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Original Article

Pathology Section

Alternative Rapid Methods for Coverslip Removal: A Comparative Study

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ABSTRACT

Introduction: In 21st century, the focus is to develop method which yields outcome in shorter tenure, and are rapid, safe and obtain quicker results. In this study, non-traditional methods were used for removal of coverslip and was compared with traditional methods.

Aim: To compare the time taken for coverslip removal of old faded slides with five different methods.

Materials and Methods: Faded slides of one year to more than 10-year-old were included in the study. Total of 90 slides were subjected to five different methods of coverslip removal like xylene at room temperature, xylene at 56° Celsius (C), Petrol, Diesel and Freezing method. Time taken for each method was noted.

Results: The mean time taken for removal of coverslip was least with freezing method followed by xylene at 56°C, Petrol, xylene at room temperature and diesel.

Conclusion: Alternative methods are safe, rapid and has less turn around time compared to routine use of xylene. Hence, can be successfully used for removal of coverslip.

Keywords: Freezing, Petrol, Restaining, Xylene

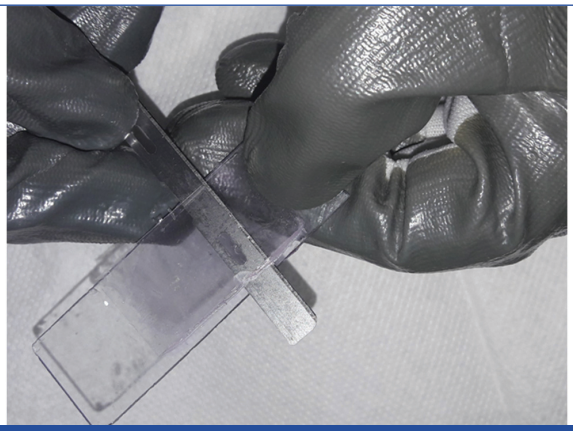
INTRODUCTION

Rapid restaining of archival slides is impossible without decreasing the time spent on coverslip removal [1-3]. Most often, old H&E stained slides show fading of stains over the period of time, unable to view for diagnosis or review. The archival slides may contain diagnostic sections of rare, extinct, interesting or classical cases which might not be easily available nowadays. Old slides can also be used in research, project, dissertation study, IHC, special staining or molecular study [4-6]. In our institute, we undertook the process of restaining of faded slide from the collection boxes meant for postgraduate study material which comprised of histopathology, haematology and cytology slides. Slides were ranging from one year to 22-year-old. The slides were divided into 40 slides/batch and were subjected to restaining. It took eight months for restaining 1200 slides and more than one third time was spent for removal of coverslip. Average time taken for restaining procedure is 100-120 hours which includes 36 to 48 hours for removal of coverslip. There is need of the hour to cut down the turn around time for coverslip removal. Hence, the aim of this study was to use alternate methods for coverslip removal which were safe, reliable and with available resources and faster as compared to routine xylene method.

MATERIALS AND METHODS

Total 90 archival slides were chosen for the prospective study which was conducted in Department of Pathology for the duration of eight months. Distyrene, Plasticizer and Xylene (DPX) were the mounting media in all slides. They were grouped according to duration into three major group as 1 to <5-year-old, 5 to <10-year and >/=10-year-old. According to coverslip size, they were divided into three subgroups; one pair of slide was taken with coverslip size 22x22 mm, 22x30 mm and 22x40 mm. In total six slides were included in each of these subgroups and total 18 slides under each major group. Five methods used for removal of coverslip were; Freezing, Xylene at room temperature, Xylene at 56°C, Petrol and Diesel. The chemicals were taken into glass beaker which were labeled and had proper lid for closure. The slides were labelled with diamond pencil and a unique number was allotted to each slide. First alphabet designated method used, middle number represented duration of slide and last number represented size of coverslip. Number 0, 1, and 2 were designated for slide duration 1 to <5-year-old, 5 to <10-year and >/=10-year-old, respectively. Similarly, number 0, 1, and 2 were given for coverslip size 22×22 mm, 22×30 mm and 22×40 mm. For example, F00 slide number means Freezing method was used, slide is 1 to <5-year-old and coverslip size is 22×22 mm.

In freezing method, the slides were kept for 10 minutes in freezing chamber (Temperature 0° to -4°C) of domestic refrigerator with coverslip facing downwards. Then after every five minutes disposable microtomy blade was passed in between the slide and coverslip to check whether coverslip was popping up easily or not. Time was noted from slide kept in freezer compartment and coverslip popped out easily. To avoid cut related injury cut resistant gloves were worn [Table/Fig-1]. For xylene at 56°C, water bath was used. The slides were kept in a glass jar and immersed inside the water bath maintained at a temperature of 56°C. Time was noted immediately after inserting jar containing slides in water bath till coverslip fell off from slides. For petrol and diesel, plastic airtight jar with cap was used. The time was noted immediately after immersing slide in chemical until coverslip detached out themselves. After the coverslips got separated, the slides were taken for restaining. Broken slides, slides with broken coverslip and slides with tissue loss during processing were excluded from study.



