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NCI Biospecimen Evidence-Based Practices (BEBP) - Nucleic Acid Extraction from Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue

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This document contains guidance that is intended to facilitate the development of evidence-based standard operating procedures.

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Disclaimer

This document contains guidance that is intended to facilitate the development of evidence-based standard operating procedures.

Abstract

This evidence-based, expert-vetted best practice document is applicable for nucleic acid extraction from standard-size FFPE blocks containing human tissue, although such guidance may also be applicable to tissue from mammalian animal models. Specifically, recommended procedures are suitable for standard cassette-sized, non-decalcified tissue biospecimens that were fixed in 10% neutral buffered formalin (NBF) for less than 48 h before paraffin embedding. Biospecimens processed under these procedural guidelines are suitable for analysis by polymerase chain reaction (PCR), methylation analysis, Sanger sequencing and next-generation sequencing, and microarray hybridization. Much of the evidence presented in this document pertains to mRNA molecules and DNA, thus shorter RNA molecules such as microRNAs (miRNAs) may require additional consideration and optimization. This document is not applicable for analysis of biospecimens by *in situ* hybridization.

Attachments

PDF

NCI BEBP_Expert-vett...

1,000KB

Guidelines

9.1.1 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings (CDC, 2007): http://www.cdc.gov/hicpac/2007IP/2007isolationPrecautions.html

9.1.2 NIOSH Pocket Guide to Chemical Hazards (CDC): **http://www.cdc.gov/niosh/npg/**

9.1.3 CLSI IL-28A: Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline—Second Edition S Hewitt, personal communications, draft CLSI IL-28a

9.1.4 QIAGEN (June, 2012) QIAamp® DNA FFPE Tissue Handbook, Valencia CA

9.1.5 QIAGEN (February, 2013) RNeasy® FFPE Handbook, Valencia CA

9.1.6 Life Technologies (2011) Ambion RecoverAll™ Total Nucleic Acid Isolation Kit, Carlsbad CA

9.1.7 QIAGEN (2012) AllPrep® DNA/RNA FFPE Handbook, Valencia CA

9.1.8 TrimGen, WaxFreeTM RNA User Manual, Sparks MD

9.1.9 Biorepositories and Biospecimen Research Branch, National Cancer Institute, National Institutes of Health. NCI Biospecimen Evidence-Based Practices, Formalin-Fixation and Paraffin Processing of Tissue Biospecimens. In Preparation.

9.1.10 Broad Institute Genomic Services (2016) Nucleic Acid Extractions: Product Data Sheet, http://genomics.broadinstitute.org/data-sheets/DTS_Extractions_1Page_5-2016.pdf

Materials

5.1 RNase removal spray or wipes
5.2 Microtome
5.3 Ice block
5.4 Pre-labeled RNase-free microcentrifuge tubes
5.5 Plastic-backed absorbent bench paper
5.6 Xylene
5.7 Ethanol
5.8 Microcentrifuge
5.9 An experimentally-validated extraction kit of the user's choice. Acceptable methods for DNA extraction include silica, magnetic bead, heating, focused-ultrasonication, resin-filter, or salting-out-based methods, but organic extraction should be avoided (See Section 7.8 in the Attached PDF for Literature Evidence). Acceptable RNA extraction methods include silica, magnetic bead, organic focused-ultrasonication or salting-out-based methods, but generally, resin-filter and heat-based methods should be avoided (See Section 7.9 in the Attached PDF for Literature Evidence).
5.10 Real-time PCR machine
5.11 Spectrophotometer
5.12 Fluorometric DNA quantification method of choice

Safety warnings

Guidelines on precautions to prevent the transmission of infectious agents should be consulted and can be used for all phases of organ/tissue dissection and handling (Reference 9.1.1).

The National Institute for Occupational Safety and Health (NIOSH) Pocket Guide to Chemical Hazards should be consulted for all steps including formalin, xylene, and other chemicals (Reference 9.1.2).

