (6, 10, 11, 13). Paraffin baiting was successfully applied to clinical specimens by Mishra and Randhawa (15), who demonstrated that it was significantly more efficacious than the conventional culture technique for isolation of *N. asteroides* from sputum. These observations were extended and confirmed by Mishra et al. and other investigators (9, 12, 16, 18, 20, 21). Although the technique has also received attention in several textbooks and laboratory manuals (1, 2, 17, 22, 24), it does not seem to have been widely exploited by diagnostic microbiology laboratories. In view of the recently increasing reports on systemic nocardiosis, especially in immunocompromised hosts, including patients with the acquired immunodeficiency syndrome (8, 23), we are prompted to focus again on paraffin baiting as a more efficacious laboratory diagnostic procedure than the conventional culture technique for the isolation of *N. asteroides* from sputum.

The material for study comprised 1,510 sputum samples collected from 1,016 patients with bronchopulmonary disorders investigated at the Tuberculosis and Chest Diseases Hospital, Amritsar, during July 1979 to January 1983. Freshly expectorated sputum samples were examined by direct microscopy and culture. The smears were stained by the modified techniques of Gram and Kinyoun (3, 19). For culture, sputum samples were first homogenized by shaking with sterile glass beads and then processed by the conventional culture technique and paraffin baiting. In the former case, sputum was streaked liberally on Sabouraud dextrose agar plates which were incubated at 37°C for up to 3 weeks. The paraffin baiting procedure used was the same as that described by Mishra and Randhawa (15). About 2 ml of homogenized sputum was added to a test tube (150 by 25 mm) containing 5 to 7 ml of McClung sterile carbon-free broth (13). To each of these test tubes was introduced a paraffin-coated glass rod. The baited culture tubes were incubated at 37°C for up to 4 weeks. In the case of positive sputum samples, cream- to orange-colored growth generally appeared in about a week on the paraffin-coated rod just about at the level of the broth (Fig. 1). Serial dilutions of a suspension prepared from the growth were streaked on

Sabouraud agar for separation of single colonies morphologically compatible with *Nocardia* species. The isolates were further tested for various biochemical characteristics as described by Gordon and Barnett (4), Gordon et al. (5, 7), and Mishra et al. (14).

Of 1,510 sputum samples investigated from 1,016 patients with bronchopulmonary diseases, *N. asteroides* was cultured from 67 samples by paraffin baiting, as compared with only 30 by the conventional technique of direct culture on Sabouraud dextrose agar. The average time taken for detection of *Nocardia* growth on the paraffin bait ranged from 7 to 12 (average, 10) days. As shown in Table 1, the isolations of *N. asteroides* were restricted to 16 of the patients, 14 of whom revealed gram-positive and partially acid-fast, thin (1 μm wide) branching filaments compatible with *Nocardia* sp. by direct microscopy of stained sputum smears. Cultures for mycobacteria were negative in all of the nocardia-positive patients, but patient 1 had previously been found to have

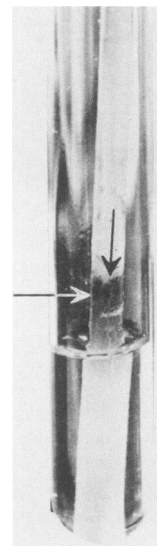


FIG. 1. Isolation of *N. asteroides* by paraffin baiting from sputum of a patient with bronchopulmonary nocardiosis. Note the dark (actually tan-colored), heavy growth of the organism on the paraffin-coated glass rod above the surface of the carbon-free broth mixed with sputum as dispensed in a test tube and seen after 4 weeks of incubation at 37°C.

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TABLE 1. Comparative efficacy of paraffin baiting and conventional technique of direct culture on Sabouraud agar in the isolation of *N. asteroides* from sputum in 16 of 1,016 patients with bronchopulmonary diseases

Patient serial no.	No. of sputum samples examined	No. of samples showing thin, branched mycelium	No. yielding <i>N. asteroides</i> by:	
			Paraffin baiting	Conventional technique
1	5	5	5	0
2	5	3	5	0
3	7	3	6	4
4	6	4	6	2
5	5	3	5	3
6	5	4	4	2
7	6	4	5	4
8	6	4	6	4
9	3	3	3	3
10	6	3	4	2
11	5	2	5	3
12	4	2	4	2
13	4	3	4	1
14	5	3	3	0
15	4	0	1	0
16	4	0	1	0
Total		46 (57.5%)	67 (83.7%)	30 (37.5%)

sputum smear positive for some acid-fast bacilli on a solitary occasion. It is apparent from the data that paraffin baiting was significantly more efficacious than the conventional culture technique for the isolation of *N. asteroides* from sputum ($P < 0.001$). In no case did paraffin baiting fail when the results were positive by the conventional culture technique. The inadequacy of the conventional culture technique is well illustrated by the isolation data of three patients (serial numbers 1, 2, and 14; Table 1) from whom *N. asteroides* could not be isolated by this method in spite of five repeat attempts made in each case. But for the use of paraffin baiting, the laboratory diagnosis of nocardiosis in these patients would have been missed. The results of this study uphold the advocacy of paraffin baiting by Mishra and Randhawa (15) for more efficacious isolation of *N. asteroides* from sputum, which frequently harbors contaminant microbes likely to interfere with its isolation. It may be pertinent to mention here that the greater success of paraffin baiting in the isolation of *N. asteroides* from sputum has been attributed primarily to the ability of this organism to utilize paraffin, in contrast to most other organisms, as a sole source of carbon. In addition, the larger amount of clinical sample utilized for baiting may itself enhance the recovery of the organism. Finally, the application of paraffin baiting in the present study led to an unequivocal diagnosis in 14 of the patients of bronchopulmonary nocardiosis, the clinical aspects of which will be presented elsewhere.

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