Reviewer's report

**Title:** An evaluation of methods for the isolation of nontuberculous mycobacteria from patients with cystic fibrosis, bronchiectasis and patients assessed for lung transplantation

**Version:** 2 **Date:** 14 Dec 2018

**Reviewer:** Tavs Qvist

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Stephenson et al. have performed a large study of RGM medium finding it suited to isolate pathogenic NTM species, the most important being M. abscessus. They collected respiratory samples from 676 patients and compared Löwenstein-Jensen medium and MGIT to RGM medium at two temperatures. RGM at 30 degrees proved more sensitive than AFB culture, but several other NTM species of questionable importance were also isolated. The method seemed to miss M. simiae and to a lesser degree M. xenopi.

The study is large and well thought-out. The clinical question is important and NTM do indeed pose a threat to these vulnerable patient populations. A faster and easier isolation method for particularly M. abscessus is in demand. The high isolation rate of other NTMs is a manageable problem. The authors make a compelling case for exploring a medium like RGM. It is a shame that Burkholderia cepacia plates are not also included, but they are mentioned and the context is properly presented. It is a weakness of the study that not all MGIT samples were incubated for 6 weeks as recommended. The manuscript is well written and will interest readers.

I only have minor comments

Page 10 line 189 Any isolate of NTM that was only recovered by RGM at one incubation temperature (or was only isolated using AFB culture) was re-inoculated onto two plates of RGM medium and two plates of RGM medium without antibiotic supplement that were incubated at 30°C and 37°C for up to 28 days. How did these count in the overall test of diagnostic sens. and spec. (table 5). Were only the initial culture result counted?

Page 12, line 219: "Overall, the data suggested little added benefit of using RGM at 37°C and that incubation temperature of 30°C should be recommended for routine use, as originally proposed [18]." This belongs under Discussion, not results.

Page 14 The sensitivity for detection of MAC was also higher than that of AFB culture (sensitivity 83% vs. 70.2%; P = 0.21) - I would recommend reformulating without the use of the word sensitivity here, as the difference is not statistically significant: for example more MAC was detected with RGM30 than with AFB, but the difference was not statistically significant.

Page 15, line 224: Comment: This demonstrates that if you know what you are looking for, NTM are much easier to find. This is among other things related to having an experienced lab tech, who can spot and scoop single colonies. This could be a challenge when implementing a system
like RGM in the clinical routine outside of specialized labs. The skills of the lab tech should not be underestimated.

Page 15, line 255 Colony counts are important as stated here - a bit more elaboration on this issue in the discussion would be welcome.

Page 26, line 479 colonization is mentioned. In the case of M. gordonae possible lab contamination should be considered.

Page 33, line 613 Competing interests: The authors should consider whether any patents, past present or pending could be relevant to declare here.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
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Yes

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Yes

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