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PEER-REVIEW REPORT

Name of journal: *World Journal of Methodology*

Manuscript NO: 87046

Title: Urine Exosome mRNA-based test for monitoring kidney allograft rejection: effects of sample transportation and storage, and interference substances

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05123031
Position: Editorial Board
Academic degree: Doctor, MD, PhD
Professional title: Associate Professor
Reviewer's Country/Territory: China
Author's Country/Territory: United States

Manuscript submission date: 2023-07-21

Reviewer chosen by: AI Technique

Reviewer accepted review: 2023-08-16 02:04

Reviewer performed review: 2023-08-20 08:00

Review time: 4 Days and 5 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No novelty
Creativity or innovation of this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No creativity or innovation

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Scientific significance of the conclusion in this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No scientific significance
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Manuscript ID: 87046 Title: Urine Exosome mRNA-based test for monitoring kidney allograft rejection: effects of sample transportation and storage, and interference substances Materials and Methods 1. Could you provide more details about the Small box cat #56519 used for urine specimen transportation? What were its specific characteristics that made it suitable for this purpose? 2. How were the gel packs (12 oz Gel Pack #PP12) utilized in the transportation box for refrigerating urine specimens? What role did these gel packs play in maintaining the appropriate temperature during transportation? 3. Could you explain the ExoLution protocol used for isolating exosomes from human urine samples? What are the key steps involved in this protocol, and how does it ensure the effective isolation of exosomes? 4. What was the rationale behind using RNA purification after exosome isolation? How does this purification process contribute to obtaining high-quality exosomal RNA? 5. Could you elaborate on the reverse transcription process? How does the SuperScript® VILO™ cDNA Synthesis Kit contribute to the conversion of exosomal RNA into cDNA, and why is this step necessary for downstream analysis? 6. What was the purpose of the pre-amplification

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step? How does the TaqMan™ PreAmp Master mix facilitate this step, and how does it contribute to the accuracy of the subsequent qPCR analysis? 7. In the qPCR analysis, you mentioned using TaqMan™ Fast Universal PCR Master Mix. What are the advantages of using this master mix in qPCR, and how does it contribute to the reliability of the results? 8. Could you explain the significance of testing interference substances, particularly medications commonly prescribed to transplant patients? How does assessing potential interference help ensure the accuracy of the assay results? 9. Among the interference substances tested, could you discuss any findings related to their impact on the assay? Were there any substances that showed potential interference with the assay, and how was this addressed? 10. The concentrations of the interference substances were mentioned in relation to the expected urinary excretion levels in transplant patients. Could you provide more context on why these concentrations were chosen and how they relate to the potential impact on the assay? Results and Discussion 1. Could you explain the significance of exploring the diagnostic value of urinary exosomes for kidney allograft rejection, especially in the context of non-invasive post-transplant monitoring? 2. Could you elaborate on the specific analytical method used in this study to detect and stratify kidney allograft rejection based on exosomal RNA markers? How does this method work, and what is its novelty in the field? 3. In the study, you mentioned exploring the stability of exosomal mRNA upon urine transportation at different temperatures. What were the key findings in terms of mRNA stability under varying temperature conditions, and how do these findings impact the clinical implementation of the diagnostic test? 4. Could you clarify the role of urine freezing and thawing on exosomal mRNA integrity? How did multiple freeze/thaw cycles affect mRNA degradation? 5. Could you elaborate on the significance of centrifugation of urine specimens before analysis? Why is this step crucial for ensuring consistent and reproducible results in the exosome-based molecular assay? 6. Regarding the

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transportation of urine specimens, what was the optimal gel pack volume and storage conditions identified to maintain optimal refrigeration and specimen temperature for 24-48 hours? 7. The study explored the stability of exosomal mRNA upon prolonged urine storage at different temperatures. What were the implications of storing urine samples at +4°C for up to one week versus storing samples at -80°C? 8. The study also evaluated the effects of interference substances on the qPCR assay. Could you explain the significance of testing medications commonly prescribed to transplant patients and the impact of blood-derived components, like hematuria, on assay performance? 9. The study emphasized that blood-derived components, including blood cells and debris, can interfere with the assay. How does centrifugation effectively address this interference? Could you explain the mechanism behind this process? 10. Based on the study's findings, how do you suggest optimizing the preanalytical process for urine-derived exosome molecular assays to ensure accurate and reproducible results? How can these findings contribute to the clinical implementation of this diagnostic test for kidney allograft rejection?