Original Article

Role of dry ice in decoverslipping of microscopic slides: A new insight

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Abstract

Background: The process of decoverslipping is often required in a laboratory to review or examine an older slide which tends to fade over time, making it almost impossible to use it for research or study purposes. The sections then need to be re-stained which can only be done after removing the coverslip. The traditional method of decoverslipping using xylene is a time-consuming process. Various methods have been used in the past; however, none were found to be completely effective. Dry ice, the solid form of carbon dioxide, is an easily available, cheap cooling agent with a low freezing temperature (−78.5°C) which was evaluated for its efficacy in decoverslipping process, as an alternative to xylene.

Materials and Method: 64 faded haematoxylin and eosin (H&E)–stained histopathology slides were randomly selected and segregated, according to duration of year, into eight major groups. Each group was further divided into four subgroups according to the time that the slides were subjected for decoverslipping. The slides were placed on dry ice and the time was set. Once the coverslip was removed, the slides were placed in xylene to remove any residual mountant. The tissue sections were evaluated for physical disfigurement followed by re-staining with H&E to check for any change in tissue morphology.

Result: The mean time taken for removal of coverslip using dry ice was 35 seconds.

Conclusion: This technique is easy, fast, and effective, with no tissue loss or compromise in staining quality, thereby preventing xylene toxicity and its effect on the environment.

Keywords: Decoverslipping, dry ice, histopathology, laboratory techniques, microscopic slides, xylene

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INTRODUCTION

The use of microscopic (or histology/histopathology) slides in learning the microanatomy, patient diagnosis, and even years later for research, teaching purposes, or at times for patient review, is undeniable. The older slides stored in the anatomy/pathology department archives are often required to study various rare and unique cases; the classical case slides may be used for teaching purposes and they may be required for research projects, immunohistochemistry (IHC) studies, dissertation projects for postgraduate students, special staining, or molecular studies.[1] It is well known that after staining of tissue

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sections on the microscopic slides, they are covered with glass cover slips (coverslipping) using a mounting media, usually DPX (Dibutylphthalate Polystyrene Xylene), for protection of the stained tissue section. In many instances, the older slides tend to fade owing to age of the slides or long contact of the section with the mounting media, making it almost impossible to use it for research or study purposes. Therefore, the archival slides are often subjected to the process of de-staining and re-staining in order to view the sections, in cases when the original paraffin block containing the tissue is no longer available for making newer sections.[2] This process of de-staining followed by re-staining is only possible once the coverslips are removed (decoverslipping). The traditional method of coverslip removal is a time-consuming process which entails immersion of the slides in xylene until the mountant dissolves and the coverslip falls off. Additionally, xylene is also known to have several health and environmental hazards. The use of xylene in a laboratory setup is widespread and affects various systems in the body including the nervous system, eyes, nose and throat, skin, lungs, blood, and gastrointestinal and the reproductive systems. Chronic exposure is also known to cause cancer. Besides occupational exposure, xylene may also be found in the environment (soil, air, surface, and ground water) via various sources, in turn jeopardizing the environment and human health.[3]

The time required for decoverslipping using xylene can range anywhere from one to five days depending on the age of the slides.[2] Various methods have been used for decoverslipping in the past, including ultrasonic vibration, liquid nitrogen, freezer method, petrol, and xylene at high temperature.[2,4] However, none of the above methods were found to be completely effective. Amongst all those methods, however, liquid nitrogen showed considerable result owing to its low freezing temperature (−196°C to −210°C) but had certain shortcomings. Therefore, it was essential to develop a method which was faster, cheap, reliable, and which preserved the quality of the section.

