Original Article

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Pathology Section

Comparison of Cell Preparations between Commercially Available Filter Cards of the Cytospin with Custom Made Filter Cards

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ABSTRACT

Introduction: Cytological evaluation of body fluids is an important diagnostic technique. Cytocentrifuge has contributed immensely to improve the diagnostic yield of the body fluids. Cytocentrifuge requires a filter card for absorbing the cell free fluid. This is the only consumable which needs to be purchased from the manufacturer at a significant cost.

Aim: To compare the cell density in cytocentrifuge preparations made from commercially available filter cards with custom made filter cards.

Materials and Methods: This was a prospective analytical study undertaken in department of pathology of a tertiary care centre. A 300 GSM handmade paper with the absorbability similar to the conventional card was obtained and fashioned to suit the filter card slot of the cytospin. Thirty seven body fluids were centrifuged using both conventional and custom made filter card. The cell density was measured as number of cells per 10 high power fields.

The median cell density was compared using Mann-Whitney U test. The agreement between the values was analysed using Bland Altman analysis.

Results: The median cell count per 10 High power field (HPF) with conventional card was 386 and that with custom made card was 408. The difference was not statistically significant (p = 0.66). There was no significant difference in the cell density and alteration in the morphology between the cell preparations using both the cards.

Conclusion: Custom made filter card can be used for cytospin cell preparations of body fluids without loss of cell density or alteration in the cell morphology and at a very low cost.

Keywords: Body fluids, Cell density, Cytocentrifuge, Cytospin, Filter card

INTRODUCTION

Cytological evaluation of the body fluids has a significant role in diagnostic cytopathology [1]. Determining the pathologic nature of the sample fluid contributes significantly patient management. The varying concentration of the pathological cell population in the body fluid makes the diagnostic cytopathology a challenging task. Cytological analysis can be done by various techniques such as conventional smear and cell block preparation [2].

A good cell preparation technique will concentrate the cells, results in even distribution of these cells, and helps in better appreciation of the cell morphology. Cytocentrifuge is one such cytological technique of concentrating the cells in the sample fluid which also results in better preservation of the cell morphology [3].

Cytospin technique is successfully used for analysis of body fluids with malignant & non-malignant cells and tissue diagnosis using monolayer technique [4,5].

Koh has described the technique of preparation of cells for microscopy using cytospin technique [6]. The only consumable in the cytocentrifuge is the filter card which is made up of cotton and cellulose material. This has excellent absorbent capacity and is sufficiently strong facilitating easy removal of the filter card from the slide. The cost per box containing 200 pieces for Thermo Shandon cytospin is around Rs 5000, which is approximately Rs 25 (approximately 40 US cents) per card.

In a busy tertiary care hospital like ours, we analyse around 10-15 body fluids per day. On several occasions, we had no supply of these filter cards due to lack of availability with the local supplier. This had resulted in cytocentrifuge being unused. In order to meet this challenge, we developed a cytofilter card with locally available paper [7].

In this study, we compared the cellular concentration of body fluids in cytospin preparations, between commercially available cards with custom made filter card. The custom made filter card is prepared from the 300 gsm handmade paper with comparable absorbability at a significantly lower cost.

AIM

To compare the cell density in cytocentrifuge preparations made from commercially available filter cards with custom made filter cards.

MATERIALS AND METHODS

This was a prospective analytical study conducted in department of pathology of a tertiary care centre in southern India. This study was conducted in the month of August 2015 and was approved by the institutional ethical committee.

Sample size: Prior data with 10 samples indicated that the difference in the response of matched pairs had a standard deviation of 20. We assumed the difference in the mean between two groups to be of clinical significance, if it were to be more than fifteen. We needed 16 pairs of subjects to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. As this was a pilot study we planned to have at least 30 samples for comparison (Sample size calculated using online calculator Power & sample size Ver 3.1.2, http://biostat.mc.vanderbilt.edu/PowerSampleSize).

Inclusion Criteria

All the body fluids excluding urine sample which had more than 25 cells/mm^3.

Exclusion Criteria

Haemorrhagic and purulent fluids were excluded due to practical difficulty in preparing the cytocentrifuge smear.

The samples obtained during the day time were included in this study. Once the sample was received and initial identification formalities completed, cell count was performed on Neubauer counting chamber. Later the sample was divided into two portions and stored in different containers to be analysed by two different pathologists. Each portion was centrifuged using the cytospin. The first attempt was with the conventional filter card and the second was with the custom made filter card. Thus two cell preparations were obtained from the same fluid. Smears were made from these cell preparations and examined under both low and high power fields. The cell density was calculated as the number of cells counted in 10 high power fields after staining. One of the authors studied the smear prepared from conventional card and the other studied the smear prepared from custom made filter card. Results were entered and analysed using Microsoft Excel 2013.

Patient Consent

No patient consent was taken specifically for this study. All the patients had given general consent for relevant investigations at the time of admission. As this was a quality improvement study, ethical committee approved this study without need for specific patient consent.

RESULTS

Total number of fluid samples analysed was 37, out of which 17 were ascitic, 19 were pleural and 1 was cerebrospinal fluid. All the samples analysed had only inflammatory and mesothelial cells. There was no malignancy detected in the study sample.

