

REVIEWER'S REPORT

Manuscript No.: JNHM-159

Title: *Evaluation of Serum STING Levels as a Potential Discriminatory Biomarker for Neisseria gonorrhoeae Infection.*

Recommendation:

Accept as it is

Accept after minor revision.....

Accept after major revision ✓

Do not accept (*Reasons below*)

Rating	Excel.	Good	Fair	Poor
Originality		✓		
Techn. Quality			✓	
Clarity			✓	
Significance		✓		

Reviewer's Name: Dr. Sahil Srivastava

Detailed Reviewer's Report

Overall Assessment

The manuscript evaluates serum STING as a potential biomarker for laboratory-confirmed *Neisseria gonorrhoeae* infection in 120 participants (60 cases and 60 controls). STING levels were higher in patients, with an AUC of 0.928, indicating promising discriminatory potential.

Major Comments

- Study design:** The manuscript inconsistently describes the study as both case-control and cross-sectional. The authors should clearly define the design and use consistent terminology throughout.
- Sample size:** A formal sample-size or power calculation is not reported. The authors should provide the statistical assumptions and justification for enrolling 60 cases and 60 controls.
- ROC interpretation:** The reported AUC of 0.928, sensitivity of 88.3%, and specificity of 85% are based on comparison with healthy controls only. These findings demonstrate preliminary discrimination but do not establish gonorrhea-specific diagnostic accuracy. Disease-control groups should be discussed as an important limitation.
- Statistical analysis:** Table 3 appears to use one-way ANOVA, but "T test" is reported. The statistical test and post-hoc analysis should be clearly stated. Confidence intervals for AUC, sensitivity, and specificity should also be provided where possible.
- STING interpretation:** Serum STING concentration should not be equated directly with intracellular cGAS-STING pathway activation because functional markers such as phosphorylation, cGAMP, TBK1, IRF3, or IFN- β were not measured. The manuscript should consistently distinguish these concepts.
- ELISA methodology:** The biomarker assay requires greater methodological detail, including detection range, sample dilution, replicates, assay variability, storage conditions, and quality-control procedures.

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7. **Infection-site analysis:** The pharyngeal subgroup contains only nine participants. Site-specific findings should therefore be interpreted cautiously. The authors should also clarify how participants with multiple anatomical infection sites were classified.
8. **Limitations and conclusion:** A dedicated limitations section should address the small, single-center sample, purposive sampling, lack of disease controls/external validation, small pharyngeal subgroup, and possible confounding. The conclusion should be moderated to state that STING showed an association and preliminary discriminatory performance rather than proving it to be a gonorrhea-specific diagnostic biomarker.

Minor Comments

1. The manuscript requires comprehensive English language and grammatical editing.
2. Several sentences contain unclear or scientifically inappropriate terminology and should be rewritten after verification of the original sources.
3. Table 3 should replace “**Age Sub-groups**” with “**Infection Site.**”
4. The reference list requires careful verification. In particular, the Gallego-Marin et al. citation appears unrelated to gonococcal infection, and the Andrade and Goyette-Desjardins references appear potentially duplicated.
5. Tables, figures, formatting, terminology, and citation style should be standardized according to journal requirements.

Final Recommendation - Accept after major revision.