Reply to Ancillary Techniques on Direct-Smear Aspirate Slides

A Significant Evolution for Cytopathology Techniques

We thank da Cunha Santos et al for their interest in our commentary,[1] and agree that the use of FTA cards is most likely a sound choice for long-term DNA storage. In our experience, the use of Diff-Quik–stained cytologic smears, stored for years, is also satisfactory for the isolation of high-quality DNA. Several points deserve clarification regarding these methods.

As the authors point out, the breakage of slides during transport is a potential issue; nonetheless, careful packing of slides can prevent this and we do not believe that this is a serious drawback to the use of smears.

With regard to the possible problem with the stability of unstained smears, we acknowledge heat and humidity as potential problematic issues. This can be circumvented by storing the slides in climate-controlled rooms. In our practice, any remaining air-dried, unstained slides are stained with Diff-Quik, coverslipped, and stored long term after ancillary tests have been completed. If additional DNA is needed in the future, one of the extra Diff-Quik–stained slides can be decoverslipped for tumor cell microdissection and DNA purification. The authors fail to provide convincing data that filter papers are any less susceptible than unstained smears to heat and humidity. Because it is possible that the filter papers can attract moisture, a side-by-side comparison would be interesting.

More clarification is needed regarding the authors' insistence that analysis of a corresponding stained cytospin slide obtained from the same needle rinse as the FTA card helps ensure the identity of the material in the FTA card. When assessing tumor purity in a specimen, or whether tumor cells are even present, there is no better substitute than the ability to examine the actual cells to be extracted with the microscope. This is possible with stained slides. Slides are not homogenous; they often display contaminating, benign cells as well as areas of relatively pure tumor cells. Microdissection of tumor-enriched areas is possible with smears whereas this is not possible with FTA cards. The use of cytospin preparations to infer tumor cellularity and purity on FTA cards is still an extrapolative exercise; extrapolation is not necessary with smears because "what you see is what you get."

More data are needed to conclude whether FTA cards or stained slides provide higher quality nucleic acids on extraction. Molecular testing methodology is constantly evolving, and the requirements of nucleic acid quality will require constant evaluation. During this very exciting era, cytopathologists are starting to investigate various cytopreparatory platforms (smears, FTA cards, ThinPrep slides, etc.) and slide staining conditions for the isolation of nucleic acids. We believe that no method is perfect and each can be continually improved. A major advantage of cytologic sample processing lies in the versatility of the sample slide preparation methods available, and we believe these different platforms are complementary. Collaborative efforts to continuously optimize nucleic acid purification from various specimen preparations is essential for cytopathologists worldwide to better serve their patients in this constantly evolving era of precision medicine.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

REFERENCE

1. Knoepp SM, Roh MH. Ancillary techniques on direct-smear aspirate slides: a significant evolution for cytopathology techniques. *Cancer (Cancer Cytopathol)* 2013;121:120-128.

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