Rapid removal of cytology slide coverslips for DNA and RNA isolation

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ABSTRACT

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INTRODUCTION

Due to constant increase in molecular targets for diagnosis and treatment, more ancillary tests are being developed for cytology specimens (1,2,3). One time-consuming step in this procedure is glass coverslip removal by xylene (4). Here we describe a method for rapid coverslip removal using liquid nitrogen. Methods: Direct smears were prepared from residual pleural fluid, Diff-Quik stained, then covered with a glass coverslip using mounting medium. Coverslips were then removed by either (1) immersing the slides in xylene for 3 to 4 days, or (2) prying off with razor blade after rapidly freezing the slides in liquid nitrogen for 30 seconds. The DNA or RNA was extracted by the Pinpoint Slide Isolation system (Zymo Research Corp) or by RNeasy FFPE kit (Qiagen), respectively. The quantities of DNA and RNA from the two different procedures were evaluated by Qubit dsDNA BR or RNA HS Assay kit. The amplifiability of the DNA was evaluated by real time PCR with primer pairs for hemoglobin beta (HBB) genomic DNA sequences, and amplifiability of RNA was also evaluated by real time RT-PCR with housekeeping gene primers for mitochondrial ribosomal protein L19 (MRPL19) after converted into cDNA.

Real time RT-PCR result shows that the DNA isolated from liquid nitrogen (LN) and xylene (XYL) procedures can be converted to cDNA, and has similar cycle threshold (Ct) values for the amplification of housekeeping gene MRPL19. (P = 0.95 t-test)

Conclusions: By removing the coverslip from the cytology slides using the liquid nitrogen method, DNA and RNA can be extracted from the slides without affecting the quantity and quality of the nucleic acids, and will be equivalent to the xylene coverslip removal method.

REFERENCES