Ethics statement

Protocols developed using this Biospecimen Evidence-Based Practice may require approval by the user's institutional review board (IRB) or an equivalent ethics committee prior to implementation.

Before start

The purpose of this document is to provide evidence-based and expert-vetted guidance on nucleic acid extraction from formalin-fixed and paraffin-embedded (FFPE) human tissues. This guidance is intended to support the development and execution of evidence-based Standard Operating Procedures (SOPs) for DNA and RNA extraction from FFPE biospecimens.

Recording of biospecimen pre-acquisition, acquisition and processing data

1 Whenever possible, extensive data should be recorded relating to preacquisition, acquisition and processing conditions that may affect the integrity of the biospecimen. Such data may consist of patient information (including age, gender, diagnosis, and treatment), details relating to surgery and biospecimen acquisition (including the use of anesthesia, warm ischemia time, and surgical procedure and duration), and FFPE processing (including fixation duration, temperature, embedding medium) and the duration of block storage (Guideline 9.1.9).

Preparation of bench space

2 Pre-label microcentrifuge tubes.

3 Clean work area and metal equipment including microtome with a xylene-free deparaffinization reagent (See Section 8.3.2 in the Attached PDF for Expert Recommendations).

4 Wipe down all equipment, work area and all tools with RNase removal wipes or spray.

Sectioning paraffin blocks

5 Place paraffin blocks face-down on a cold plate for a minimum of 00:05:00 or at room temperature if blocks were stored at -20°C. The 5 min incubation may be reduced if the block has been stored under recommended conditions (See Section 7.1 in the Attached PDF for Literature Evidence). Placement of paraffin blocks on wet ice is not recommended (See Section 8.3.2 in the Attached PDF for Expert Recommendations).

6 A new microtome blade should be used for each biospecimen.

7 Position the FFPE tissue block in the specimen clamp of the microtome.

8 Cut sections that are 5-10 µm thick (Guideline 9.1.4) (See Section 7.2 in the Attached PDF for Literature Evidence). Allow individual sections to roll up naturally. After discarding the

first 3-4 sections, place the number of sections required to obtain the desired volume of tissue needed for extraction directly into a sterile RNase-free microfuge tube using RNase-free forceps. For reference, 1-8 sections will be required for a block with a tissue surface area of 250 mm2 (Guideline 9.1.10). However, the optimal number of sections for extraction is based on the tissue surface area and cellularity (determined by H&E staining) of a given block and the extraction kit used; thus, the number of sections or tissue area needed for extraction should be experimentally validated for each extraction kit and standardized within a specimen cohort (See Section 8.3.3 in the Attached PDF for Expert Recommendations).

9 Paraffin sections should be processed for extraction immediately, optimally within 24 h. While available literature is limited, evidence suggests that when necessary, sections may be stored for up to 90 days at room temperature (See Section 7.3 in the Attached PDF for Literature Evidence) or 4, -20 or -80˚C in a sealed microcentrifuge tube (See Section 8.3.4 in the Attached PDF for Expert Recommendations) for some analyses.

Deparaffinization of sections

10 Add 1 ml of xylene to the microcentrifuge tube or slide and incubate at Room temperature for 00:10:00 to deparaffinize sections. Acceptable alternative deparaffinization methods are discussed in Sections 7.4 (Literature Evidence) and 8.3.5 (Expert Recommendations) in the Attached PDF. For DNA extraction, acceptable alternatives include use of proprietary buffers, mineral oil, lysis buffer heated to 98-120°C, focused-ultrasonication, heptane or the xylene substitute HemoClear. For RNA extraction, acceptable alternatives include use of proprietary buffers, heptane, lysis buffer heated to 95-98°C, focused-ultrasonication or the xylene substitute HemoD. Microwave heating should be avoided (See Section 7.4 in the Attached PDF for Literature Evidence). The acceptable duration of deparaffinization will depend upon the method employed (See Section 7.5 in the Attached PDF for Literature Evidence).

