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.

1. **Novelty**: The study is not novel and is an already validated method for rapid bacterial diagnosis

    ◦

2. **Clarity and Detail**:

- Overall, the results are presented clearly, indicating that both DNA extraction methods yielded the same PCR assay results, amplifying all target genes without non-specific bands.
- Some of the sentences are very lengthy and ambiguous. Consider revising such sentences

- For example, the sentence: "The present study highlights that DNA extraction is **he central dogma** for PCR analysis". Rewrite the sentence as central dogma, not suited here

1. **Data**:

- The inclusion of specific genes detected (ctxA, tcpA, wbe, toxR, ace, zot, rst, ctxB1, ctxB7) provides detailed insight into the study's findings.

    ◦ As various methods of DNA isolation are compared, it would be apt to include the agarose gel image to validate your results.
    ◦ It has been stated that: The weakness of the enzymatic lysis method is that "**commercially available enzymes may be contaminated** with microbial DNA". "Quote the suitable reference

4. **Specificity and Sensitivity**:

    ◦ The specificity of the template DNA is well-demonstrated, emphasizing that the extracted DNA did not yield amplicons from other species, thus confirming its specificity for V. cholerae.
    ◦ The sensitivity of the method, calculated at 1.5x10^3 CFU per assay, is mentioned but lacks comparative data to highlight its significance. Providing a comparison with other methods would underscore the improvement.

5. **Comprehensive Coverage**:

    ◦ The coverage of both V. cholerae O1 and non-O1/non-O139 strains in detecting specific genes adds robustness to the study.

- The consistent results between both DNA extraction methods reinforce the reliability of the new method.

Discussion and Analysis

1. **Contextualization**:

   - The discussion effectively places the findings within the broader context of existing DNA extraction methods, highlighting the weaknesses of enzymatic, chemical, and mechanical methods.
   - The explanation of these drawbacks (e.g., contamination, toxicity, equipment requirements) provides a strong rationale for developing a new method.

2. **Comparison with Existing Methods**:

   - The discussion contrasts the new method with traditional broth boiling, outlining the limitations of the latter, such as cost, time, and potential for DNA overharvest.
   - This comparative analysis strengthens the argument for the new method's advantages.

3. **Advantages of the New Method**:

   - The advantages of the new DNA extraction method are clearly enumerated: rapid processing time (24 hours), avoidance of purification steps, no need for RNase, robustness, and cost-effectiveness.
   - The practical implications of these advantages, particularly for early diagnosis during cholera outbreaks, are well articulated.

Improvements

1. **Statistical Analysis**:

   - Including statistical validation of the results would enhance the credibility of the findings. Specific statistical measures (e.g., confidence intervals, p-values) should be mentioned to substantiate the claims of sensitivity and specificity.

2. **Quantitative Comparisons**:

   - Providing quantitative comparisons with other methods regarding cost, time, and sensitivity would better illustrate the improvements made by the new method.
   - For instance, specifying the cost savings in using sterile distilled water instead of broth medium would make the argument more compelling.

3. **Structured Presentation**:

   - Structuring the discussion into clear subsections (e.g., "Specificity and Sensitivity," "Comparison with Existing Methods," "Practical Implications") would enhance readability and focus.
   - A brief summary or conclusion section at the end of the discussion would help in recapitulating the key findings and

their significance.