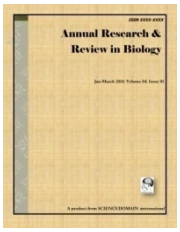

Annual Research & Review in Biology
4(20): 3139-3149, 2014

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Comparison of ThinPrep® and Conventional Preparations for Peritoneal and Pleural Cytology Smears

Nasar Yousuf Alwahaibi[1*], Nasra Said Alnoumani[1] and Usha Rani Bai[1]

[1]Department of Pathology, College of Medicine and Health Sciences, Sultan Qaboos University, P.O. Box 35, Postal Code 123, Muscat, Oman.

Authors' contributions

This work was carried out in collaboration between all authors. Author NYA designed the experiment and wrote the manuscript. Authors NSA and URB carried out the preparation and staining processes and analyzed data. All authors read and approved the final manuscript.

Original Research Article

Received 14th March 2014
Accepted 10th May 2014
Published 2nd June 2014

ABSTRACT

Aims: To compare the performance of ThinPrep® 5000 processor with the conventional method of preparing pleural and peritoneal smears as well as to study the possibility of replacement of the conventional method with ThinPrep® 5000 system.
Methodology: Forty-one samples of serous fluid comprising 17 pleural fluids and 24 peritoneal fluids, were analyzed. Smears were prepared using ThinPrep® 5000 and conventional methods. All the slides were stained by the Pap method.
Results: 98% of all cases showed monolayer architecture with minimal overlapping using ThinPrep® 5000 method. However, 73% of cells, prepared by conventional method, were either crowded, overlapped or both. In addition, the cytomorphology of cells prepared by ThinPrep® 5000 method was better preserved (61%) than those with conventional method (41%). However, conventional smears were cellular in 73% of the cases whereas with ThinPrep® 5000 method, high cellularity was only seen in 2%.
Conclusion: The findings of this study support the use of ThinPrep® 5000 system in the diagnosis of pleural and peritoneal samples.

Keywords: Conventional method; cytology; liquid-based cytology; peritoneal fluid; pleural fluid; ThinPrep® technique.

**Corresponding author: Email: nasar@squ.edu.om;*

1. INTRODUCTION

Poor fixation, thick smears, air-drying artifacts, and obscuring organisms, blood and inflammatory cells are the main drawbacks of conventional method of preparing smears from gynaecological specimens**.** In addition, false negative results are another concern [1]. The Papanicolaou stain is still the method of choice in staining cytological smears, which are prepared by either liquid-based cytology method or conventional method. ThinPrep® processor was approved by the U.S Food and Drug Administration (FDA) in 1996 and was used as a replacement for the conventional Pap smear. ThinPrep® has been used extensively in cervical pathology and the results showed a satisfactory increase in specificity, sensitivity, morphology and specimen adequacy compared with the conventional Pap method [2-7]. However, the use of ThinPrep® method (Hologic, Marlborough, MA) or other similar methods such as Autocyte Prep®, from South American system, Tripath imaging, INC, Burlington, North Carolina, Dnacitoliq from Digene Brazil, Sa Paulo, Brazil, and CellprePlus® from Biodyne, Seongnam, Korea, in non-gynecological specimens is not common in Asia, particularly in Oman [8]. In general, serous fluids such as pleural, peritoneal or pericardial, are less received specimens compared with the cervical smears.

ThinPrep® 5000, which was introduced in 2008, is a processor used in the liquid – based cytology, to produce uniform, thin layer preparation of cells from cell suspension collected in methanol fixative. Gynaecological and non - gynaecological specimens can be prepared using this processor. In addition, its major application to overcome the limitations of cytological interpretation of conventional smears. ThinPrep® system consists of three main steps; dispersion, cell collection and cell transfer.

The main aim of this study was to compare the performance of ThinPrep® 5000 processor with the conventional method in preparing pleural and peritoneal smears as well as to study the possibility of replacement of the conventional method with ThinPrep® 5000 system.

2. MATERIALS AND METHODS

This study was ethically approved by the Medical Research Committee and Ethics Committee (MREC # 412) from the College of Medicine and Health Sciences, Sultan Qaboos University, Sultanate of Oman.

