Indigenous Technique as an Effective Liquid-Based Cytology Tool for Multiple Single-Layered Cell Preparations

Shelly Sharma, Pranab Dey
Department of Cytology and Gynec Pathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

Abstract

Aims and Objectives: The aim of this work was to study the application of Modified Millipore technique (MMT) as a liquid-based tool to have multiple monolayer cellular smears for the routine cytology and Immunocytochemistry. Materials and Methods: In this study, we included 32 effusion fluid samples and 30 fine-needle aspiration cytology (FNAC) samples. From each of the samples, at first routine conventional smear was prepared. The residual samples were processed by MMT to make multiple smear preparation. Both the conventional and MMT of the monolayer cells were evaluated. The various cytomorphological features including the cellularity, background information, nuclear morphology, and cytoplasmic preservation were compared in these two techniques. Result: There were a total of 15 cases of fluid samples and 27 cases of FNAC. Statistical analysis of Mann–Whitney U test showed that the monolayer preparation by MMT and liquid-based cytology of fluid and FNAC smears are of same quality (\(P > 0.01\)). Conclusion: Monolayer preparation by MMT is cheap and effective. This technique can be used in routine laboratory for multiple monolayer cell preparations.

Keywords: Monolayer preparation, Liquid based cytology, Millipore, Single-Layered cell

Introduction

Multiple monolayer smears are often needed in cytology.\(^1\) Presently there are a few commercially available liquid-based cytology (LBC) preparations in this field.\(^2,3\) These techniques are sophisticated and very expensive. We need indigenous, cheap, and effective technique for developing countries. Millipore technique is used in cytology to collect the cells on the filter paper.\(^4\) In this study, we tried to use the modified Millipore technique (MMT) to make multiple monolayer smear preparations.

Materials and Methods

Ethical justification

This prospective study was based on the residual material after the routine procedure. Permission was taken from each patient, and the identity of the patients was hidden. All the fine-needle aspiration cytology (FNAC) was done after taking proper consent. The study followed the Helsinki accord and ethics were maintained.

This is a prospective study which comprises of 15 effusion fluid samples, and 27 cases of FNAC. The body fluid was collected in anticoagulant solution (ammonium oxalate 1%; fluid sample as 1:9 ratio). The sample of the fluid was processed for routine preparation by Sure Path, and after that the residual part of the sample was used for the smear preparation by MMT.

Similarly in case of FNAC, after the smear preparation by routine method, the other residual part within the syringe was rinsed in 0.85% physiological saline and used for MMT. Papanicolaou’s stain was used for the MMT in both fluid samples and FNAC smears, whereas May Grunwald Giemsa, hematoxylin, and eosin stain and Papanicolaou’s stains were used for routine and SurePath fluid sample and FNAC smears. The smears from both the techniques (conventional and MMT) were compared by the competent cytopathologists (PD). The following features were studied and graded semi-quantitatively:

Address for correspondence: Prof. Pranab Dey, Department of Cytology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh - 160012, India. E-mail: deypranab@hotmail.com

How to cite this article: Sharma S, Dey P. Indigenous technique as an effective liquid-based cytology tool for multiple single-layered cell preparations. J Cytol 2020;37:122-5.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPedknow_reprints@wolterskluwer.com
Modified Millipore technique for fluid samples

The fluid specimen was centrifuged at 1500 rpm for 10 min. The supernatant was discarded. The cells were washed with phosphate-buffered solution (PBS) at 1500 rpm for 5 min. The cell deposit was re-suspended by adding 1–2 mL of phosphate-buffered water. Then the sample was vortexed at 3000 rpm for 15 ± 5 s. The final volume was made up to 25 mL with phosphate-buffered water. The suspension of fluid was filtered through Millipore filter paper which was premoistened with 0.85% normal saline at 30 mm Hg negative pressure. The Millipore filter paper was made of cellulose acetate (Merck Life Science, Bangalore, India). The pore size of the filter paper was 5 µm to allow the subcellular particles to pass out and to retain the cells of interest. The volume of material to be put for filtration was inversely proportional to the amount of cell deposit. It should be noted that the fluid should remain on the filter paper throughout the filtration process. The deposit on the filter paper was imprinted on poly-L-lysine-coated slides and immediately fixed in 95% alcohol for staining. If more than two slides are needed, the filter paper can be cut into four parts immediately with surgical blade, and imprint smears made. The fluid suspension can also be split into two parts to make multiple slides for special/immunocytochemistry staining. If the fluid specimen was hemorrhagic, then the cell deposit was treated with 1% glacial acetic acid and then two to three washings were done in PBS before diluting the specimen with phosphate buffered water prior to the filtration process.

