Maximizing the Utility of Archival Formalin-Fixed Paraffin-Embedded Blocks for Nucleic Acid Analysis

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LABORATORIES, ARCHIVES, and biorepositories are filled with old formalin-fixed paraffin-embedded (FFPE) biospecimens. They may be physically intact or melted or mouse chewed; some may be unidentifiable while others may have years of associated clinical, proteomic, and histological data. Archival FFPE blocks, particularly those with rich associated data, can be extremely valuable to research. For example, such blocks, coupled with their associated data, can potentially be used to identify molecular biomarkers of cancer progression, survival, and treatment response, important goals of the U.S. Cancer MoonshotSM initiative.¹ The ambitious aims of the Cancer MoonshotSM to accelerate cancer research by increasing both the number of therapies and their availability to patients, to improve cancer prevention, and to improve early stage detection will necessitate maximizing all available biospecimen resources. Although there is a clear need for prospectively collected biospecimens, a wealth of information may be gained from archival biospecimens.

Archival FFPE blocks from the National Cancer Institute's (NCI's) Surveillance, Epidemiology, and End-Result (SEER) program^{2,3} represent one such valuable research resource. The SEER program has >45 years of data on tumor characteristics, survival, and, in some cases, treatment. Over the past 15 years, the SEER program has collected residual tissue biospecimens that correspond to SEER data on >60,000 cancer patients; many are FFPE blocks. As such, the SEER biospecimens and the associated data are an irreplaceable resource and must be utilized in a manner that maximizes the quantity and quality of the data produced.

In most cases, although valuable archival FFPE blocks may come with a treasure trove of valuable clinical data, there is little to no annotation available about the pre-analytical steps undertaken when preserving and processing the tissues. How long had the biospecimens been subjected to cold ischemia before being placed in formalin? What was the composition of the formalin fixative solution—buffered or aqueous? How long did the biospecimens sit in formalin? How was the tissue further processed to paraffin? Further confounding the use of archival blocks, FFPE processing under any conditions introduces artifacts including protein cross-linking, fragmentation, and the addition of methylol groups that may affect downstream DNA and RNA analysis; this has been demonstrated in experiments showing variable

concordance between FFPE and frozen biospecimens.⁴ Many such effects can be attenuated (but not eliminated) by proper biospecimen collection, handling, and storage,⁵ through demodification during nucleic acid extraction and modified analytical workflow,⁶ or resolved through changes to the bioinformatics pipelines.⁷ In the case of archival FFPE biospecimens, we cannot control processing and storage—but throwing away those specimens can mean losing an irreplaceable and potentially valuable resource.

Despite these challenges, researchers are pressing forward with genomic analyses of archival FFPE blocks and developing approaches to mitigate the unknowns. An important element of an effective genomics research approach for archival FFPE blocks is utilizing harmonized nucleic acid isolation procedures designed to mitigate as many of the processing-related artifacts as possible and not further compromise the quality of the resulting data. The extent to which the effects of processing are mitigated together with the extraction protocol utilized are assay- and target dependent.

In this issue of *Biopreservation and Biobanking*, we introduce a Biospecimen Evidence-Based Practice (BEBP) focused on nucleic acid extraction from FFPE tissue. This represents the second in NCI's BEBP series and offers evidence-based, step-by-step guidance that may serve as a template for individual laboratory's standard operating procedures (SOPs). The steps in the BEBP are backed by both a referenced literature summary and expert input. In addition to the links to the research articles cited, there are links to original curations of the articles in the NCI Biospecimen Research Database (BRD).⁸ The input from a panel of experts provides added value to the BEBP as it ensures that the methods stated are practical and up to date for today's laboratories. The expert input also includes information from the experts' laboratory method optimization and daily workflow, information that in many cases has not been published. In addition, the BEBP includes information on fit-for-purpose quality markers that can serve as a guide in decision-making about which biospecimens and assays are most likely to yield reliable data, because once nucleic acids are extracted from FFPE biospecimens, it can be difficult to determine whether it is worth pursuing costly analysis of the resultant nucleic acids, and/or which analysis methods are

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most likely to yield results. Such a thoughtful approach may help ensure the best use of valuable research funds and irreplaceable biospecimens.

The most important goal of the BEBP series is to provide needed evidence-based guidance for SOP development and thereby improve biospecimen quality and optimal use of existing biospecimens. It has been reported that 48% of researchers report difficulty in obtaining high-quality biospecimens and that 60% of researchers have questioned their findings based on biospecimen quality.⁹ There is a wealth of information available on preanalytical and analytical factors that affect biospecimen quality, but it is distributed through hundreds of journal articles, protocols, laboratory guidelines, and the experience of experts. As a consequence, many researchers use SOPs without being confident whether the method they are using is based on evidence. By analyzing the evidence, consolidating it into one document, making the recommendations flexible and vetting them with experts, and placing the document in the publicly accessible BRD, we hope to hasten the transition to evidence-based SOPs.

As we pursue research studies using archival FFPE tissues, let us think about the unique information each may hold about disease states and progression, and maximize the impact of our research with evidence-based approaches to FFPE analysis.

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