A Clinical Evaluation of Thermo Scientific Anaerobe Recovery and Isolation Agar (A.R.I.A. medium)

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Overview

Purpose: To evaluate the efficacy of Thermo Scientific™ Anaerobe Recovery and Isolation Agar (A.R.I.A.™ medium) (Thermo Fisher Scientific), for isolation of clinically relevant anaerobic bacteria.

Methods: One hundred clinically relevant anaerobes and 42 clinical specimens were inoculated onto A.R.I.A. medium with 5% horse blood (with and without 75 µg/ml neomycin) and Thermo Scientific™ Fastidious Anaerobe Agar (FAA) with 5% horse blood (with and without 75 µg/ml neomycin). A 5 µg metronidazole disc was added and plates were incubated anaerobically for up to 6 days. Plates were observed for growth, β-haemolysis, zone of inhibition around metronidazole disc and fluorescence.

Results: A.R.I.A. media (figures 1 and 2) performed very well when compared with the FAA equivalent for the isolation of anaerobic bacteria both in pure culture and directly from clinical specimens.

Introduction

Anaerobic bacteria are important pathogens that can cause a variety of infections in humans[1]. Accurate species determination of anaerobes from clinical samples has become increasingly important with the re-emergence of anaerobic bacteraemia and prevalence of multiple-drug-resistant microorganisms[2]. Any culture media used for this purpose should be able to isolate and provide good recovery of a wide range of anaerobic bacteria commonly found in an extensive number of clinical samples.

Methods

The study was conducted in two stages:

Stage 1

A panel of 100 clinically relevant anaerobes covering a broad range of genera and species (originally isolated and identified from clinical material by the Anaerobe Reference Unit using standard procedures (including partial (~450bp) 16S rRNA sequence analysis) was selected. Isolates were cultured from frozen beads onto FAA with 5% horse blood and incubated in an anaerobic chamber for 24-48 hr. at 36 ±1°C prior to examination for viability and purity.

Isolates were suspended in 0.9% sterile saline to McFarland 0.5 standard and 10 µl loop of suspension was streaked with a sterile loop onto A.R.I.A. medium with 5% horse blood (A.R.I.A.), A.R.I.A. medium with 5% horse blood & 75 µg/ml neomycin (A.R.I.A.-N), FAA with 5% horse blood (FAA) and FAA with 5% horse blood & 75 µg/ml neomycin (FAA-N) in random order. A Thermo Scientific™ metronidazole (5 µg) antimicrobial susceptibility testing disc (Thermo Fisher Scientific) was placed at the junction of the first and second rows of streaks away from the inoculum. Plates were incubated immediately (in an anaerobic chamber) in an atmosphere of 10% H_2, 10% CO_2, 80% N_2 at 36 ±1°C.

Plates were examined after 24 hr., 48 hr., and 72 hr. incubation. Growth, colony size, colony shape, colony colour, β-haemolysis, zone of inhibition around the metronidazole disc and long-wave (365 nm) fluorescence were observed and recorded. Cultures of slow-growing or potentially pigmented organisms were further incubated and were examined for each of the above-listed parameters after a total of six days incubation.

Stage 2

Forty two fresh clinical specimens (29 assorted pus/fluid samples and 13 blood cultures) were obtained from the on-site clinical microbiology laboratory. A 10 µl loop of specimen was streaked onto each of the four media. Application of a metronidazole disc, incubation and examination were as described for stage 1.

Results

A total of 98 pure isolates (comprising 59 Gram-positive- and 39 Gram-negative organisms) were recovered on FAA and A.R.I.A. media. The remaining two isolates were non-viable on any medium. Twenty four of the 98 organisms isolated (comprising 20 Gram-positive- and four Gram-negative organisms) yielded no growth on FAA-N and A.R.I.A.-N. Of the 42 clinical specimens examined, 13 pus/fluid samples yielded no anaerobic growth after 6 days incubation. Ten pus/fluids and six blood cultures yielded facultative organisms only after 6 days. The remaining 13 specimens (7 blood cultures, 6 pus/fluids) yielded a total of 22 anaerobes/microaerophiles. Four of these also yielded facultative organisms. Up to six distinct organisms were isolated from individual specimens.

FIGURE 1. A.R.I.A. medium

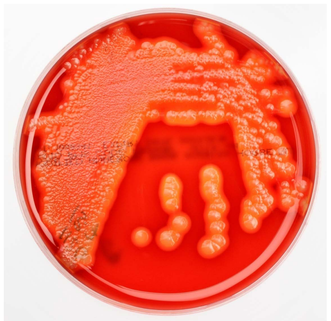
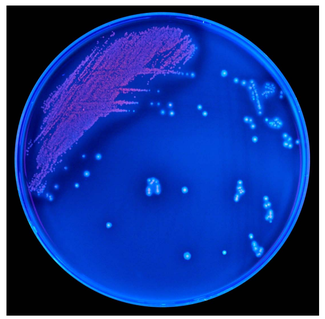


FIGURE 2. A.R.I.A. medium under fluorescent light



A number of anaerobes failed to grow on either neomycin-containing agars (24 pure isolates and 5 isolates from clinical specimens). This highlights the risk of failing to isolate relevant anaerobic organisms if a suitable non-selective agar is not included in the primary culture of clinical specimens.

Quantity of growth and colony size on A.R.I.A. and A.R.I.A.-N correlated well to that seen on FAA and FAA-N. Very good correlation was demonstrated for metronidazole disc zones (data not shown). Excellent correlation between A.R.I.A. and FAA and between A.R.I.A.-N and FAA-N was demonstrated for colony shape, colour and β-haemolysis.

Although FAA and FAA-N showed more typical fluorescence colour, the intensity of fluorescence was far greater on A.R.I.A. and A.R.I.A.-N. Fluorescence is known to be a highly unpredictable feature, subject to variables such as media constituents, bacterial growth stage, operator interpretation, exposure to oxygen and prolonged anaerobic incubation[3]. Therefore, fluorescence is rarely used as a definitive aid to identification but as one of a set of tests for identification.

Conclusion

Overall, performance of A.R.I.A. and A.R.I.A.-N was comparable to FAA and FAA-N in terms of growth characteristics, though slight differences in fluorescence were observed for some organisms. A.R.I.A. and A.R.I.A.-N can be recommended as a suitable alternative to FAA and FAA-N for the isolation of anaerobic bacteria both in pure culture and directly from clinical specimens.

References

1. Brook, I. (1988). Recovery of anaerobic bacteria from clinical specimens in 12 years at two military hospitals. Journal Clinical Microbiology 26(6): 1181-1188.
2. Simmon, K. E., Mirrett, S., Barth Reller, L., Petti, C.A. (2008). Genotypic Diversity of Anaerobic Isolates from Bloodstream Infections. Journal Clinical Microbiology 46(5), 1596-1601.
3. Hall, V. (2012) unpublished.

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