

## **TITLE** VALIDATION OF SPECIFIC METHOD FOR CULTURE OF *Nocardia* sp

**AUTHORS** Bressan, E A RI.; Lemo, ME B; Freitas, NC; Souza, CL; Almeida, L P.; Lazari, C S; Cappellano, P; Granato, C; Sampai o, J. L M

**INSTITUTION** GRUPO FLEURY S A, SÃO PAULO SP (AV. GENERAL VALDOMIRO DE LIMA, 508)

### **ABSTRACT:**

The genus *Nocardia* is composed of Gram positive bacilli commonly found in soil, decaying organic matter and surface of vegetables. The highest incidence of nocardiosis occurs in adults and immunocompromised patients. Culturing clinical samples for *Nocardia* presents many difficulties, including the slow growth of this microorganism which allows the proliferation of commensal bacteria in samples from non-sterile sites. The present study aimed at validating culture media and a sample preparation method, known to be recommended for *Legionella*, for isolation of *Nocardia* species from clinical samples from non-sterile sites. Twelve strains from seven different species (*N. farcinica*, *N. cyriacaegeorgica*, *N. nova*, *N. abscessus*, *N. testacea*, *N. araoensis* and *N. asiatica*) from our culture collection were used for the validation process. Initially suspensions (0.5 Macfarland) were prepared from recent growth obtained on sheep blood agar. These suspensions were added to bronchoalveolar lavage samples containing other bacterial types in order to simulate a clinical sample with *Nocardia*. Subsequently, a serial dilution of this bacterial suspension was performed in saline followed by quantitative plating on sheep blood agar. In parallel, this bacterial suspension was inoculated into bronchoalveolar lavage (BAL) samples and these BAL samples were treated with acidic solution of KOH + HCl for 5 min in order to eliminate commensal bacteria. After acid treatment, the sample was plated on Thayer Martin (TM) agar, BCYE agar and BCYE plus cefotin and vancomycin agar. Plates were incubated for fourteen days in ambient air at 37°C. The number of colony forming units (CFU) was determined for each media and compared.

The average CFU for sheep blood agar, BCYE agar, BCYECV agar and TM agar were, respectively 68, 69, 34 and 43. BCYE agar failed to support growth of one strain of *N. niiae*. BCYECV agar failed to support the growth of *N. niiae* and *N. testacea* strains, while TM agar failed to support the growth of one strain of *N. testacea*. Note, the *N. testacea* strain grew only on BCYE agar and *N. niiae* grew only on TM agar, both after acid treatment.

Acid treatment efficiently eliminated commensal bacteria. The best approach to isolate *Nocardia* species is the combined use of sheep blood agar plating without treatment and plating on BCYE agar and TM agar after acid treatment.

**Keywords:** bacterium culture, *Nocardia*

**Development Agency:** Microbiology Fleury Group, Hospital Beneficência Portuguesa