Dry ice, the solid form of carbon dioxide, also has a low freezing point (−78.5°C) and is cheap, easily available, and is primarily used as a cooling agent. It has wide usage in the medical, pharmaceutical, and food industry, laboratory refrigerant, storage and transportation of medical supplies, vaccines and perishable goods, in agrochemical industry and to create special fog effects, etc.[5] Considering the properties, low freezing temperature, and existing widespread applications of dry ice, its efficacy as a decoverslipping agent was thought to be evaluated, as to the best of our knowledge, dry ice has never been used for this purpose. Hence, this study was carried out to evaluate the efficacy of dry ice for decoverslipping of histopathology slides as an alternative to traditional xylene.

MATERIALS AND METHODS

Sixty-four random histopathology slides were selected from the archives of the department of oral pathology between the years 1995 and 2019 for the decoverslipping procedure. These archival slides showed faded H&E staining and were all mounted with DPX as the mounting media. The slides were then segregated according to duration into eight major groups as 1 to <3 years, 3 to <6 years, 6 to <9 years, 9 to <12 years, 12 to <15 years, 15 to <18 years, 18 to <21, 21 to ≤24 year-old slides. Thus, each group consisted of eight archival slides from the predetermined years. Each group was further divided into four subgroups according to the time that the slides were subjected to the decoverslipping technique: 60 s, 45 s, 30 s, and 15 s. The subgroups, consisting of two slides each, were evaluated against the aforementioned time intervals.

The experiment was conducted in a well-ventilated area in the histopathology lab. The slides were labelled with a diamond glass marking pencil with a unique identification code, following which they were placed on the dry ice (−78.5°C) inside a thermocol ice box with proper lid closure; the thermocol box placed on a solid surface. The coverslip side of the microscopic slide were kept facing the dry ice [Figure 1]. The slides were handled after wearing thick rubber gloves due to the extreme low temperature of dry ice. Two slides per group were assessed at one time according to the predetermined duration (i.e. 60 s, 45 s, 30 s, 15 s) with the help of a stopwatch. Following that, they were removed and placed

Figure 1: Placement of microscopic slides on dry ice

on a metal tray. A disposable microtome blade was then inserted between the microscopic slide and the coverslip to check whether the coverslip was easily popping off the slide [Figure 2]. Once the coverslip was removed, the slides were placed inside the Coplin jar containing xylene for 2 min to remove any residual mountant that may have been present on the slide [Figures 3 and 4]. The tissue sections were evaluated for physical loss or disfigurement after this procedure using a stereo microscope. The remaining dry ice was left to undergo sublimation in the thermocol box in a well-ventilated area in the histopathology lab. Care was taken not to dispose in air-tight containers, garbage/trash cans, or dump it in the sink drain or toilet.

Following this, the faded sections were re-stained with routine H&E staining to further check for any tissue loss or change in the morphology of the section [Figures 5 and 6]. Slides with a broken coverslip, broken slides, or slides with tissue loss were excluded from the study.