Forty-one samples of serous fluid comprising 17 pleural fluids and 24 peritoneal fluids, were obtained from Sultan Qaboos University Hospital, Sultanate of Oman.

All samples were centrifuged at 1500rpm for 5 minutes. The supernatant was poured off and the sediment of each sample was used to prepare conventional and ThinPrep® 5000 (Hologic, INC, Marlborough, USA) smears.

For the conventional preparation of pleural and peritoneal samples, direct smears were made and fixed immediately in 95% ethyl alcohol. In direct smears, small drop of the sediment of the specimen was transferred onto a labeled frosted end-slide. The side edge of a second slide was placed at a 45º angle on the first slide. The opposite edge was lowered slowly on the drop of the specimen. The second slide was then pulled gently and quickly down the first slide.

Sediment was not washed with CytoLyt solution. For the ThinPrep® 5000 preparation, the sediment of pleural and peritoneal samples were added to the PreservCyt® solution vial and allowed to stand for 15 minutes before processing. This would ensure that the morphology of the cells is preserved. The sample vial with its corresponding microscope slide and blue filter (5.6µm pore size) were loaded into a carousel for processing. During the process, the vial and filter were picked up by the machine. The vial was placed in the disperser to be dispersed after uncapping. After placing the slide on the cell transfer station, the filter was introduced to the vial to collect the cells. The cells were transferred on the slide which was then dropped into a fixative bath. The filter was disposed into the filter waste bin. The vial was then recapped and returned to the carousel.

All the slides prepared from both methods, were further fixed in 95% alcohol and stained by the Pap method. All the slides were examined independently by two cytotechnologists. The following criteria were used to evaluate the two methods:

- Cellularity: No cells, low (less than 10 cells per field), moderate (10 to 20 cells per field) or high (more than 20 cells per field).
- Blood cells: No blood cells, low, moderate or high.
- Inflammatory cells: No inflammatory cells, low, moderate or high.
- Background: Clear, blood stained, proteinaceous or blood stained proteinaceous material.
- Architecture: Single layer, overlapped, crowded, overlapped and crowded.
- Cytomorphology: Fair or good.

3. RESULTS

All the slides were well preserved and stained. Table 1 shows that ThinPrep® 5000 prepared slides had evenly distributed cells. Where mono layer architecture was seen in 98% of all cases. On the other hand, 73% of conventional smears were either crowded, overlapped or both (Figs. 1 and 2).

Table 2 shows ThinPrep® 5000 technique was more valuable in producing clear background by reducing the background-obscuring materials such as blood and inflammatory cells. Only 12% of ThinPrep® slides were proteinaceous compared to 27% conventional smears (Figs 3,4,5 and 6). In addition, the cytomorphology of cells prepared by ThinPrep® 5000 method was more preserved (61%) than those with conventional method (41%) (Fig. 7).

Table 1. Architecture feature of both conventional and ThinPrep® 5000

Architecture		Single layer	Overlapped	Crowded	Overlapped and Crowded
	ThinPrep® 5000	98% (40)	0% (0)	2% (1)	0% (0)
	Conventional method	27% (11)	27% (11)	34% (14)	12% (5)

(*The number of cases is indicated in parentheses)*

Table 2. Features of both conventional method and ThinPrep® 5000

Background		**Clear**	**Bloody**	**Proteinaceous**	**Bloody and Proteinaceous**
	ThinPrep® 5000	78% (32)	5% (2)	12 % (5)	5% (2)
	Conventional method	17% (7)	51% (21)	27% (11)	5% (2)
Blood cells		Low cells	Moderate cells	High cells	
	ThinPrep® 5000	100% (41)	0% (0)	0% (0)	
	Conventional method	56 % (23)	27% (11)	17% (7)	
Inflammatory cells		Low cells	Moderate cells	High cells	
	ThinPrep® 5000	78% (32)	22% (9)	0% (0)	
	Conventional method	10% (4)	44% (18)	46% (19)	

