# RECOMMENDATIONS FOR A STANDARD BIOSPECIMEN RESEARCH EXPERIMENTAL PROTOCOL BY THE ISBER WORKING GROUP ON BIOSPECIMEN SCIENCE

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Validation of clinically relevant biomarkers should take into account the potential impact of preanalytical variations on each specific biomarker. This validation process is the focus of Biospecimen Research. Because a comprehensive study of all of the possible combinations of each type of preanalytical factors would require huge numbers and volumes of samples, one single research group cannot perform all of the necessary testing. Establishment of a standard experimental design will thus allow scientific collaborations and comparisons of results, with each individual laboratory performing tests on parts of the variation factors and available options.

The benefits of biospecimen research based on standardized options include:

Harmonization of Biospecimen Research and comparability of results obtained by different Biospecimen Research scientists, since the same standard options will be examined.

Possibility of elaboration of standard nomenclature of sample collection / processing / storage procedures for biorepositories, with common codes corresponding to different levels of variation factors.

Identification and validation of new quality control biomarkers / tools for biorepositories to address actual biospecimen "Quality Control issues".

A standard experimental protocol for evaluating the impact of preanalytical variations is presented by the ISBER Biospecimen Science Working Group, for application in the following situations:

1. During the "preclinical exploratory" phase of a biomarker(s) screening study, in order to identify and exclude biomarkers which are susceptible to the preanalytical variations which have been or will be inflicted on the study samples.
2. During the "clinical assay validation" phase in order to confirm the robustness of a target biomarker (or surrogate biomarkers) and allow selection or optimal design of the sample collection and processing protocols involved with "retrospective longitudinal" or "prospective screening" phases, respectively.

This protocol contains standardized options to evaluate the most relevant and generally encountered preanalytical variation factors for different types of samples.

**Table 1**
shows the baseline processing of the "reference" **plasma** sample, highlighted in **rose**. For each type of preanalytical variation factor, the predefined levels of variability that can be studied by a Biospecimen Research scientist are indicated.

**Pre-centrifugation:**

- Type of tube: **EDTA**, Heparin, ACD, Citrate, Sodium Fluoride/ Sodium Oxalate, Micro Tube with gel
- Delay time 1 (Δt1): **<2h**, 2h, 4h, 8h, 24h, 48h, 72h
- Temperature: 20-25°C, **3-7°C**, 35-38°C

**Centrifugation:**

- Temperature: **20-22°C**, 3-7°C
- Number of centrifugations: 1, **2**

**Post-centrifugation:**

- Secondary container: **Polypropylene tube**, plastic straw, glass vial
- Delay time 2 (Δt2): **<1h**, 2h, 4h, 8h, 24h, 48h, 72h
- Pre-aliquoting temperature: 22-25°C, **3-7°C**, 35-38°C
- Storage temperature: -20°C, **-80°C**, vapor-phase liquid nitrogen
- Number of freeze-thaws: **1**, 2, 4, 10, 20, 30
- Storage duration (years): **1.5**, 3, 6, 9, 12, n

**Table 2**
shows the baseline processing of the "reference" **urine** sample, highlighted in **yellow**. For each type of preanalytical variation factor, the predefined levels of variability that can be studied by a Biospecimen Research scientist are indicated.

**Pre-centrifugation:**

- Type of collection: First void, Later void, 12h, **24h**
- Type of container: With protease inhibitors, **Without protease inhibitors**
- Delay time 1 (Δt1): **<2h**, 2h, 4h, 8h, 24h, 48h
- Temperature: 20-25°C, **3-7°C**, 35-38°C, -80°C

**Centrifugation:**

- Temperature **20-22°C**, 3-7°C
- g, Delay time 2 (Δt2) : 1000g 10min, **2000g 10min**, 4000g 10min
- Number of centrifugations: 1, **2**

**Post-centrifugation:**

- Secondary container: **Polypropylene microtube**, Plastic straw, Polypropylene 5ml tube
- Delay time 3 (Δt3): **<2h**, 2h, 4h, 8h, 24h, 48h, 72h
- Pre-aliquoting temperature: 22-25°C, **3-7°C**, 35-38°C
- Storage temperature: -20°C, **-80°C**, vapor-phase liquid nitrogen
- Number of freeze-thaws: **1**, 2, 4, 10, 20, 30
- Storage duration (years): **1**, 3, 6, 9, 12, n

**Table 3**
shows the baseline processing of the "reference" **solid tissue** sample, highlighted in **blue**. For each type of preanalytical variation factor, the predefined levels of variability that can be studied by a Biospecimen Research scientist are indicated.

**Pre-fixation:**

- Warm ischemia time: 0, **≤10min**, 15-20min, 25-30min, 50-60min
- Cold ischemia time (delay time 1 (Δt1) before fixation): 2min, 15min, **30min**, 60min, 120min

**Fixation:**

- Cryopreservation: **Snap Freezinng**, OCT embedding
- RNA-Later
- No cryopreservation: **Buffered formalin**, methanol/chloroform/acetone, aldehyde-based, non-aldehyde/non-acetic acid-based, non-aldehyde/acetic acid-based

**Post-fixation:**

- Delay time 2 (Δt2) from OCT embedding to freezing: 0, 15min, **4-8h**, 24h, 48h, 72h
- Storage temperature (for cryopreservation): **-80°C**, vapor-phase liquid nitrogen
- Storage duration (years): **1**, 3, 6, 9, 12, n