Original Article

Intraoperative Surgical Pathology Consultation (Frozen Section) in Resource-Limited Setting

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Abstract

Background: Intraoperative consultation aims at guiding decision during surgeries. Frozen section (FS) technique is a valuable tool used to rapidly prepare slides from tissues for microscopic interpretation. Over the past 100 years, FS diagnosis of surgically resected tumors and tissue has become a well-established practice in developed countries. On the other hand, developing countries only relatively recently got introduced to this technique. However, it remains unpopular due to a number of factors. This study strives to show that an alternative means in terms of reagents can easily be sought when the challenge of resource limitation is encountered. To the best of our knowledge, such a study has not been conducted in Nigeria. **Methods:** This was a prospective study over 12 weeks in which a comparison was made from surgical biopsy specimens received for intraoperative consultation. Using commercially available cryocompound, optimal cutting temperature (OCT) and readily available, less expensive, easy to handle alternative to embedding media: water and office glue. Staining was by conventional hematoxylin and eosin. Both groups were then viewed with the light microscope and findings were reported and compared. **Results:** Fifty-seven cases were studied, of which 24 were positive for tumor. Thirty cases were negative for tumor, and three cases had features consistent with metastatic tumor. Freezing time was 1–2 min for OCT and the glue alternative, while a third group was in frozen water for 2–3 min. The slides' quality was satisfactory for tissues processed in the OCT and glue; however, those processed in water had the worst outcome in terms of artifacts. Freeze and staining artifact were minimal in tissues processed using OCT and glue, whereas tissues processed using water was worst. **Conclusion:** Office glue is a readily obtainable alternative medium for FS and can provide satisfactorily comparable results to the conventional OCT in a resource-limited setting and is in the opinion of the authors, a good alternative.

Keywords: Frozen section, intraoperative, limited resource, office glue, optimal cutting temperature, water

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Introduction

Intraoperative consultation is often the cornerstone for optimizing surgical management of the disease. Information obtained from the procedure can dramatically alter the course of surgery. Frozen section (FS) technique is a valuable tool used to rapidly prepare slides from tissue for microscopic interpretation.[1] Numerous research applications rely on the FS technique to prepare microscopic slides utilizing a host of sophisticated morphologic, immunohistochemical, and molecular methods. Preparation of FS slides is a complex technical process requiring the development of refined technical skills, as well as an understanding of the histology, microanatomy, and pathology of the tissues, being examined.[2] Whether used for intraoperative consultation or in research, the results will hinge on the ability to achieve a high-quality preparation.[3] The procedure relies heavily on the use of the cryocompound, optimal cutting temperature (OCT). A search on its composition (especially as stated on the product information leaflet)[4] showed that it is made of:

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1. Polyvinyl alcohol 10.24%
2. Polyethylene glycol 4.26%
3. Nonreactive ingredients 85.5%.

A major handicap in its use in resource-poor settings its sustained supply, especially due to relatively high cost. Thus, there is a need for an alternative which must have composition similar to OCT as much as possible. The office glue, which has varying brands and different compositions, which are often proprietary was considered for use. Our favored brand (because it was chemically closest to OCT) had the following composition:

1. Acrylic polymer 40%
2. Water 40%
3. Sodium stearate 10%
4. Polyethylene glycol 3%
5. Polyoxyethylene 2%
6. 2-amino isobutanol
7. Sodium hydroxide 0.3%.

Therefore, it is a potential substitute to OCT.

On the other hand, water (which was used as negative control) has the following properties:

1. Molar mass of 18.01528 (33) g/mol
2. Density 999.9720 kg/m^3 (liquid) and 917 kg/m^3 (solid)
3. Boiling point 100°C (212°F; 373 K)
4. Melting point 0.00°C (32.00°F; 273.15 K).

A cryostat is a cooled chamber or cabinet that houses an instrument to section frozen samples; it contains a rotary microtome and knife (or blade) holder, and a means to freeze samples.[3] The normal working chamber temperature varies from 0°C to −35°C, but the recommended setting is −27°C 2.[5]

Despite many recent advances in ancillary techniques, intraoperative consultation remains one of the most diagnostically and technically challenging areas of surgical pathology. Therefore, pathologists must render a diagnosis quickly, despite the pitfalls and artifacts associated with FS preparation.[3,6-8]

The aim of this study is to provide the easy to handle, readily available, and affordable alternative consumables in limited resource settings. To the best of our knowledge, no such study has been conducted in Nigeria.