(*The number of cases is indicated in parentheses)*

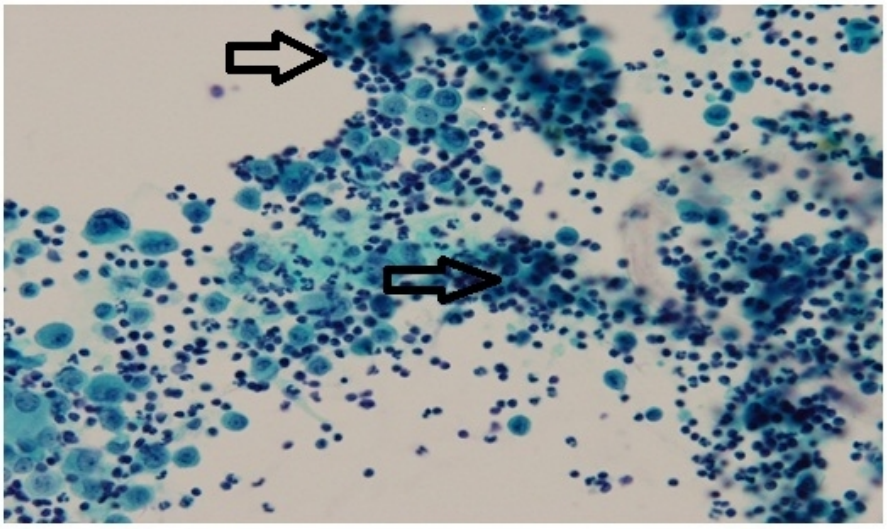

Fig. 1. Conventional pleural fluid smear with high cellularity, overlapped and crowded cell layer (arrows) (Papanicolaou stain, x40)

In terms of cellularity of the slides, conventional smears were cellular in 73% of the cases whereas with ThinPrep® 5000 method, high cellularity was only seen in 2% of all cases (Fig. 8).

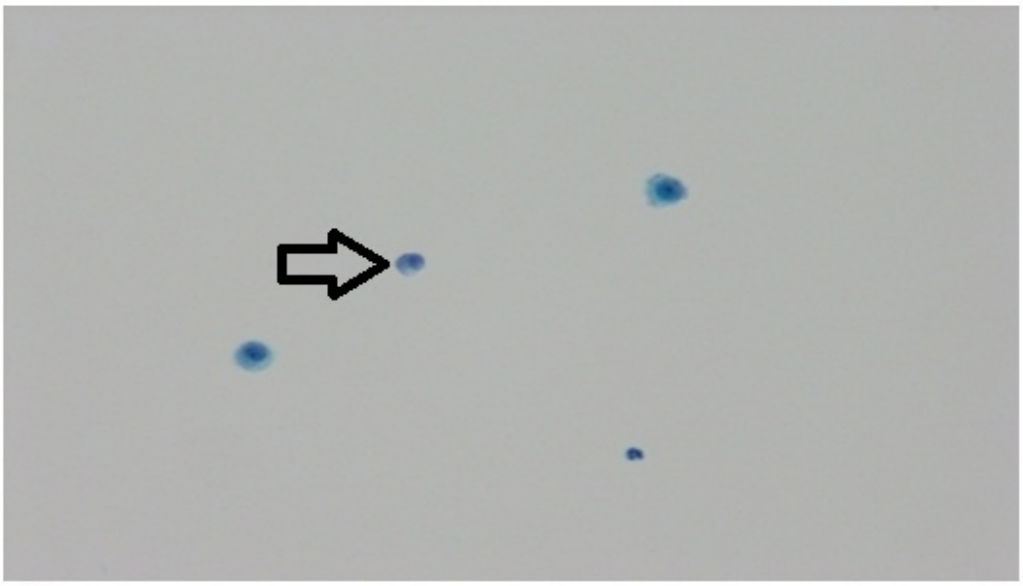

Fig. 2. ThinPrep® 5000 produced thin layer smear of the same sample in figure 1 showing low cellularity and clear cytomorphology (arrow) (Papanicolaou stain, x40)