11 Centrifuge tubes for 00:02:00 at maximum speed (approximately 16,000-20,000 x g) (Guidelines 9.1.4, 9.1.5, 9.1.6, and 9.1.7) and discard the supernatant.

12 Wash the pellet/slide in 100% ethanol, vortex, and centrifuge at maximum speed at room temperature. Discard the supernatant. A second ethanol wash may be necessary for some deparaffinization techniques and specimens of low quality (See Section 8.3.5 in the Attached PDF for Expert Recommendations).

13 Ensure the pellet is completely dry. Ethanol may be removed from the pellet by air-drying at Room temperature or with the use of a speed vacuum.

Proteinase K digestion

14 Add lysis buffer containing proteinase K at the volume and concentration specified by the extraction kit and incubate at 55-56°C for 2-48 h for DNA or for 15 min - overnight for RNA. The optimal duration of digestion is dependent upon multiple factors, including buffer composition and enzyme concentration, and must be determined experimentally. Other digestion durations and lower temperatures may also be acceptable (In the Attached PDF, see Sections 7.6 (Literature Evidence) and 8.3.6 (Expert Recommendations) for alternatives).

15 Heat tubes to 90°C for 10 min-2 h for DNA extraction or to 80°C for 0-15 min for RNA extraction. Optimal durations of demodification will be dependent upon the extraction kit used (See Section 7.7 in the Attached PDF for Literature Evidence). This step may be unnecessary for some analyses, in which case it can be omitted. For alternate temperatures and durations see Sections 7.7 (Literature Evidence) and 8.3.7 (Expert Recommendations) in the attached PDF.

DNA and RNA extraction

16 Continue to DNA or RNA extraction using the method specified by the experimentally validated nucleic acid extraction kit (See Sections 7.8 and 7.9 in the Attached PDF for Literature Evidence). Acceptable methods for DNA extraction include silica, magnetic-bead, heating, resin-filter, ultrasonication or salting-out based methods, but organic extraction should be avoided (See Sections 7.8 and 8.3.8 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). Acceptable RNA extraction methods include silica, magnetic bead, organic, ultrasonication, or salting-out based methods, but generally resin filter and heat-based methods should be avoided (See Sections 7.9 and 8.3.8 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively).

Post-extraction treatment

17 Remove contaminating nucleic acids by RNase treatment of DNA or DNase treatment of RNA is advised but not required (See Sections 7.10 and 8.3.9 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively).

Nucleic acid quantification and quality assessment

18 Quantify DNA and RNA using a fluorometric or qPCR-based method. While spectrophotometry should be avoided for DNA quantification as it overestimates yield, it can provide valuable information on DNA purity and quality. Spectrophotometry may be acceptable for RNA quantification, although inconsistent and overestimated yields have

been observed with incomplete DNAse treatment (See Sections 7.11 and 8.3.10 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively).

19 Verify DNA integrity using fit-for-purpose quality metrics. Suggested DNA quality guidelines are organized by analytical platform in Table 1 in the attached PDF (See Sections 7.12 and 8.3.11 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively).

20 Verify RNA quality using the percentage of RNA fragments >200 nt (DV200) and/or a combination of DV200 and real-time qRT-PCR amplification of products from the 5′ and 3′ end of the transcript. RIN is not an informative method of RNA quality assessment for FFPE specimens and should be avoided. Suggested RNA quality guidelines are listed by analytical platform in Table 2 in the attached PDF (See Sections 7.13 and 8.3.12 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively).

Protocol references

References considered during the development of this NCI BEBP document are listed below (also See Section 9.2 in the Attached PDF) and include hyperlinks to the PubMed abstract and NCI Biospecimen Research Database curation where applicable. References are cited within the Summaries of Literature Evidence (See Section 7.0) in the Attached PDF.

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