[Table/Fig-1]: Removal of coverslip in freezing method with disposable microtomy blade.

RESULTS

In conventional xylene method (Room temperature), the mean time for removal of coverslip was 52 hour 10 minutes. Mean duration in which

coverslip came-off was 13 minutes 7 seconds by freeze method, 14 hours 7 minutes with xylene at 56°C and in 20 hours 2 minutes with petrol method. Slide with duration 1 to <5-year old, 5 to <10-year and >/=10-year-old, coverslip came off in 9 minutes 2 seconds, 15 minute 8 seconds and 15 minute 8 seconds with freezer method while with xylene at room temperature it took 41 hours 20 minutes, 56 hours and 59 hours respectively and 9 hours, 12 hours 5 minutes and 39 hours respectively with petrol method. The duration of slides and time taken to remove coverslip is shown in [Table/Fig-2]. While the mean time taken for coverslip coming off the slide is shown in [Table/Fig-3]. With the diesel method, coverslip did not come out even after 120 hours. Hence, they were excluded from the study group.

	Duration (Hours: Minutes: Seconds)			
Method	1 to <5 yr	5 to <10 yr	>/=10 yr	Mean±SD
Freezing	00:09:02	00:15:08	00:15:08	00:13:07±00:03:01
Xylene (RT)	41:20:00	56:00:00	59:00:00	52:10:00±7:08:00
Xylene (56 deg)	14:30:00	15:20:00	14:50:00	14:07:00±00:04:00
Petrol	9:00:00	12:05:00	39:00:00	20:02:00±13:04:00

[Table/Fig-2]: Mean time taken to remove coverslip with different methods and duration of slides.

Method	Mean time (hrs:min:sec)
Freezing	00:13:07
Xylene (RT)	52:10:00
Xylene (56 deg)	14:07:00
Petrol	20:02:00

[Table/Fig-3]: Mean time taken to remove coverslip with different methods.

DISCUSSION

Traditionally, xylene is widely and most commonly used solvent for coverslip removal [1]. The turn around time with xylene is more, sometimes it might take 72 to 94 hours for coverslip removal [7,8]. Most common need for coverslip removal is for extraction of DNA for molecular analysis for research purpose. In the era of molecular testing where there is demand for target therapy, even a smaller specimen can be useful. Determining mutations in Epidermal growth factor receptor, BRAF and KRAS genes which is useful for target therapy [1,3]. Sometimes the archival cytological slides may be the only source for such studies like in lung adenocarcinomas, thyroid lesions and other soft tissue tumors. Other research fields where coverslip removal is needed is for neuronal cultures [5,9,10].

There is paucity of literature regarding untraditional methods for coverslip removal. In this study, methods which are easily available and feasible were used. This is the first kind of extensive study which has described the usage of different methods for removal of coverslip. Few studies have also described use of liquid nitrogen, ultrasonic vibrations and scratching coverslip along with application of ice block for coverslip removal.The problem with these methods are either they are not easily available at the setup, like liquid nitrogen or may cause damage to the tissue section like in ultrasonic or with scratching method [3-6].

Mechanism of action of Diesel, Petrol and Xylene at room temperature is same i.e., they all are solvents and dissolve the DPX. Heating aids in penetration as well as fastens melting of DPX. Impurities and high density of diesel might be a reason for poor penetration as well as ineptitude in dissolving DPX. On the contrary, Petrol which is also a product of crude oil, has low density and undergoes fractional distillation, which might be a reason for robust solvent properties compared to diesel.

In the present study, it was found out that the most efficient and fastest method for coverslip removal was freezing method. The mean time in which the coverslip popped out was 13 minutes 7 seconds. For 5 to <10 years and more than or equal 10-year-older slide it took 15 minutes 8 seconds each, while for less than five-year-old slide it took 10 minutes. Similar findings were seen in the study conducted by Treece AL et al., and Da Cunha Santos G et al., where they used freezer method for coverslip removal [1,2].

In other methods, for 1 to <5-year-old slide, coverslip came out within 9 hours with petrol method. Mean time was 20 hours 2 minutes, as it took more time for >10-year-old slides and with Xylene at 56°C, the mean time in which coverslip came out was 14 hours 7 minutes. In both of these methods, turn around time was significantly less compared to routine xylene method. No significant changes in terms of quality of staining was found on microscopic examination of slide.

LIMITATION

Further studies should be carried out to determine the loss of any cells with different methods of coverslip removal by molecular analysis and immunohistochemistry which was constraint of this study. However there was no loss of part/whole tissue in any of the methods in this study.

CONCLUSION

Alternative methods especially freezing, xylene at high temperature and petrol method can be used for removal of coverslip. These methods are reliable within available resources and remarkably reduce the turn around time for restaining of faded old stained slides.

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