The median cell count with conventional filter card was 386 and with custom made card, it was 408 cells. The spread of the data and comparison is depicted in [Table/Fig-1].

Cells /10 HPF	Median	Quartile 1	Quartile 3
Conventional	386	214	505
Custom	408	281	543
U = 644	Z = -0.43	p = 0.66*	

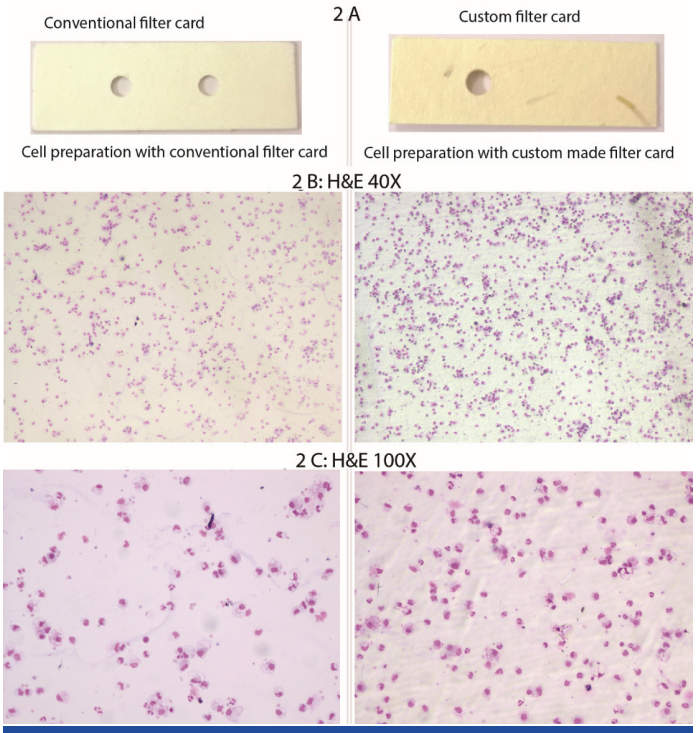
[Table/Fig-1]: Comparison of cell density after cytospin preparation between conventional and custom filter card.
*Mann-Whitney U test

Comparison of cell morphology between two filter cards is depicted in [Table/Fig-2].
The difference in the cell density after cell preparation with conventional and custom made filter card was not significant with p value of 0.66. Bland Altman analysis for the 95% limits of agreement was 10 cells /HPF [Table/Fig-3].

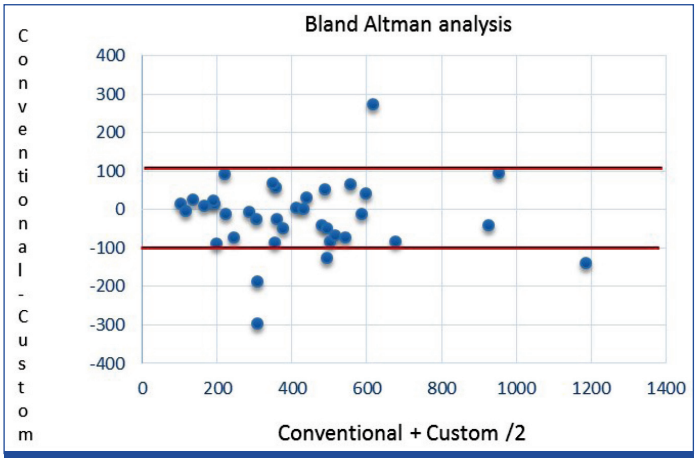
DISCUSSION

The cytocentrifuge has been an indispensable technique for body fluid analysis in the advanced laboratories. Filter card is the consumable which needs to be purchased from the manufacturer at a significant cost. Purchase formalities in the hospital, transport issues might jeopardise use of cytospin at times.

A locally available alternative without compromising the quality of the cell preparation could be very helpful in such situations [Table/Fig-2a]. We could as well demonstrate that there was no significant difference in the cell density between conventional and custom made cards [Table/Fig-2b&c]. In addition to this, there is a significant cost advantage. In our study, we could prepare the custom made filter cards at quarter of a Rupee (approx. 2 US cents) compared to Rs 25 (approx. 40 US cents) per card sold by the manufacturer.



[Table/Fig-2]: (A) Photographs showing conventional and custom made filter card. (B) Comparison of cell preparation of ascetic fluid between conventional and custom made filter cards – (H&E 40 X). (C) Same as above at 100 X.



[Table/Fig-3]: Agreement of the cell density values after cytospin cell preparation between conventional and custom filter card.

In our earlier study, we have elaborated on the technique of preparing custom made filter card in house [7]. This is the first study comparing the effectiveness of custom made filter card with conventional filter card. Hence, we did not find any literature for comparison. However, our study has limitations such as small sample size and not having malignancy in the samples studied. We are planning to address this issue in our upcoming study.

CONCLUSION

A custom made filter card for cytospin using 300 gsm handmade paper is equally effective as conventional filter card in cell preparation of the body fluids. The cost of this consumable can be significantly reduced.

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