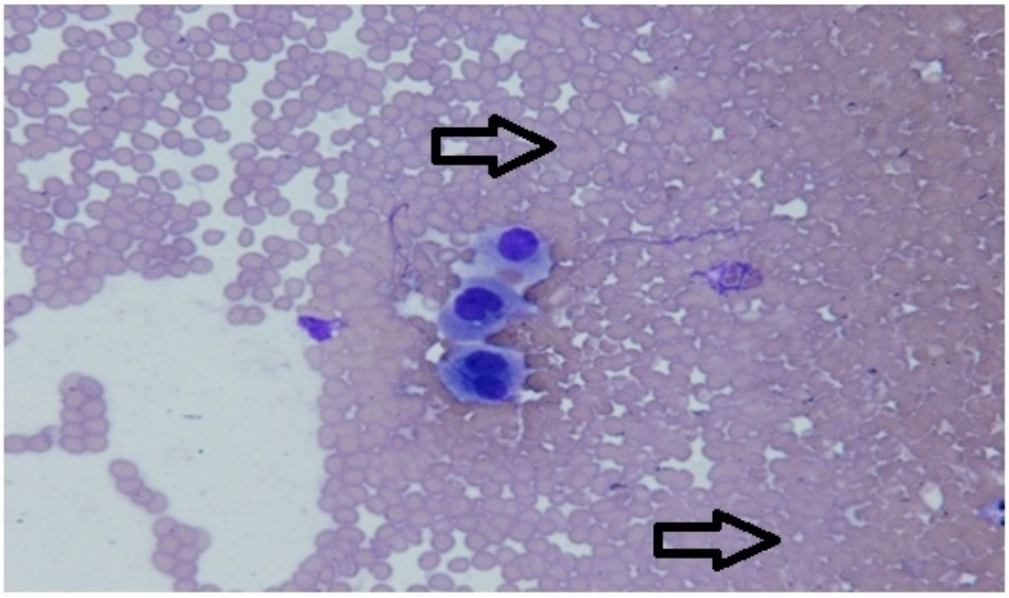

Fig. 3. Conventional pleural fluid smear in which the background is obscured with red blood cells (arrows) (Papanicolaou stain, x40)

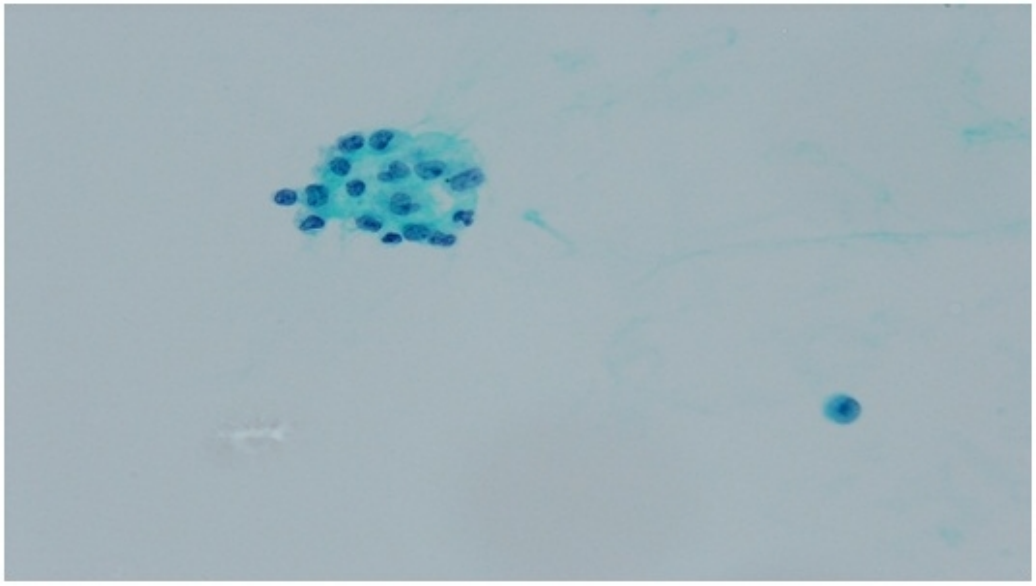

Fig. 4. The red blood cells were decreased when processing the sample in figure 3 with ThinPrep® 5000 (Papanicolaou stain, x40)

