Importance of pH of Fixatives Used for Fixation of Histopathology Specimens – An Un-Recognized Issue

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Abstract
Objective: Adequate fixation of the surgical specimens is very important to ensure optimal histopathology, immunohistochemistry and genetic tests results. To achieve these results, it is very important to use good fixatives with pH maintained at 7. We measured pH values of the histopathology specimens received from different parts of country to determine whether fixative being used is appropriate or not.

Material and Methods: It was a descriptive cross sectional study carried out at Armed Forces Institute of Pathology, Rawalpindi, Independent Medical College and Mian Muhammad trust hospital Faisalabad on one thousand consecutive samples received for histopathology reporting. To assess the pH, fixative of the sample was taken out in the kidney tray and pH meter was dipped in it and value of pH noted.

Results: The specimen pH was recorded. The mean value came out to 6.1 ranging from 1.3 - 8.8. The maximum number of readings fell between 6.1 -7.0 (n 530 cases). The second highest group was 5.1- 6.0 which comprised of 269 cases. About 366 specimens have pH less than 6 and 104 specimens above 7, whereas for ideal fixation, the ideal recommended pH is 7.

Conclusion: Unfortunately, most of the specimens received did not have ideal pH. It is very important to educate medical and paramedical staff, who deal with the surgical specimens, about the importance of pH and also how to ensure proper fixation of the specimens. This will help in achieving ideal histopathology results.

Key Words: Histopathology, Fixatives, pH

Introduction
In histopathology, tissues are fixed before they are examined microscopically. Fixation is the foundation for the subsequent stages in the preparation of sections through to the making of diagnosis.

sensitive techniques are being introduced in histopathology which have very strong impact on diagnosis. So, a good fixation is not only necessary for light microscopic examination but also for more advanced and sensitive techniques like special stains, immunohistochemistry and genetic studies like polymerase chain reaction and in-situ hybridization.

There are many fixatives commercially available and each has its own advantages and disadvantages. An ideal fixative should have maximum advantages and least number of disadvantages. One such fixative is formaldehyde. It is widely used in our operation theatres and histopathology departments for transport and fixation of surgical specimens. It is suitable for most of the tests carried out at histopathology department like light microscopy, special stains, immunohistochemistry and genetic studies. There are many important factors involved in fixation like hydrogen ion concentration (pH), depth of penetration, osmolarity, concentration, duration and temperature. Among these factors, the one which is very important and easily measurable is hydrogen ion concentration (pH). pH values of different fixatives vary. In general pH is usually adjusted to the physiological range by use of a suitable buffer. Satisfactory fixation occurs between pH 7 and 8. Outside this range there are changes which are detrimental to the ultra-structure of tissue. After being surgically removed, pH within the tissue cells is known to decrease, due to anoxia. As many normal tertiary and quaternary structures of most proteins are stable over a limited pH range about neutrality i.e. pH of 7, many buffer systems are available to decrease this acidic environment and to preserve cellular proteins. The most commonly used in our laboratories is phosphate buffer.

Although there are many issues which need to be addressed regarding specimen handling but the one which is the most basic, easily controllable and of utmost importance is adequate hydrogen ion concentration of the fixative. From our experience we have noted that many times morphological details are compromised due to use of poor fixative in operation theatre. Whenever the pH of these fixatives is retrospectively checked it is not up to the mark. Most of the time it is towards the acidic range. The rationale of this study is to determine the frequency of pH values of
fixatives used to fix surgical specimens received at AFIP Rawalpindi, Independent Medical College and Mian Muhammad Trust Hospital Faisalabad from different medical centers of our country.

Material and Methods
It was a descriptive cross sectional study carried out at Armed Forces Institute of Pathology, Rawalpindi, Independence Medical College and Mian Muhammad Trust Hospital Faisalabad on one thousand consecutive samples received for histopathology reporting. Request forms of these samples were retrieved and department identification numbers and name of referring hospitals were noted. Hydrogen ion concentrations (pH) of fixatives used for fixation of surgical specimens were measured with the help of an instrument called pH meter. To assess the pH, fixative of the sample was taken out in the kidney tray and pH meter was dipped in it and value of pH noted. Data was entered in SPSS version 17. Mean, median and mode were calculated for the pH values.

