J Forensic Sci, Jan. 2003, Vol. 48, No. 1 Paper ID JFS2002258_481 Published 11 Dec. 2002 Available online at: www.astm.org

Authors' Response

Sir:

In the Letter to the Editor, Subhash Arya comments on the utility of GenoFixTM for preserving DNA in tissues at room temperature for extended periods of time. Arya further notes that fixatives like GenoFixTM would be of "immense value to surgeons and allied health care personnel in developing countries in preserving numerous surgically excised tissues awaiting different DNA or RNA based molecular investigations even at far-off locations. In such countries, adverse environment seriously affects even the ordinary biochemical or immunological assays."

It is worth pointing out that GenoFixTM was developed as a tissue fixative that also stabilizes DNA. As such, fixed tissues can be examined by microscopic techniques such as immunohistochemistry but also by DNA/RNA-probing techniques such as *in situ* hybridization. In this respect, it is different than RNA*later*TM (Ambion), which appears to be designed to optimize stability and recovery of RNA. Indeed, GenoFixTM is expected to have much wider application than only for "practitioners of forensic sciences," as Arya has suggested.

Arya also discusses the problem of tissue RNA preservation. In our experience, most of the problems encountered in dealing with tissue RNA are caused by degradation of RNA prior to putting the tissue into a preserving solution. That is, acidic lysosomal enzymes, including ribonucleases, become activated once tissue is cut off from its blood supply. Once degraded, even dropping the tissue into liquid nitrogen would, of course, not restore the integrity of the RNA. Our experience with GenoFixTM (as yet unpublished) is that RNA, usable for RT-PCR analysis, can be extracted for over three months at room temperature if surgical samples are put into GenoFixTM as soon as possible after removal. We have not tested

storage at -20° C, but would expect that stability would be even greater. Tissues fixed in GenoFixTM, embedded in paraffin and then stored at room temperature for up to seven years, have yielded good real-time PCR results.

Because of its alcohol/aqueous content, GenoFixTM is expected to be compatible with aqueous-based RNA preservatives such as RNA*later*TM, but this has not been tested directly. It is worth noting that GenoFixTM, like RNA*later*TM, is now a commercial product. This should enable more investigators to obtain samples and begin developing their own experiences (http://www.DNAGenotek.com).

Again, the study was targeted to address the demands of forensic applications and was not extended to cover all potential medical applications. However, since similar circumstances conceivably apply to clinical samples, we certainly encourage other users to evaluate the benefits of the method and we look forward to learning more about the utility of this new approach to preserving tissues and enabling DNA and RNA analyses.

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