FTA Cards for Preservation of Nucleic Acids for Molecular Assays

A Review on the Use of Cytologic/Tissue Samples

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Context.—Traditional methods for storing histologic and cytologic specimens for future use in molecular assays have consisted of either snap-freezing with cryopreservation or formalin-fixing, paraffin-embedding the samples. Although snap-freezing with cryopreservation is recommended for better preservation of nucleic acids, the infrastructure and space required for archiving impose challenges for high-volume pathology laboratories. Cost-effective, long-term storage at room temperature; relatively easy shipment; and standardized handling can be achieved with formalin-fixed, paraffin-embedded samples, but formalin fixation induces fragmentation and chemical modification of nucleic acids. Advances in next-generation sequencing platforms, coupled with an increase in diagnostic, prognostic, and predictive molecular biomarkers have created a demand for high-quality nucleic acids. To address issues of the quality of nucleic acid and logistics in sample acquisition, alternatives for specimen preservation and long-term storage have been described and include novel universal tissue fixatives, stabilizers, and technologies.

Objective.—To collect, retrieve, and review information from studies describing the use of nucleic acids recovered from cytologic/tissue specimens stored on Flinders Technology Associates (FTA, GE Whatman, Maidstone, Kent, United Kingdom) cards for downstream molecular applications.

Data Sources.—An electronic literature search in the PubMed (National Center for Biotechnology Information, Bethesda, Maryland) database allowed the selection of manuscripts addressing the use of FTA cards for storage of cytologic samples for molecular analysis. Only articles published in English were retrieved.

Conclusions.—The use of FTA cards is a versatile method for fostering multicenter, international collaborations and clinical trials that require centralized testing, long-distance shipment, and high-quality nucleic acids for molecular techniques. Studies with controlled temperature are required to test the quality of recovered RNA after long-term storage.

The rapidly evolving area of diagnostic, prognostic, and predictive molecular biomarkers coupled with advances in next-generation sequencing platforms have created a demand for high-quality nucleic acids. In addition, guidelines and recommendations to standardize and improve the quality of molecular research using biospecimens have highlighted the importance of tissue preservation and storage.\textsuperscript{2,3} Traditional methods for storing histologic and cytologic specimens for future use in molecular assays have consisted of either snap-freezing with subsequent cryopreservation or collection in a fixative or preservation solution, most frequently in the form of formalin-fixation, paraffin-embedding, which is the standard preservation procedure for routine clinical diagnostics in pathology laboratories.

High-quality nucleic acids and proteins are retrieved from cryopreserved samples and are suitable to most downstream analytic assays; however, the architectural and morphological details are not totally preserved. In contrast, cellular morphology is usually not compromised for formalin-fixed, paraffin-embedded (FFPE) samples, but formalin fixation induces cross-links of proteins as well as the fragmentation and chemical modification of nucleic acids, which affects the quality of the DNA, RNA, and proteins extracted and potentially interferes with the results of a variety of analyses.\textsuperscript{4-5} A recent study concluded that the extent of DNA damage directly confounds the determination of somatic variants in large public databases.\textsuperscript{6} In addition, cost-effective long-term storage at room temperature; relatively easy shipment; and standardized handling can be achieved with FFPE samples, significantly contrasting to frozen samples, which require special infrastructure.
Abbreviation: FFPE, formalin-fixed, paraffin-embedded.

with highly controlled conditions and maintenance with consequent high costs involved with handling, shipping, and long-term storage (Table).

Snap-freezing with cryopreservation is still the recommended method for better preservation of nucleic acids and biobanking; thus, storage of tumor samples in a high-volume surgical pathology laboratory has become cumbersome and an expensive operation if every solid tumor specimen undergoes some type of molecular testing. In addition, most private-based and independent laboratories do not routinely save tissue for molecular tests and, with current increased incorporation of those tests to our practice, methods to preserve nucleic acids from clinical specimens must be convenient, reliable, and cost effective to be adaptable to the nonacademic surgical-pathology laboratory. Recently, to address issues of the quality of nucleic acids and logistics in sample acquisition, alternatives for specimen preservation and long-term storage have emerged and include novel universal tissue fixatives, stabilizers, and technologies, with special filter paper cards, more specifically the Flinders Technology Associates (FTA) cards, being a versatile method.

