# Review of: "A Rapid and Robust DNA Extraction Method for PCR-Based Diagnosis of V. cholerae"

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Potential competing interests: No potential competing interests to declare.

H. Kumar Khuntia and collaborators describe a validation study of a simple, rapid, and cheap DNA extraction method for the PCR-based diagnosis of *V. cholerae*, proposing it as a valid alternative to culture methods or other DNA extraction methods currently available, to be applied during outbreaks and in rural hospitals without advanced laboratories or particularly experienced staff. The validation study compares the results obtained from 44 stock samples of *V. cholerae* O1 strains and 20 rectal swab samples, containing known strains of *V. cholerae*. The 64 samples were tested by PCR, following the application of two alternative methods of DNA extraction by boiling. In particular, the authors compared the levels of sensitivity and specificity obtained by performing PCR on DNAs extracted directly by boiling bacterial suspensions in distilled water, without a preliminary enrichment, to the levels of sensitivity and specificity obtained with a classic method of extraction by boiling the bacterial suspension in LB, following a 48-hour cultivation in specific solid and liquid media. The two methods reportedly revealed equivalent outcomes, with comparable levels of sensitivity and specificity.

I agree with the authors' assertion that a less expensive and more practical method of DNA extraction, in terms of the facilities required, compared to chemical or column-based methods, is desirable in territories subjected to frequent outbreaks and equipped with laboratories without adequate infrastructures and advanced machinery. However, in my opinion, the validation study described is incomplete, and the description of methods and results shows inaccuracies and weaknesses.

First of all, the comparison of the two boiling methods was performed on 64 samples (44 stock samples and 20 clinical samples), for which it is not clear whether these were strains preserved after isolation or more complex matrices (e.g., faeces) for which the diagnostic outcome was already known. Hence, the text should provide clearer information about the samples. Additionally, in the first case, it would be desirable to verify the effectiveness of the extraction method on more complex matrices. In both cases, it would be useful to increase the number of samples analyzed. Finally, considering that the authors report disadvantages for each of the extraction methods currently available, it would be desirable to compare the effectiveness of the boiling extraction method also with other extraction methods among those mentioned in the discussions.

Also, I suggest providing information such as DNA concentration and quality (e.g., purity) obtained from both extraction methods, for better comparison of the “newly proposed” method to the commonly used one.

I would not mention the possibility of contamination by exogenous DNA among the drawbacks of the enzymatic extraction method, first of all because we are talking in any case about extraction methods of the total DNA contained in the sample, therefore including the DNA from the patient himself and other microorganisms that may be present in the subject. The DNA of interest for a correct diagnostic outcome can be usefully discriminated from the rest of the genetic material by applying a robust amplification protocol with highly specific primers. Referring to primers, I recommend revising Table 1: primer sequences in lines 1-3 lack any description of the related target gene, expected amplicon size, and reference. The whole table should be improved for forward and reverse primer differentiation and the corresponding amplicon size expected for each target gene from column 1. Figure 1 is not available.

On the whole, the purpose of the work described is well formulated, but the study performed is incomplete. Improvements are needed referring to the sample selection, the methodology application, the results' description, and argumentation.