SANAMED
2025; 20(3): 241–242
ISSN-1452-662X

DOI: 10.5937/sanamed0-62870
UDK: 577.113.8
ID: 183828745
Letter to the Editor

REASSESING RIBONUCLEIC ACID ISOLATION FROM HUMAN MONONUCLEAR CELL CULTURE WITH MAGNETIC BEADS PRE-ENRICHMENT FOR MOLECULAR ANALYSIS

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Primljen/Received: 18. 11. 2025. Prihvaćen/Accepted: 13. 12. 2025. Online First: 23. 12. 2025.

Dear Editor,

It is with considerable interest that I have perused the recently published contribution by Bhatia, entitled *"Ribonucleic Acid Isolation from Human Mononuclear Cell Culture with Magnetic Beads Pre-enrichment for Molecular Analysis,"* set forth within *Sanamed*, volume 19(1). The author addresses a conundrum of pronounced import for those engaged in molecular analyses and the development of immunotherapies. As such, the established difficulty of extracting ribonucleic acid (RNA), particularly from mononuclear cell (MNC) cultures exceeding a few months in age, is well documented within the author's own laboratory experience, having persisted, as stated, for over two years of consistent failure.

The developed methodology, centered upon the pre-enrichment of the cultured MNCs utilizing Cluster of Differentiation 45 (CD45)-specific magnetic beads antecedent to the customary mini column isolation, proves efficacious where previous attempts faltered. The successful isolation of RNA from cultures exceeding six months in duration—confirmed through spectrophotometric yield measurements and subsequent conventional and real-time Polymerase Chain Reaction (PCR) assays for the beta-actin housekeeping gene—is indeed a notable technical advancement. The assertion that this study constitutes the inaugural demonstration of isolating RNA from aged human MNC cultures via specific magnetic beads is, moreover, a claim that warrants careful consideration.

Notwithstanding the demonstrated success of this technique, one might cast an inquiring gaze upon certain aspects of the execution and presentation. Firstly, the affiliation of the esteemed author with Genekam Biotechnology AG, which entity serves as the sole source for the crucial magnetic beads, the mini column isolation kit, the PCR kits, and the specialized magnetic rack, invites circumspection. Whilst proprietary methods often feature in novel protocols, the near-total reliance upon reagents and apparatus supplied by the author's own commercial interest renders the protocol less immediately accessible or generalizable for laboratories not possessed of the aforementioned instruments and supplies. The efficacy of the method, therefore, appears for the present to be closely associated with this particular commercial supply chain.

Secondly, whilst the author reports that the initial method failed consistently over two years, and that isolation without magnetic beads was not achieved, the results presented lack detailed quantitative metrics comparing the failed isolations to the successes. The presentation of "failure" in Table 1 illustrates the necessity of the pre-enrichment step, yet does not afford the readership an optimal means to gauge the precise extent of nucleic acid degradation or inhibition encountered previously—data which would further illuminate the magnitude of the technical challenge overcome.

Finally, the discussion alludes to further consequential applications of the isolated RNA—namely, its conversion to complementary DNA (cDNA) and subsequent use for other purposes—yet these vital data are deferred for "future publications." Herewith, a more comprehensive elucidation of the robustness of the isolated material might have been advantageously reserved for this singular publication, ensuring that the full scope of the methodology's utility is presented forthwith (1).

In essence, whilst the developed magnetic bead pre-enrichment methodology provides a much-needed solution for obtaining RNA from refractory MNC cultures, it is sincerely hoped that future work will include more extensive comparative data and endeavor to ascertain the protocol's viability utilizing reagents sourced from diverse suppliers, thereby further affirming its widespread applicability in molecular diagnostics and therapeutic development. This issue merits further investigation. We thank Bhatia et al. (1) for their valuable study on RNA isolation from human MNC culture with molecular analysis.

Abbreviations

MNC – Mononuclear Cells
CD45 – Cluster of Differentiation 45
PCR – Polymerase Chain Reaction
RNA – Ribonucleic Acid
cDNA – Complementary DNA

Funding: No funding was available.

Conflicts of Interest: None declared.

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Note: Artificial intelligence was not utilized as a tool in this study.

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***How to cite this article**:* **Sengul I, Sengul D.** Reassessing Ribonucleic Acid Isolation from Human Mononuclear Cell Culture with Magnetic Beads Pre-enrichment for Molecular Analysis. Sanamed. 2025; 20(3): 241-242. doi: 10.5937/sanamed0-62870.