



SDI Review Form 1.5

PART A:

Journal Name:	British Microbiology Research Journal
Manuscript Number:	MS: 2012/BMRJ/1605
Title of the Manuscript:	Assessment of antigen preparation methods for polyclonal antibody production against <i>Streptomyces</i> spp.

(Note: Title of different sections as proposed below may differ in case of review paper)



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PART B: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part and write here 'Corrected' if not agreed, give suitable justifications)
General comment:	Good concept, though lacking on applicable data and characterization of the polyclonal antibodies. More could be described and considered.	
Specific comments:		
Title and abstract	- Title was consistent with paper content - Abstract could be worked on further. Would not call the study design, a cross-sectional study and not sure of the relevance of place and duration of study in the abstract, this information should go to the end of the paper.	Corrected. We have revised the study design and place of study in accordance with the reviewer's comments.
Introduction (Is the problem/objective of this study original, important and well defined?)	- Problem statement is not clear. - What are the methods currently used, only state that these are time-consuming and intensive. What value does an immunological method afford should be stated clearly ie how much time would be saved, would there be better specificity.	Corrected. In accordance with the reviewer's comments, we have provided more information and supporting references (Section 1, Page 2, line 31-38).
Are all the references cited relevant, adequate? Are there any other suitable current references authors need to cite?		
Materials & methods (Kindly comment on the suitability of the methods. Sufficient details of the methods should be provided to allow peers to evaluate and/or replicate the work)	Could have used soil sample and added in sonicated sample, to assess recovery of method. Since in results section clear that soil sample does not score in the assay.	We added different concentrations of sonicated antigen to the soil sample. This experiment was performed in triplicate. The <i>Streptomyces</i> antigen was not detected by either anti- <i>Streptomyces</i> antibodies, even when a high antigen concentration (25 µg) was used. We conclude that antigen recovery is not possible by this method. We have amended the manuscript accordingly (Section 2.9, Page 4, line 126-130).
Results & discussion (Kindly comment on: 1. Are the data well controlled and robust? 2. Authors should provide relevant references during discussion. 3. Discussion and conclusions should be based on actual facts and figures. Biased claims should be pointed out. 4. Are statistical analyses must for this paper? If yes, have sufficient and appropriate statistical analyses been carried out?)	Line 110-The sonication method showed more protein in the sample, not necessarily higher resolution profile. Line 114-115, if this method has been used before, what is different and unique about this paper?	Corrected. We have revised the manuscript accordingly (Section 3.1, Page 4, line 138-139). Sonication has been used previously (Kirby and Rybicky, 1986), but we performed sonication under different conditions to those described by Kirby



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	Line 117-sentence does not make sense Fig 1 assume the antigen load has been normalized, though there is no place this is mentioned. Line 151-152 the difference in Ab titer may be due to animal variability and cannot be associated to the antigen Line 152-what are the possibilities for the Ab titer dropping 3-5 wks following the last boost? Line 170-171 data not shown for the lack of cross-reactivity, should be stated and suggest describe the method Section 3.3 cross reactivity to other Strep isolates could be written better. If cell wall antigens are specific then were any attempts made to centrifuge the cell wall and isolate the walls from the cytoplasm? Or purify the antisera over a ligand column differentially purifying the anti-cell wall antigens versus the anti-cytoplasmic antigens. Section 3.4 There's no mention of the denaturation by autoclaving and boiling antigen versus sonication. What inhibitors could be present in soil, what are other possibilities	and Rybicky. Corrected. We have revised this section of the manuscript to make it clearer (Section 3.1, Page 4, line 145-148). Corrected. In Fig. 1, the antigen load was normalized. This is now mentioned in Section 2.2, page 2, line 69-70. Corrected. We have revised the manuscript and included a reference to support this statement (Section 3.2, Page 5, line 172-173). Corrected. We have revised the manuscript and provided a reference to support this statement (Section 3.2, Page 5, line 173-175). Corrected. We have revised the manuscript accordingly (Section 3.4, Page 6, line 193-213). Corrected. We have revised the manuscript (Section 3.4 page 6, line 193-213). Corrected. In section 3.5, we added only sonicated antigen to the soil sample. Possible inhibitors are mentioned in Section 3.5, page 7, line 224-227.
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Are there any ethical or competing interest issues?		
Conclusion (Is the conclusion supported by the data, discussed inside the manuscript? Conclusions should not be biased and should be based on the data, presented inside the manuscript only)	Section 4, how much faster is this method? Suggest giving some context of the current methods. If the Ab are not suitable this makes this approach weak unless you can provide some better data sets.	Corrected. We have revised the manuscript in accordance with the reviewer's suggestions (Section 4, Page 8, line 237-240).
References		