Methods

This was a prospective study, in which a comparison was made from surgical biopsy specimens received for intraoperative consultation that is fresh unfixed tissues were subjected to FS using commercially available cryocompound, OCT and readily available, less expensive, easy to handle alternative to embedding media, represented by clean water and office glue. Other materials used were 10 ml syringe, dispensing slide and nontoothed forceps, and the Cryostat machine. Conventional hematoxylin and eosin (H and E) stain was done, and surgical slides produced using the different consumables were reported and compared. The freezing time, quality of sections in terms of artifacts, staining, and diagnosis were compared for the three materials used. Fifty-seven cases were received over 12 weeks. The cases were mainly received from surgical subspecialties, which included general surgery, urologic surgery, ear, nose and throat surgeries, gynecological, and neurosurgeries.

Slide A [OCT, Figure 1a] and Slide B [office glue, Figure 1b] tissue was cut at 2 cm × 2 cm × 1 cm, it was transferred to the cryostat machine and face-down embedding was done and sectioning was at (why listing figures in methodology?) −27°C and subsequently stained using H and E methods, Figure 2a and b. The freezing time was within 1–2 min.

Slide C [Figure 1c] (clean water) tissue was cut at 2 cm × 2 cm × 1 cm, it was transfer to the cryostat machine, face-up embedding was done on a chuck with cold water (that was chilled in a refrigerator) dispense gently on the tissue in the cryostat machine until it frozen and sectioning was at −27°C and subsequently stained using H and E methods [Figure 2c]. The freezing time was within 1–2 min. This served as the control group.

Slide A (OCT), Slide B (Office Glue), and Slide C (Clean water), it was single blinded slides review and three pathologist independently reviewed the slides and give the report of the legions which was compared later.

Bony tissues, formalin-fixed tissue, and tissues in any form of fixative were excluded from the study.

Results

FS where commonly freezing time for OCT and glue was 1–2 min and clean water was 2–3 min, the quality of the sections was good. Freeze and staining artifacts were minimal on the tissue processed using OCT and glue, whereas tissue processed using water was worst. Of 57 cases studied, 24 cases were positive for tumor, 30 cases were negative for tumor (benign), and three cases had features consistent with metastatic carcinoma [Table 1].

Discussion

In the present study, we look at the alternative to OCT, which is not readily available and where available is costly. The alternative of choice was office glue, which has fairly similar

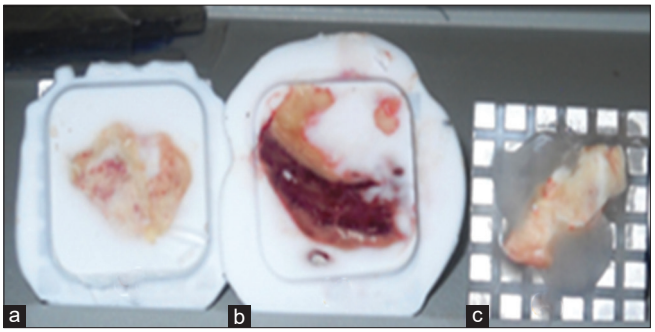

Figure 1: Frozen tissue blocks for: (a) optimal cutting temperature, (b) office glue, and (c) water