Results
The specimen pH was recorded. The minimum pH was 1.3 and maximum pH was 8.8. The mean value came out to be 6.1. The mean, median and mode were 6.18, 6.3 and 6.4 respectively. The maximum number of readings fell between 6.1 -7.0 (n = 530 cases). The second highest group was 5.1-6.0 which comprised of 269 cases. About 366 specimens have pH less than 6 and 104 specimens above 7, whereas for ideal fixation, the ideal recommended pH is 7 (Table 1).

<table>
<thead>
<tr>
<th>pH groups</th>
<th>Number of specimens</th>
</tr>
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<tbody>
<tr>
<td>1.0-2.0</td>
<td>1</td>
</tr>
<tr>
<td>2.1-3.0</td>
<td>4</td>
</tr>
<tr>
<td>3.0-4.0</td>
<td>8</td>
</tr>
<tr>
<td>4.1-5.0</td>
<td>84</td>
</tr>
<tr>
<td>5.1-6.0</td>
<td>269</td>
</tr>
<tr>
<td>6.1-7.0</td>
<td>530</td>
</tr>
<tr>
<td>7.1-8.0</td>
<td>103</td>
</tr>
<tr>
<td>8.1-9.0</td>
<td>01</td>
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</tbody>
</table>

Discussion
Fixation is a chemical process by which biological tissues are preserved from decay and prevented from autolysis and putrefaction. Fixation prevents biochemical reactions, and increase the mechanical strength or stability of the treated tissues. The proper pH of the fixative reagent is required for proper fixation. Histopathology specimens should be fixed in 10% Neutral Buffered Formalin (NBF). Other factors affecting fixation are osmolarity, size of specimen, volume of fixative, temperature, duration of fixation and time of removal of the specimen to start of fixation. The pH should be kept at or near 7 to get maximum good results. A good fixation helps in preparing good quality slides, carrying out proper immunohistochemical stains and cytogenetic studies which can give us best results. This is especially important in morphologically difficult lesions in which immunohistochemical and genetics studies have to be done. This study was carried out to find out the pH of fixative used to fix histopathological specimens which were received from different parts of country. The pH is an important factor to determine fixative ability to fix the specimens properly.5,6,7 Unfortunately the lack of education regarding fixation of specimens in doctors results in poor fixation of specimens. This badly effects proper processing of the tissue and also subsequent ancillary techniques. It is evident from the results of this study that 366 specimens had pH less than 6 and 104 specimens above 7, whereas for ideal fixation, the ideal recommended pH is 7.

This study enlightens the importance of the fixative pH. All doctors and paramedical staff related to surgical specimens need to know the importance of proper pH. It can be easily determined from the results of this study that most submitted specimens have pH either below or above the recommended pH. This means we do not know how to make proper fixative. This can have serious implications on the biopsy results. This should be made a permanent part of curriculum of medical students and staff to educate them how to process a surgical specimen once surgery is done. We cannot deny the fact that once histopathology specimens are poorly fixed, we cannot revert back the changes which had already been occurred in specimen due to poor or bad fixative pH. This can be controlled at first sight by allowing surgical specimens to fix in proper pH fixative. This will further affect the interpretation of immunohistochemistry, cytogenetic and PCR studies.8-12

Conclusion
The poor fixation due to inappropriate pH can affect the processing, staining including special staining and immunohistochemistry, cytogenetic studies and also their interpretation which can lead to wrong diagnosis and treatment of patient. In order to overcome all these problems, medical and paramedical staff involved in dealing with surgical specimens should be properly educated regarding the handling and fixation of surgical pathology specimens.13,14

Conflict of interest
This study has no conflict of interest declared by any author.

References