Modified Millipore technique for FNAC

FNAC sample was collected, and routine smears were made. After making the routine smears, the needle was rinsed in 0.85% physiological saline, and the sample was collected in 1% ammonium oxalate (1–2 mL). Homogenization was done by pipetting the sample several times. The sample was vortexed at 3000 round per minute (rpm) for 15 ± 5 s. The aspirated sample was suspended by adding 1–2 mL of phosphate-buffered water. The sample was vortexed again for 10 ± 5 s. The final volume of the sample was made up to 15 mL by adding buffered water. The FNAC suspension was filtered by using Millipore filter paper, which was premoistened with normal saline at 10 mm Hg negative pressure. It must be noted that some amount of fluid should remain on the filter paper till the end of filtration process. Imprint smears were made on poly-L-lysine-coated slides and immediately fixed in 95% alcohol.

RESULT

The distribution of the cases is shown in Table 1. The majority of the fluid samples (16/32) and FNAC (13/30) were diagnosed as metastatic adenocarcinomas.
routine smears as well as for immunocytochemistry. The MMT technique took only 10–15 min. It was relatively rapid and easy to perform.

These techniques had several advantages, such as:
1). Monolayered preparation,
2). No cell loss during processing,
3). Well preserved cells,
4). The simple and economical technique, and good for limited-resource settings,
5). A useful tool for immunocytochemistry staining and
6). Suitable for scanty aspirates and fluid samples having low cellularity.

The MMT had several advantages over the cell block techniques such as:
1). A rapid procedure as cell block takes time for fixation, processing and cutting and involves multiple steps (1–2 days),
2). No hindrance of formalin for immunocytochemical staining,
3). Use of plasma in cell blocks gives background which can result in non-contributory results,
4). Cost-effective as no thrombin is required,
5). Cells are well preserved in filtration technique as the cells do not undergo treatment in multiple reagents such as alcohols and toluenes.
6). Cell blocks with scanty material often results in very low/no cellularity as some material is lost during processing, orientation and cutting,
7). Cell blocks often result in congestion of cells whereas in imprint smears we get monolayer smears.

Nasser A et al.[5] used Millipore technique in the thyroid FNAC samples. They noted that Millipore technique was more useful than cell block in case of thyroid FNAC samples. In comparison to their study, we have performed this technique in different types of samples, including effusion fluid and FNAC of the various lesions of the body.

In conclusion, to the best of our knowledge, this is the first kind of such study. We hope that the technique described by us will help to make multiple monolayer preparation of cellular smears that can be used for routine diagnosis.

Table 2: Total scores in Modified Millipore technique (MMT) and liquid-based cytology (LBC) by SurePath

| Type          | Number of cases | Mean rank | Sum of ranks |
|---------------|-----------------|-----------|--------------|
| Cellularity   | MMT 32          | 32.81     | 1050.00      |
|               | LBC 32          | 32.19     | 1030.00      |
|               | Total 64        |           |              |
| Nuclear details| MMT 32          | 28.44     | 910.00       |
|               | LBC 32          | 36.56     | 1170.00      |
|               | Total 64        |           |              |
| Background    | MMT 32          | 30.80     | 985.50       |
|               | LBC 32          | 34.20     | 1094.50      |
|               | Total 64        |           |              |

Mann–Whitney U test: cellularity: $P > 0.881$, nuclear details $P > 0.30$, background $>0.368$. No significant difference was found between the two groups

Table 3: Total scores in modified Millipore technique (MMT) and fine-needle aspiration cytology (FNAC) smear

| Type          | n   | Mean rank | Sum of ranks |
|---------------|-----|-----------|--------------|
| Cellularity   | MMT 30 | 30.37     | 911.00       |
|               | FNAC 30 | 30.63     | 919.00       |
|               | Total 60 |         |              |
| Nucleus      | MMT 30 | 30.28     | 908.50       |
|               | FNAC 30 | 30.72     | 921.50       |
|               | Total 60 |         |              |
| Background    | MMT 30 | 29.72     | 891.50       |
|               | FNAC 30 | 31.28     | 938.50       |
|               | Total 60 |         |              |

Mann–Whitney U test: cellularity: $P > 942$, nuclear details $P > 0.915$, background $>0.670$. No significant difference was found between the two groups
Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Singh P, Rohilla M, Dey P, Comparison of liquid-based preparation and conventional smear of fine-needle aspiration cytology of lymph node. J Cytol 2016;33:187-91.
2. Linder J, Zahniser D. The thinprep pap test. A review of clinical studies. Acta Cytol 1997;41:30-8.
3. Kirschner B, Simonsen K, Junge J. Comparison of conventional Papanicolaou smear and SurePath liquid-based cytology in the Copenhagen population screening programme for cervical cancer. Cytopathology 2006;17:187-94.
4. Del Vecchio PR, De Witt SH, Borelli JI, Ward JB, Wood TA Jr, Malmgren RA. Application of Millipore filtration technique to cytologic material. J Natl Cancer Inst 1959;22:429-31.
5. Nassar A, Cohen C, Siddiqui MT. Utility of Millipore filter and cell block in thyroid needle aspirates: Which method is superior? Diagn Cytopathol 2007;35:34-8.