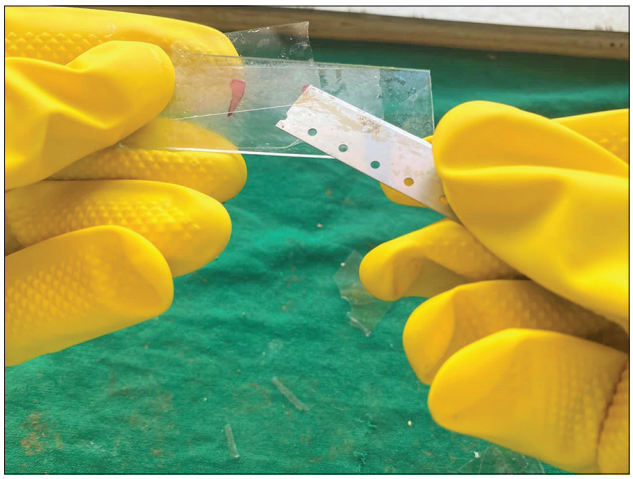

Figure 2: Decoverslipping using disposable microtome blade after 35 s

Figure 4: Residual mounting media removed from the slide

RESULTS

The mean time taken for removal of coverslip using dry ice was 35 s. The time taken to remove the coverslip of slides with a duration of 1 to <3 years, 3 to <6 years, 6 to <9 years, 9 to <12 years, and 12 to <15 years was 30 seconds; while for the slides with duration of 15 to <18 years, 18 to <21 years, and 21 to ≤24 years was 40 seconds [Table 1]. The time duration for removal of coverslip was further reduced to 15 s after it was successfully removed at 30 s. It was noticed that at 15 s, the coverslips became brittle and although they could be removed, they broke into multiple fragments with some tissue loss. Those slides were excluded from the study and it was deduced that 15 s would not be a favourable time duration for the decoverslipping method.

DISCUSSION

Histology/Histopathology slides are often required to be viewed after several years of initial staining and

Figure 3: Placement of decoverslipped slide inside a Coplin jar with xylene

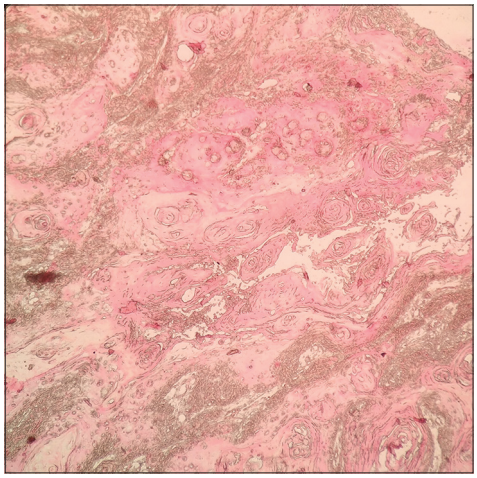

Figure 5: Old slide with faded H&E-stained section

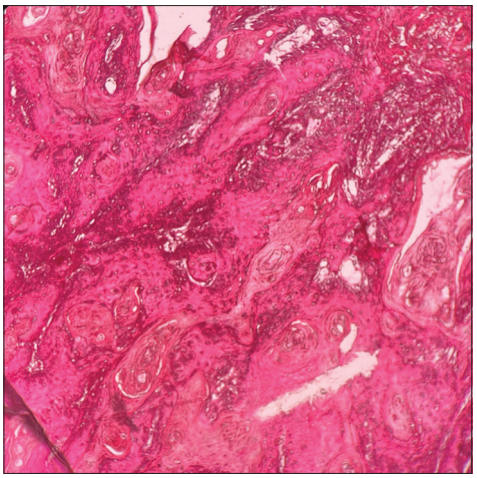

Figure 6: Faded H&E-stained section re-stained after decoverslipping with dry ice. No loss of tissue or deterioration in staining quality seen

Table 1: Data showing the number of slides in each group segregated according to their age and the time required for the decoverslipping process

Age of slides in years (1995-2019) in 8 groups	No. of slides per group	Time duration for decoverslipping in seconds
1-3	8	
3-6	8	
6-9	8	30
9-12	8	
12-15	8	
15-18	8	40
18-21	8	
21-24	8	

coverslipping. Often the older slides stained with the regular H&E stain or other special stains tend to fade over time, making observation of the tissue details extremely difficult. In such cases, when the original tissue block is unavailable for further section-making, the existing faded sections need to be re-stained, which can only be done after removal of the coverslip (decoverslipping) placed on those sections.

