Fixation Parameters Investigated

The influence of biospecimen handling and fixation parameters on molecular, histological, and morphological results was investigated using existing BRD content and infrastructure. The following search criteria was constant for all data-mining exercises:

- Biospecimen type: Cell or Tissue
- Preservative type: Formalin
- Paper type: Nonreview

The experimental factor, which was selected in conjunction with the above search criteria, was dependent on the variable of interest. Experimental factors are organized by classification, corresponding to stages in the lifecycle of a biospecimen.

**RATIONAL**

The Biospecimen Research Database (BRD), under development by the National Cancer Institute’s Office of Biorepositories and Biospecimen Research, is a searchable web-based literature database populated with peer-reviewed published research and review articles relevant to human biospecimen science. In order to assess the utility of the BRD as a vehicle for meta-analysis, representative variables relevant to formalin fixation and paraffin embedding of biospecimens were investigated.

The goals of the data mining exercise were to:

1. Obtain a functional assessment of the BRD in terms of existing infrastructure and curation platforms;
2. Appraise the current BRD paper population and identify specific and underrepresented variables;
3. Summarize consensus in reported biospecimen fixation and handling when applicable;
4. Ascertain whether the present literature base is accurately capturing the state of the science.

**METHODS**

**BRD Experimental Factor Classifications**

- Preacquisition
- Acquisition
- Biospecimen Aliquots & Components
- Biospecimen Preservation
- Storage
- Analyte Extraction & Purification
- Platform-specific Methodology

**RESULTS**

**Consensus**

Conclusions supported by 3 or more papers populating the BRD.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>BRD Papers</th>
<th>Consensus</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>3</td>
<td>PCR results of small biospecimens (2-10 mm diameter) were favorable to larger biospecimens.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>PCR analysis was optimal in biospecimens fixed for 2-48 h, with adverse effects observed after fixation for &gt;72 h.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Evidence of RNA degradation was observed in specimens fixed for 1-72 h.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Protein immunoreactivity was stable in biospecimens fixed for 6-8 d. The antigens investigated included: p27Kip1, ER, PR, AR, c-erb-B2, HER-2/neu, EGF, MMR-1, VEGF, p53, PCNA, Ki-67.</td>
</tr>
<tr>
<td>RNA</td>
<td>4</td>
<td>PCR success and efficiency (80-90% of bp fragments) were not impacted by paraffin block archival at room temperature for 1 wk.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>RNA degradation was more extensive in blocks stored for 3.5-17 y compared to those stored for 1 y or less.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>RT-PCR success rate decreased by 6-20% after 1-10 y, 30-50% after 10-30 y, and 60% after 40 y of paraffin block archival compared to fresh blocks, although amplicon length also influenced RT-PCR success. The analytes investigated included hepatitis C, beta-actin, C-8-CR.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Real-time qRT-PCR analysis of paraffin blocks archived for 1-8 y was successful; while analysis was impaired for blocks stored for 11 y or longer. The analytes investigated included UDH, RPL12, beta-actin, RPL13A, RPLD, GUS, TBP, TFRC.</td>
</tr>
<tr>
<td>Protein</td>
<td>3</td>
<td>Immunostaining was altered in slides stored for 3.5-17 y at room temperature compared to freshly cut sections. Alterations in immunostaining intensity and duration threshold were antigen-specific. The antigens investigated included: ER, PR, HER-2, Chromogranin, CD3, Vimentin, EGFR.</td>
</tr>
</tbody>
</table>

**Evaluation of BRD Search Terms**

<table>
<thead>
<tr>
<th>Fixation Parameter</th>
<th>Corresponding Experimental Factor</th>
<th>Studies Returned (k)</th>
<th>Relevant Studies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature delay prior to fixation</td>
<td>Time at room temperature</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td>Size of the fixed specimen</td>
<td>Aliquot size/volume Biospecimen collection method</td>
<td>11</td>
<td>45%</td>
</tr>
<tr>
<td>Temperature of fixation</td>
<td>Temperature of fixation</td>
<td>3</td>
<td>100%</td>
</tr>
<tr>
<td>Method of fixation delivery</td>
<td>Method of fixation delivery</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Time in fixative</td>
<td>Time in fixative</td>
<td>34</td>
<td>85%</td>
</tr>
<tr>
<td>Embedding Reagents</td>
<td>Embedding media Embedding reagents</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>Duration of specimen archival</td>
<td>Storage duration</td>
<td>26</td>
<td>73%</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

**Variables Excluded from Meta-analysis**

**Fixation Parameters**

- Under-represented in the BRD
  - Room temperature delay pre-fixation
  - Temperature of fixation
  - Embedding reagents
  - Method of fixation delivery
  - Post fixation of frozen specimen sections

**Fixation Parameters Absent from the BRD**

- Refrigerated delay prior to fixation
- Fixative pH
- Fixative source
- Fixative age
- Biospecimen size / fixation volume ratio

**Relationships Among Preanalytical and Analytical Variables**

Although definitive conclusions were not possible for many of the experimental variables investigated, several relationships among variables became evident. Fixation temperature directly affected the duration of fixation required, as did accelerated fixation (injection, immersion, ultrasound acceleration). Further, RT-PCR success was influenced by the size of the mRNA fragment of interest and its corresponding amplicon, as RNA fragmentation was observed with prolonged fixation or storage.

**Fixation temperature**

- **Fixation duration**

**Accelerated fixative delivery**

- **Fixation duration**

**Conclusions and Suggested Improvements**

The BRD was successfully used as a data-mining tool. While conclusions were limited, principally restricted by paper abundance, the data-mining exercise successfully identified:

1. Consensus for five subjects of interest
2. Specific biospecimen handling and fixation variables for future literature searches; and,
3. Potential relationships among biospecimen handling and analytical variables.

**Generation of publishing guidelines**
- Biospecimens handling methods
- Analytical details

**Specific literature searches**
- Capturing inter-dependent variables

**Expansion of search capabilities**
- AND/OR
- Free-text
- Multiple Experimental Factors