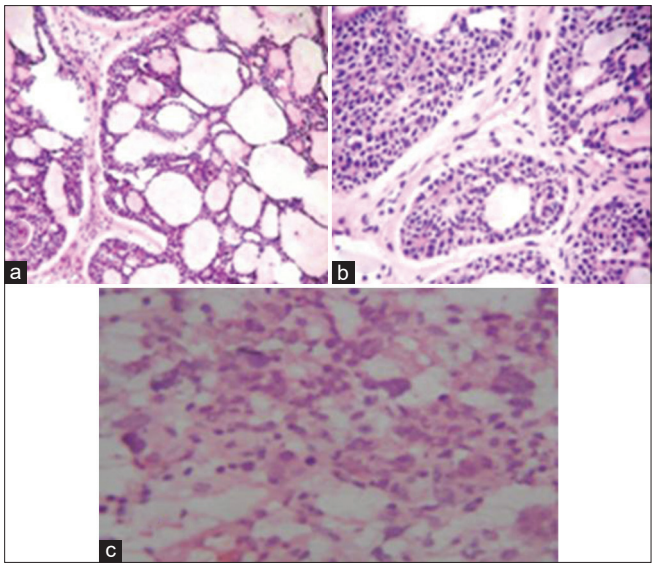

Figure 2: Frozen section H and E stains (×100) for: (a) optimal cutting temperature, (b) office glue, and (c) water

chemical composition to OCT but with different relative percentage. The composition of OCT as previously mentioned comprise polyethylene glycol 4.26% and the nonreactive ingredients 85.5%,[9] whereas for office glue, the polyethylene glycol is 3% and water 40%.[4] This might explain the similarity in freezing time between these two fluids, which is the optimal freezing time for the FS.[2,7,8] We noticed that the freezing artifacts were minimal for tissues processed using OCT and glue; this is probably due to the presence of polyethylene glycol, which offers some protection from freezing artifact.[3] It is known that saturating the tissue with cryoprotectants such as sucrose, glycerin, or polyethylene glycol offers some protection from freezing artifact.[1,3] The freezing time for clean water was 2–3 min, but this creates freezing artifact. When liquid water freezes and turns solid, it may form either hexagonal or cubic crystals, or turn to vitreous (amorphous) ice. Freezing and forming crystals expand the volume of the liquid water. Water is the only substance known to have this property of expansion when freezing. If crystals form and expand, they stretch or puncture the cell membranes. Later, after sectioning and thawing the tissue, cell contents leak out into space around the cells or over the section surface. Thus, staining for the cytosol contents may cause weaker staining in the cell and stronger background staining. If large crystals form, cells are crushed and displaced resulting in "swiss cheese artifact" (from creating multiple tiny holes in the section).[1,3,10,11] These phenomena are also possible explanations on the freezing (bubbles, compression, nuclear holes, and chromatin changes) and staining artifacts encountered in the present research. However, the freezing and staining artifacts can be reduced to the barest by understanding and manipulating the physical properties of freezing tissue. This will depend on the rate of achieving the freezing temperature.[12-14] If the rate of freezing is rapid, the water will solidify without crystal formation, as vitreous ice without crystals which does not expand on freezing. This is the basis of the often stated need to freeze biological tissue very fast to achieve the solid state as vitreous ice.[3,12-15]

Table 1: Biopsy site and frozen section diagnosis

	Benign	Malignant	Metastatic	Total
Breast	0	1	0	1
Ovary	4	2	0	6
Brain	5	3	2	10
Head and Neck	16	13	0	29
Colon	1	0	0	1
Hand	0	1	0	1
Prostate	1	3	0	4
Bladder	0	1	0	1
Testis	2	0	0	2
Chest wall	1	0	1	2
Total	30	24	3	57

In this study, request for FSs was most frequent during surgeries involving as follows: head and neck, nervous system, and female genital and urinary tracts. Of the 57 cases, 30 were negative for tumor, 24 were malignant, and 3 were metastatic carcinomas. Freezing artifact was more encountered in the nervous system followed by the head and neck, probably because they have high-water content.[1] However, rapidly freezing the tissue will reduce freeze artifact by the formation of vitreous ice crystal, which results in less artifacts microscopically as we have done in this study.

Despite inherent difficulties in processing, sectioning, staining of FSs, and microscopic evaluation using the alternative medium of office glue, it provides a fairly reliable and cheaper option.

However, the need for further studies is hereby emphasized, especially in terms of concordance and discordance rates in the use of this alternative medium.

Conclusion

Office glue as a cheaper, readily obtainable, alternative medium for FS can provide satisfactorily comparable results to the conventional OCT in a resource-limited setting (as obtains in most health-care centers in Nigeria); the challenge of cost and availability necessitates exploring alternatives to commercially available OCT. Office glue sharing similar composition with OCT is, in the opinion of the authors, a good alternative.

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Conflicts of interest

There are no conflicts of interest.

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