B211 Alternative Reducing Agents for DNA Extraction

Megan E. Grimes, MFS, 5187 Salt Pond Place, Woodbridge, VA 22193; and Mark F. Kavlick, MPhil, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will understand how reducing agents can improve forensic DNA extraction, that there are alternatives to the commonly used reducing agent Dithiothreitol (DTT), and how to easily substitute these agents into an established casework workflow.

This presentation will impact the forensic science community by introducing and describing alternative reagents to improve DNA extraction from semen samples. The reduction of disulfide bonds is a critical step in the extraction of DNA from sperm cells, which may be improved using alternative reducing agents compared to DTT.

A commonly utilized reducing agent in forensic DNA extraction is DTT, which facilitates the isolation and purification of DNA from proteins within a biological sample by reducing disulfide bonds, thereby enhancing proteinase K digestion. Additionally, an alkylating agent, such as Iodoacetamide (IAM) may be employed as a secondary step to prevent reformation of disulfide bonds.

DTT has many disadvantages as a reducing agent, in that it is unstable in air, requires refrigeration, and has an unpleasant odor. During a previous study, it was observed that alternatives to DTT *alone*, such as DTT followed by alkylation with IAM or replacement of DTT with Tris(2-Carboxyethyl)Phosphine (TCEP), improved DNA extraction yields.[1] Therefore, this study focused on identifying other alternatives to DTT to improve/enhance DNA yields from semen samples. Three alternatives to DTT and TCEP were identified and evaluated. These included Dithiobutylamine (DTBA), Glutathione (GSH), and Tributylphosphine (TBP).[2-4] Specifically the following conditions were compared: (1) DTT alone (Standard Operating Protocol (SOP)); (2) DTBA; (3) DTBA followed by IAM; (4) GSH; (5) GSH followed by IAM; (6) TBP; and, (7) TBP followed by IAM. DNA yields were measured using a nuclear DNA-specific quantitative Polymerase Chain Reaction (qPCR).

All reagents tested were water-soluble and easily incorporated into a semi-automated SOP workflow. In brief, the SOP involved lysis of the semen specimen in buffer G2, proteinase K, and DTT, followed by purification on an EZ1™ BioRobot®. Each alternative reducing agent simply replaced DTT during the initial lysis stage. Alkylation by IAM, where tested, involved a separate incubation for 30 minutes in the dark at room temperature.

Results demonstrated that the alternative reducing agents provided increased DNA yields over the SOP. DTBA, DTBA followed by IAM, GSH, and GSH followed by IAM showed significant increased yields of 25%, 25%, 65%, and 61%, respectively, compared to the SOP. In conclusion, DTBA and GSH may be considered superior reducing agents to that of DTT for forensic DNA extraction. In particular, these alternatives may prove invaluable for challenging samples, such as low template, or those which are recalcitrant to extraction, such as spermatozoa.

Reference(s):

1. Grimes M. Enhanced DNA Extraction via the Reduction and Alkylation of Disulfide Bonds by Iodoacetamide and Tris(2-carboxyethyl)phosphine. *Proceedings of the American Academy of Forensic Sciences*, 68th Annual Scientific Meeting, Las Vegas, NV. 2016.
2. Lukesh J., Palte M., Raines R. A potent, versatile disulfide-reducing agent from aspartic acid. *Journal of the American Chemical Society*. 2012, 134(9):4057-4059.

3. Chakravarthi S., Jessop C., Bulleid, N. The role of glutathione in disulfide bond formation and endoplasmic-reticulum-generated oxidative stress. *EMBO Reports.* 2006, 7(3): 271-275.

4. Humphry R., Potter J. Reduction of Disulfides with Tributylphosphine. *Analytical Chemistry.* 1965, 37(1): 164-165.

DNA Extraction, Dithiobutylamine, Glutathione