The various instances where decoverslipping may be required are extraction of DNA for molecular analysis for research purposes, in target therapy, neuronal cultures, or to study cases of lung adenocarcinoma, soft tissue tumours, and thyroid lesions.[4,6,7] Literature search reveals various methods of decoverslipping as an alternative to xylene for rapid removal of coverslip. However, to the best of our knowledge, dry ice has never been used for this purpose. Zhou *et al.* described a method for decoverslipping of cytology slides for DNA and RNA isolation from cells using liquid nitrogen.[1] However, in a study conducted by Cozma and Henwood, it was found that there was significant tissue loss by this method with cracking of the glass slides due to extremely low temperature (−196°C to −210°C) of liquid nitrogen. While some amount of tissue loss may not be detrimental in DNA and RNA isolation, it is not the same in case of histopathological evaluation where maintenance of intact tissue morphology is of prime importance.[2] Furthermore, liquid nitrogen is not easily available at the conventional laboratory setup and requires extremely careful transportation, storage, and handling techniques. Other processes published in previous reports include ultrasonic vibration technique described by Rainbow[8] and rubbing ice block over a scored coverslip to force water between the coverslip and slide. Both of these techniques show rapid and significant tissue loss after loosening of the coverslip.[2,4] Additionally, the ultrasonic vibration technique is extremely time-consuming and requires continuous monitoring by a skilled technician, making it impractical in a busy laboratory with limited manpower.[2] Another method proposed by Cozma and Henwood suggested the use of ultra-low freezer at a temperature of −80° C.[2] Although this method shows promising results when kept for 5 to 10 min, it is slightly impractical in the sense that most laboratories would not have access to ultra-low freezer temperature of −80° C. Few other methods employed for the removal of coverslips by Karigoudar *et al.*,[4] are freezing of slides at −4°C, use of petrol, and xylene at high temperature (56°C). The freezing method was observed to take a mean time of 13 min 7 s for decoverslipping, petrol around 20 h 2 min, while xylene was found to remove the coverslip in 14 h and 7 min. All of these methods are relatively time-consuming with petrol being quite expensive for day-to-day usage in laboratories.

The present study employed the usage of dry ice for the removal of coverslips, and it was found that dry ice successfully removed the coverslips in 30 s for slides aged between 1 and 15 years. For slides >15 years old, decoverslipping took 40 s. The slides were then immersed in xylene for a mere 2 min to ensure that there were no remnants present on the slides that might hinder the re-staining process.

The possible reason for dry ice to be a successful decoverslipping medium could be the following: reduction in temperature of the environment surrounding the mounting media (DPX) impairs its plasticizer property. With decreasing temperatures, the movements of the molecular chains reduce and at a certain temperature, the molecular chains become completely immobile, losing all its adhesive characteristics. The DPX thus becomes brittle thereby eliminating the ability of the material to act as a seal or glue between the slide and the coverslip.

The time taken for decoverslipping by this process is extremely less compared to conventional xylene which

may take up to 72 to 94 hours, thereby significantly increasing the turnaround time.[4] The other methods for removal of coverslip described in literature are either too technique sensitive, expensive requiring a heavy initial setup, or are extremely time-consuming compared to the process described in this study. Furthermore, it does not cause any tissue loss or disfigurement of the tissue sections which is pivotal for diagnosis of histopathological slides or teaching of histology. Additionally, there was no cracking of the glass slides using this method unlike in liquid nitrogen method,[2] which shows that the temperature of dry ice (−78.5°C) was well tolerated by the microscopic glass slide. The faded slides were also re-stained to check the quality of staining after the decoverslipping process and it showed that there was no deterioration in the quality of staining of the tissue sections.

CONCLUSION

Fading of histological slides over the years is a key challenge to proper reviewing and evaluation of old cases. In many such scenarios, the archival slides need to be re-stained, which can only be done after removal of the coverslips. The conventional method of decoverslipping using xylene may take several days. The other methods published in literature are not only expensive and time-consuming but also ineffective in a few cases. The technique (placing the microscopic slides on dry ice for a mere 30 s) proposed here is easy, fast, and effective. Furthermore, there is no loss of tissue during the procedure and the quality of the staining is not compromised. This method will thus not only make the decoverslipping process in the laboratories faster but will also prevent xylene toxicity and its effect on the environment.

Declaration

Patent application for this procedure has been published by The Patent Office, Government of India (Patent Application Number: 202141012074 A) on 26/03/2021.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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