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Growth on blood agar discriminates *Mycobacterium avium* and *Mycobacterium intracellulare*

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Mycobacterium intracellulare and *Mycobacterium avium* (MAC) require specialized culture and identification procedures. To simplify the diagnosis, we inoculated reference strains, and 85 *M. avium* and 12 *M. intracellulare* clinical isolates, on egg-based and sheep blood agar. After 5 days of culture, there were significantly more colonies on sheep blood than on egg-based agar for *M. avium* (ratio: 250.5 ± 209) but not for *M. intracellulare* (ratio: 0.44 ± 0.11). Using a ratio ≥ 20, the sensitivity of the identification of an MAC isolate as *M. avium* was 97.65%, the specificity was 100%, and the positive predictive value was 100%. Differential growth on egg-based and blood agar is an aid to the identification of MAC isolates.

Keywords *Mycobacterium avium*, *Mycobacterium intracellulare*, identification, culture

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The bacteria of the *Mycobacterium avium* complex (MAC) [1] are closely related acid-fast organisms comprising two species, *M. intracellulare and M. avium*.

The latter species has three subspecies, *avium*, *paratuberculosis*, and *silvaticum* [2,3], and there is taxonomic evidence for a third species, referred to as MAC-X strains [4]. The MAC bacteria are environmental bacteria [2], and are opportunistic pathogens primarily responsible for pulmonary infections in non-immunocompromised patients. Such infections include tuberculosis-like disease in elderly men who are heavy smokers and have underlying lung disease, and also in women who have no history of underlying lung disease [5], lymphadenitis in children [6], and disseminated disease and death in those with AIDS and other immunocompromised patients [7]. In recent years, the isolation and identification of MAC strains has become important to medical and veterinary research and for diagnostic laboratories that test for MAC infections. Indeed, the precise identification of MAC isolates at the species level is necessary to better define these different clinical entities, as well as to better assess the epidemiology of MAC infections.

The identification of MAC isolates is mainly based on molecular techniques [8], and is often left to specialized laboratories. Relying on specialized laboratories' results in diagnostic delays and additional costs, and to overcome this problem we tested whether MAC could grow on blood agar, a medium commonly used in clinical laboratories. We also compared the growth of both reference and clinical MAC isolates on egg-based and blood agar, to see whether this could differentiate the MAC species.

In a preliminary experiment, 10 μL of 10-fold serial dilutions (10^6 CFU/mL to 10^0 CFU/mL) of *M. avium* subsp. *avium* type strain (IPC 104244^T) and *M. intracellulare* type strain (IPC 104243^T) (Collection de l'Institut Pasteur, Paris, France) in Middlebrook 7BH9 broth were streaked in parallel on slants of Coletsos agar (egg-based agar) (bioMérieux, La Balme les Grottes, France) and 5% sheep blood agar in tubes (Bio Technologie

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Table 1 Sources of 97 MAC (85 *M. avium and* 12 *M. intracellulare*) isolates included in this study; *M. intracellulare* isolates were all recovered from pulmonary samples collected from HIV patients

	HIV^-	HIV^+	Total
Pulmonary	21	23	4
Blood	0	42	42
Lymph node	4	4	8
Other	1	2	3
Total	26	69	97

Appliquée, Dinan, France), and incubated at 37 °C for 9 days; colonies were then counted. After inoculation of a suspension with 10^6 CFU/mL on Coletsos agar, 10 *M. avium* colonies were detected after 6 days, and more than 10^4 colonies were detected after 9 days. After inoculation of a suspension of 10^5 CFU/mL on blood agar, 10 colonies were detected after 5 days, and up to 10^4 colonies were detected after 9 days. After inoculation of a 10^6 CFU/mL suspension of *M. intracellulare*, regardless of the culture medium, 10 colonies were detected after 5 days, and a maximum of 10^3 colonies were detected after 9 days.

Based on these preliminary results, we conducted blind testing of 97 clinical MAC isolates (Table 1), identified by 16S rRNA–probe hybridization using the Accuprobe system (GenProbe, San Diego, CA, USA), based on hybridization with the MAC probe followed by specific *M. avium* and *M. intracellulare* probes, following the supplier's recommendations. All the MAC isolates hybridized with the MAC probe; 85 of 97 (87.7%) hybridized with the *M. avium* probe and not with the *M. intracellulare* probe, and 12 of 97 (12.3%) hybridized with the *M. intracellulare* probe and not with the *M. avium* probe. Double hybridization was not observed. Isolates were coded before the growth experiment, and the technician who read the growth results was unaware of the molecular identification of the isolate. Ten microliters of a 10^4 CFU/mL suspension of each isolate in Middlebrook 7BH9 broth were streaked in parallel on Coletsos and 5% sheep blood agar in tubes, and incubated at 37 °C for 5 days before colonies were counted using a dissecting microscope. After 5 days of incubation, clinical isolates of *M. avium* produced 957 ± 196.2 (mean ± standard deviation) colonies on blood agar, and 6.62 ± 21.19 colonies on Coletsos agar (ratio: 250.5 ± 209) ($P < 10^{-3}$, Student test). *M. intracellulare* clinical isolates, however, produced 4.46 ± 1.61 colonies on blood agar, and 10.38 ± 38 colonies on Coletsos agar (ratio: 0.44 ± 0.11) ($P < 10^{-3}$, Student test). After 5 days of incubation, the average number of colonies on blood agar was at least 100 times higher for *M. avium* than for *M. intracellulare* isolates ($P < 10^{-3}$, Student test). There was, however, no significant difference observed in the growth of the two species on Coletsos agar. Using a ratio of colonies on blood agar/colonies on Coletsos agar of ≥20, we could identify an MAC isolate as *M. avium* with a sensitivity of 97.65%, a specificity of 100%, and a positive predictive value of 100%.

Our results indicate that 5% sheep blood agar supports the growth of MAC species and is significantly superior to an egg-based medium for the culture of *M. avium*. This difference most probably resulted from nutrient requirements rather than growth inhibition by egg-based medium, but further experiments may help to resolve this point. Blood agar is a basic medium routinely used in clinical laboratories that appears to enable MAC species to be distinguished. When MAC species are suspected (according to short AFB, small smooth colonies, sometimes dissociated into smooth and rough colonies, non-pigmented when young, growing in 2–3 weeks in solid media, without producing nitrate reductase or acid phosphatase as first-line quick biochemical tests), parallel inoculation on blood agar and an egg-based medium would enable easy differentiation between *M. avium* and *M. intracellulare* after a 5-day incubation. This is similar to the time that it would take for samples to be shipped to and identified in a specialized laboratory applying molecular techniques [4], such as the commercially available 16S rRNA–probe system, Accuprobe.

In summary, tools currently available for identification of MAC to the species level require either expertise or special equipment not available in every laboratory. Differential growth on 5% sheep blood and egg-based agar enables any clinical laboratory to discriminate readily between *M. avium* and *M. intracellulare*. Only basic laboratory equipment is needed, and the cost is about 3 Euros, compared to 36 Euros for molecular probing.

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