FTA CARD OVERVIEW, SAMPLE COLLECTION, AND ADVANTAGES

The FTA card (GE Whatman, Maidstone, Kent, United Kingdom) consists of a special type of filter paper impregnated with a proprietary formula containing reagents that promote cell lysis and protein denaturation with subsequent release of nucleic acids that are entrapped within the matrix of the card and stabilized at room temperature, allowing long-term storage. Originally applied to the perseveration of blood samples for forensic tests, collection of materials for genotypic analysis of microorganisms, plants, and molecular epidemiologic studies, the cards have also been used to store gynecologic and nongynecologic cytology specimens for various molecular analyses. The objective of this review was to collect and retrieve information from studies describing the use of nucleic acids recovered from cytologic/tissue specimens stored on FTA cards for downstream molecular analysis. The cards commercially available differ essentially on the color of the filter paper, the number of sample areas, and the chemicals present that will influence the protocols used for nucleic acid extraction. For the indicating cards, the color of the paper changes from pink to white on the areas that the material is spotted, and is, therefore, recommend-

ed for colorless samples (Figure). Both FTA Classic Card and the FTA Elute card have similar technologies with release of the entrapped DNA into an eluate solution, but they comprise 2 distinct chemistries. Various protocols have been used for DNA extraction. Although no significant difference in the amount of extracted DNA was found between the FTA Classic and Elute card, it was suggested that FTA Classic cards preserve DNA integrity better for molecular applications than the FTA Elute cards.

Many advantages have been described for the cards, including low-cost, simple extraction protocols; easy transportation; minimal storage space; and no special infrastructure being required. The cards can be stored at room temperature for DNA extraction. It is recommended that the cards be stored in a sealed pouch with desiccant. For RNA preservation, frozen storage can be helpful, according to manufacturer instructions. Because they are lightweight and do not require processing or snap-freezing, the cards are suitable for transportation by mail and, therefore, can be used as a complementary method for storing genetic material, particularly for collaboration within the context of clinical trials and for transporting samples to referral centers for advanced molecular technologies.

A study analyzed the risk of cross-contamination by using discs from a blank area of the FTA card after punching human papillomavirus (HPV)–16 FTA cards. No cross-contamination was identified, suggesting minimal contamination risks among samples.

Cell suspensions from fine-needle aspiration (FNA) biopsies or from cells scraped from surgical specimens have been the most often type of preparation used to apply the cells to the cards, but cervical smears, buccal swabs, sputum, and smears/touch imprints from surgical samples have also been described. Apparently, cell suspension is the optimal cell preparation method because of the absorbent nature of the cards and because they were designed, and have been mostly used, for collecting blood. For nucleic acid extraction, 2 circle punches (disks) are usually collected from the card, and approximately 25 punches (3 mm) can be obtained from the entire surface of the card.

FTA CARDS: NUCLEIC ACID STABILITY AND TIME OF STORAGE

The stability of DNA obtained from the cards, as determined by the time of storage, has been documented

* References 7–10, 12, 17, 18, 20–25, 27, 28.

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in several publications. A study using suspension of tumor cells scraped from the surface of 60 fresh surgical specimens collected on Roswell Park Memorial Institute tissue culture medium and stored on FTA cards at room temperature showed no correlation between the robustness of the polymerase chain reaction (PCR) and the length of time the cells were stored. The average storage time was 49 days (range, 10–150 days) before DNA extraction. Similarly, DNA from residual material of lymph node FNA biopsy samples, preserved in FTA Classic and Elute cards for a minimum of 1 to a maximum of 123 days (mean, 34.8), could be successfully extracted, PCR amplified, and used for high-throughput mutation analysis. In a different study, PCR analysis performed on DNA retrieved from FTA Classic cards both immediately and after 6 months of storage of 26 cell suspensions obtained from ovarian tumors revealed identical results for amplification of the CYP2C9 gene fragment containing the *6 polymorphism. Amplification was also successful on all 11 samples stored on IsoCode card (currently, the FTA Elute card), which was tested immediately and after 18 months of storage. For residual material from lymph node FNA biopsy samples stored up to 123 days (mean, 34.8), successful PCR was demonstrated in all samples using DNA extracted from FTA Classic and Elute Cards for a 500–base pair (bp) amplicon and in 95% of the samples stored on classic cards for a long amplicon (1.5-kb amplicon), showing the DNA integrity and the high-quality DNA retrieved from the cards. The PCR products were used for high-throughput mutation analysis (mass array spectrometry). Another study observed no correlations between the robustness of PCR and the collection time for FNA samples stored on the FTA Classic cards for up to 1 year.

