Reviewer’s report

Title: Accuracy of IgM antibody testing, FQ-PCR and culture in diagnosis of acute infection by Mycoplasma pneumoniae in adults and adolescents with community-acquired pneumonia

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Reviewer: Naomi J Gadsby

Reviewer’s report:

The authors describe a study comparing the performance of IgM, culture and FQ-PCR (“fluorescence quantitative” PCR) for M. pneumoniae diagnosis. Several similar studies have been previously published examining the differences between serology and/or culture and/or molecular detection for this organism but this is the first to describe the use of the FQ-PCR test.

However, it is not clear to what extent this FQ-PCR commercial test differs from real-time PCR and in what way it is quantitative as the results are expressed only qualitatively.

Comparison is made between the three test methods and the “gold-standard” of a 4-fold titre rise in IgG between acute and convalescent sera, however no satisfactory evidence or explanation is given as to why this is the gold standard. Contrary to what is stated in the introduction, Mandell et al 2007 and Waites and Talkington 2004 do not actually say or infer that the use of IgG in this way is “the most reliable method”.

The patient population is an adolescent/adult CAP cohort but no results of any other microbiological test are given. This would help in interpreting positive results from the three test methods where the gold-standard is negative.

The discussion needs to be edited to make it more concise and the whole manuscript needs an English language review for grammatical errors.

Finally, the authors have not always properly referenced statements made in the article, for example, citing review articles rather than going back to the original source, and in some cases citations appear to be incorrect.

Major compulsory revisions:

What is the nature or principle of the FQ-PCR test – is it real-time PCR-based? Do the authors know the gene target for the FQ-PCR? Results are expressed as positive or negative, but the test is described as quantitative. FQ-PCR is stated as “more rapid, convenient and sensitive” however the two supporting articles cited (Loens et al 2003, Morozumi et al 2004) do not use FQ-PCR. If the authors mean PCR in general they should clarify this.

“False positives” which were negative by the gold standard but positive by one of
the three other methods tested are noted in Table 2 but not described in the text. Were any specimens positive by all three methods but negative by the gold standard? Was there another microbiological explanation for CAP? If not, perhaps these are true positives, given that there is really no gold-standard single diagnostic method for M. pneumoniae. This should be discussed further in the discussion section.

It is unclear if the statement “although M. pneumoniae had been found to asymptptomatically colonise the respiratory tract” refers to the present study; all 125 patients were symptomatic with CAP.

Can the authors state the reason for excluding patients with an onset of more than 7 days?

Rapid culture is mentioned in the discussion but the reference cited (Liu et al 2010) does not use this method. Therefore the correct reference should be cited. Furthermore, in the last line of the discussion the authors comment that “rapid culture method could be potentially clinically applicable” but have not actually used it in the present study and do not provide any more information about it. Could they comment further on this or remove the reference to it.

Minor essential revisions

Introduction:

Cao et al 2010 is referenced for the finding that 20% CAP in adults is due to M. pneumoniae. However, Cao et al actually describe a 29.3% prevalence and refer in their own paper to a study by Liu et al 2009 in which there was a prevalence of 20.7%. The authors should correct this.

Methods:

The authors use "throat swab" and "throat wash" interchangeably in the manuscript although they are two different specimen types – could they clarify which is the one they used in this study?

What was the volume used for extraction for FQ-PCR and which Qiagen manual extraction kit was used?

The authors cite Cao et al 2010 for the method of confirming culture positives by PCR, however Cao et al cite a method published in a book without any modifications. It would be better to cite the original source in the present manuscript.

Were the U/ml cut-off values for the interpretation of the serology based on manufacturer’s recommendations or on other criteria?

Results:

What were the co-morbidities noted? These should be described in the methods section.
Discussion

The second sentence in the first paragraph appears to be almost identical to the wording of the abstract from the von Baum et al 2009 paper cited. The essence should be summarised in the author's own words.

In the second paragraph is a reference to “produced 15 days after the onset” – this is straight out of Martinez et al 2008 as cited but actually refers to other studies described in that paper, rather than Martinez’s own data. Their own data describes some patients with IgG seroconversion but no IgM however there is no analysis of timing in respect of onset.

When referring to a paper, use the author's surname not forename e.g. “Martinez et al” not “Maria et al”.

Loen et al 2010 is cited as evidence of a high re-infection rate in China, however this review paper quotes only one (paediatric) study with an incidence of 7.1%, therefore I think the authors have cited the wrong reference here.

The sensitivities of 5 commercial kits assessed in Touati et al 2009 are actually 61.9-97.6% which rounds up to 62-98% rather than the 61-97% quoted by the authors.

Touati et al 2009 states detection limits in ng DNA/uL but the authors quote 2-5 copies/uL. The authors should clarify how they have converted this.

The third paragraph of the discussion quotes a study by leven and Goosens 1997 giving 61% culture sensitivity, however the original source of this figure is the 1996 paper by leven et al cited in leven and Goosens 1997.

Table 1:

ECG abnormality is listed but not explained in the methods section (as for co-morbidities). What definition have the authors used for this? (NB: 25/125 is 20.0% not 19.2% as in the table).

Similarly no further information is given about antibiotics prior to admission in the methods section although it is in the table; how many days prior to admission was this taken from, and was the class of antibiotic recorded?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests