Bone Marrow Sample Preparation and Processing: An IQMH Survey on Laboratory Practices

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INTRODUCTION
Examination of the bone marrow, including aspirate smears, clot sections, imprint morphology and trephine biopsy are essential for correct diagnosis and management of haematological disorders.1 The Institute for Quality Management in Healthcare (IQMH) offers a bone marrow proficiency testing (PT) survey where glass slides of bone marrow aspirate or biopsy, peripheral blood smears and relevant clinical data are provided. A questionnaire was developed by the IQMH-Hematology Scientific Committee to gather information regarding practices for preparation and processing of bone marrow.

METHODS
A questionnaire consisting of 17 questions was distributed to 59 participants from July to September 2015 with the rotation slide survey to determine laboratory practices for bone marrow sample preparation and staining.

RESULTS
The calculations of per cent of laboratories is based on the number of responding participants for each question. If a participant did not reply to a question they were not included in the statistics. When a laboratory had multiple responses to a question the per cent was based on the total number of participants responding and not the total number of responses. Fifty-one (87%) participants submitted results. Presented here is a summary of the responses:

Types of Preparations
All (100%) respondents prepare bone marrow aspirate sections with the majority (92%) also performing a biopsy tissue section. Sixty-seven per cent prepare particle crush smears, 65% clot sections and 55% trephine biopsies. None of the participating laboratories indicated that they perform a buffy coat smear (Figure 1).

Faution
The majority (82%) of laboratories that perform bone marrow tissue sections place these in fixative immediately at the bedside. The most commonly reported fixative used for clot sections was buffered formalin (61%). Other fixatives reported by respondents were Acetic acid-zinc formalin (AZF) (2%), Bouin’s (2%), 80% (2%), Isopropanol barium chloride formalin (IBF) (1%).

For trephine biopsies, buffered formalin was also the most commonly reported fixative (61%). As well, 4% reported using Acetic acid-zinc-formaldehyd (Af) (2%), 80% (2%), Isopropanol barium chloride formalin (IBF) (1%).

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There was great variability in the fixation times for clot sections and trephine biopsies, with the greatest number reporting a minimum of 24 hours (35%) and 4% respectively. The variation in fixation times is summarized in Figure 2.

DISCUSSION AND CONCLUSIONS
All (100%) laboratories prepare bone marrow aspirate smears; a minority (92%) also collect a trephine biopsy. Practice regarding other types of bone marrow preparations was variable and included: particle crush smears (65%), clot sections (61%) and biopsy biopsies (55%).

The majority (82%) of laboratories place bone marrow tissues in fixative at the bedside. The International Council for Standardization in Haematology (ICSH) recommends touch biopsies be made prior to placing the trephine biopsy in fixative, the container labelled to identify the sample and time of collection to calculate the time of fixation.2 In the absence of a laboratory technique at the bedside, laboratories need to ensure this process is being followed for cases requiring touch biopsies (e.g. dry tap, poor aspirate).

There is a shift from buffered formalin, given the toxic effect of mercury. Alternatives to BS fixative may also be considered, without sacrificing histological quality.3

Fixation times for both bone marrow clot and biopsy sections vary greatly (Figure 2), potentially impacting morphologic detail and immunoreactivity. The fixation time recommended in ICSH guidelines is 8–12 hours in buffered formalin, but may vary from 2–7 hours depending on the fixative used.4 Data analysis was limited in that fixation time was not correlated with fixation method.

There was no consistency regarding the decalcification agents used or the length of time for decalcification as there are many commercial decalcification agents available (Figures 3 and 4). Heating and stirring may be considered by laboratories to improve the decalcification process. Methods that have a shorter length of time for decalcification are not recommended.5,6

Romanowsky staining of bone marrow aspirate smears was most commonly performed using Wright-Giemsa or modified Wright-Giemsa stains (Figure 5).

All (100%) of one laboratory routinely stains bone marrow biopsies with H&E as recommended by the ICSH.7 The laboratory not performing H&E uses HPS, a variation of the H&E stain that provides more colour contrast. Slightly more than half of the laboratories routinely perform reticulin stains, the remainder likely at the pathologist’s discretion.

Immunohistochemical staining of trephine biopsy sections is a valuable ancillary test for certain diagnostic indications. However, staining results are largely dependent on pre-analytical processes including fixation and decalcification.8

Variability has also been described regarding positivity controls for immunohistochemistry.9 Our survey demonstrated that only 18% (29%) laboratories reported using bone marrow biopsy controls that have undergone a similar decalcification process as patient samples. Those that do not use these types of controls have alternate sources of control material as listed in Table 1. There are several groups that have published guidelines for acceptable positive and negative controls for immunohistochemistry testing which laboratories are encouraged to review.

The results from this questionnaire demonstrate wide variability and lack of standardization amongst laboratories in preparation and processing of bone marrow specimens. This may lead to inconsistencies in the interpretation of marrow findings, and the classification of disease. These findings present an opportunity to review published guidelines to standardize current laboratory practice.

A follow-up questionnaire would be helpful in determining if laboratories have made any changes following the published results of the questionnaire.

Table 1: Controls Used for Bone Marrow Immunohistochemistry (n=28)

<table>
<thead>
<tr>
<th>Type of Control</th>
<th>No. of Respondents</th>
<th>% of Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen tissue controls treated the same way</td>
<td>2</td>
<td>7%</td>
</tr>
<tr>
<td>Frozen tissue controls treated the same way, except that has been decalcified</td>
<td>3</td>
<td>11%</td>
</tr>
<tr>
<td>Frozen tissue controls treated the same way, except that has been decalcified, and immunohistochemically stained</td>
<td>2</td>
<td>7%</td>
</tr>
<tr>
<td>Non-dye treated tissue controls and immunohistochemically stained</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Non-dye treated tissue controls</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Negative Controls</td>
<td></td>
<td></td>
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<tr>
<td>Formalin fixed formalin</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Formalin</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>4%</td>
</tr>
</tbody>
</table>