For identification of microorganisms using cytology samples, successful amplification by PCR for the detection of Mycobacterium tuberculosis was obtained from sputum specimens collected on FTA cards from patients in treatment for tuberculosis. A reproducibility analysis performed by taking a punch from the card after 1-, 6-, and 12-month storage intervals showed in 10 of the 11 samples (91%) the same pattern of HPV types. One case, over time, gradually lost 2 high-risk genotypes (types 18, 56). However, the sample was HPV multi-infected (types 16, 18, 56) and had insufficient volume of available residual material after cytology testing. The authors argued that a lower amount of specimen may have been initially placed on the FTA card, which may explain the negative results with the second and third punches.

Similarly, for blood samples, the time of storage (1 week, 2 months, and 1 year), the storage temperature (room temperature, −20°C, and −80°C), and the presence or absence of ethylenediaminetetraacetic acid had no significant effects on the amount of DNA recovered from IsoCode (FTA Elute) and Classic cards. Even DNA from postmortem blood stored on FTA cards has been demonstrated to be rather stable up to 16 years. The DNA extracted could be used for human identification purposes. A negative correlation between the FTA card storage time and DNA quantity...
was observed. DNA integrity was also affected during storage. Although complete short tandem repeat profiles were obtained for all samples, there was evidence of degradation manifested as decreased peak heights in the larger-sized amplicons. That effect should be taken into account, depending on the intended application, especially if high-quality DNA and long PCR amplicons are required.13

In terms of RNA stability, a few studies have published the results using human cells. Cell suspensions of mammalian cell samples were stable on cards stored at room temperature and 4°C for periods of up to 3 and 5 months, respectively, in the desiccated pouches after sample application. Samples stored at temperatures of −20°C or less were stable for periods of at least 1 year.15 Amplifiable RNA was retrieved for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene on 8 cell suspensions of ovarian tumors stored on FTA Classic cards tested immediately and after 6 months of sample collection.7

**PERFORMANCE OF FTA CARDS COMPARED WITH OTHER STORAGE/PRESERVATION METHODS**

Studies have compared the performance of the cards to other types of storage and to fresh samples regarding the nucleic acids recovered and the usage for downstream applications. DNA extracted from cells stored on the FTA filter paper, when compared with DNA extracted from fresh samples, showed no significant differences in the ability of the templates to produce amplicons using PCR amplification of 2 different loci. With only 3 major discrepancies found among 60 cases, the differences were attributed to the extraction of fewer cells by the FTA filter paper method.8 Further testing, for monoclonal rearrangement of the immunoglobulin heavy locus (IgH) gene in 15 lymph node specimens, revealed no difference between the results obtained with DNA from the cards and those obtained from fresh cell suspensions. The authors argued that this particular application of the cards could be extremely useful in laboratories performing flow cytometry for the diagnosis of lymphoma because a cell suspension is routinely prepared before flow cytometric evaluation, and in the event that additional molecular studies are needed, the FTA filter paper could then be sent for molecular testing. In fact, in a study using residual material from routine cell suspensions; therefore, an aliquot with a predetermined volume can be used for generating a cytospin for cellularity assessment, such as total cell count, determination of the tumor cell percentage, and other morphological analyses. An automated cell counter can also characterize the cell suspension before applying the material on the cards, thus guaranteeing comparable samples, as previously described.10 As documented in some publications, the amount of DNA recovered from 2 disks (3-mm size) was limited. However, a maximum of approximately 25 disks can be obtained from an FTA microcard, and multiple cards can be generated at the time of sample collection, which can obviate the low recovery. A few studies used RNA with few samples.7,11 Therefore, comparison analyses on the performance of the RNA recovered from the cards compared with other preservation methods and the stability of the RNA at various storage temperatures still need to be addressed.

**CONCLUSIONS**

The high quality of nucleic acid stored on the cards, the low cost of storage and handling, the ease in transporting, and simple extraction method make FTA cards a compelling, versatile alternative to traditional methods for storage of cytology samples. Studies are still needed to confirm the utility of the cards for storing various types of specimens, such as effusions and the quality of the nucleic acids, especially RNA, after long-term storage. The cards emerge as an excellent method to foster increasing multicenter,
international collaborations; more specifically, in cases of rare neoplasms, the cards maximize retrieval, and for clinical trials that require centralized testing, long-distance shipment, and high-quality nucleic acids for advanced molecular techniques, the cards can be particularly useful.

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