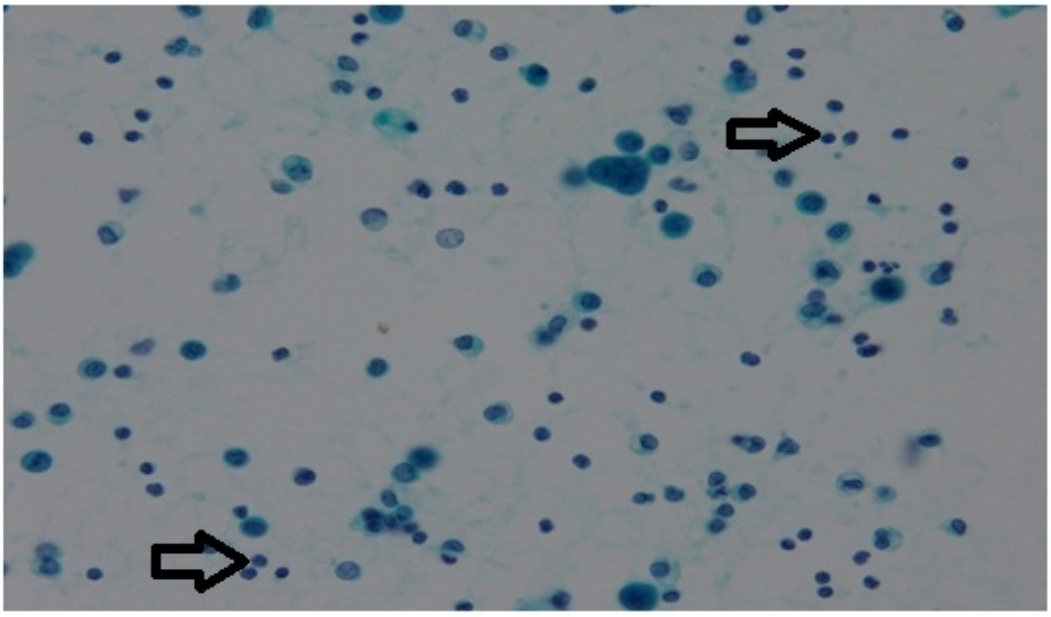

Fig. 5. Conventional peritoneal fluid smear showing increased number of inflammatory cells (arrows) (Papanicolaou stain, x40)

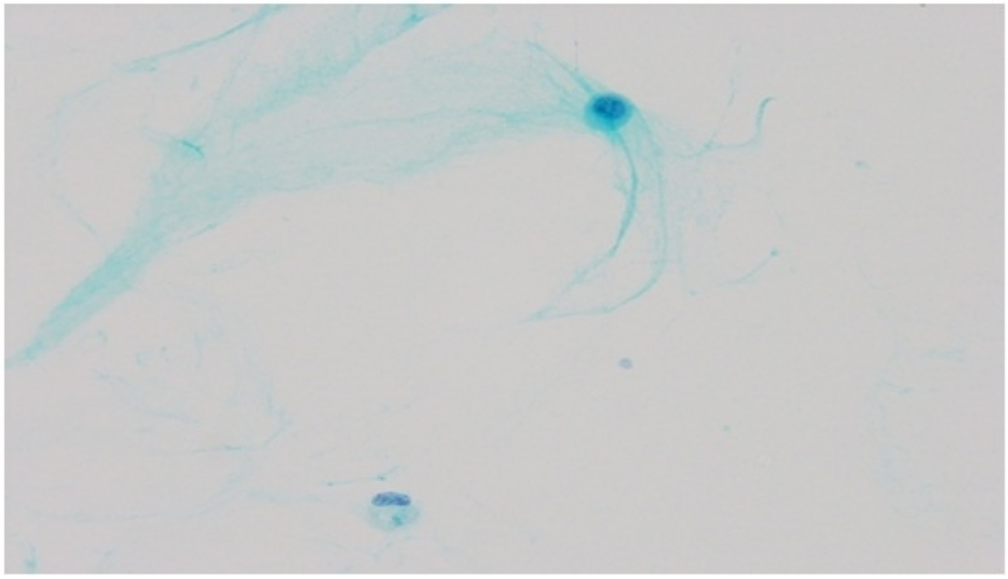

Fig. 6. ThinPrep® 5000 smear of the sample in figure 5 showing a decrease in the count of inflammatory cells (Papanicolaou stain, x40)

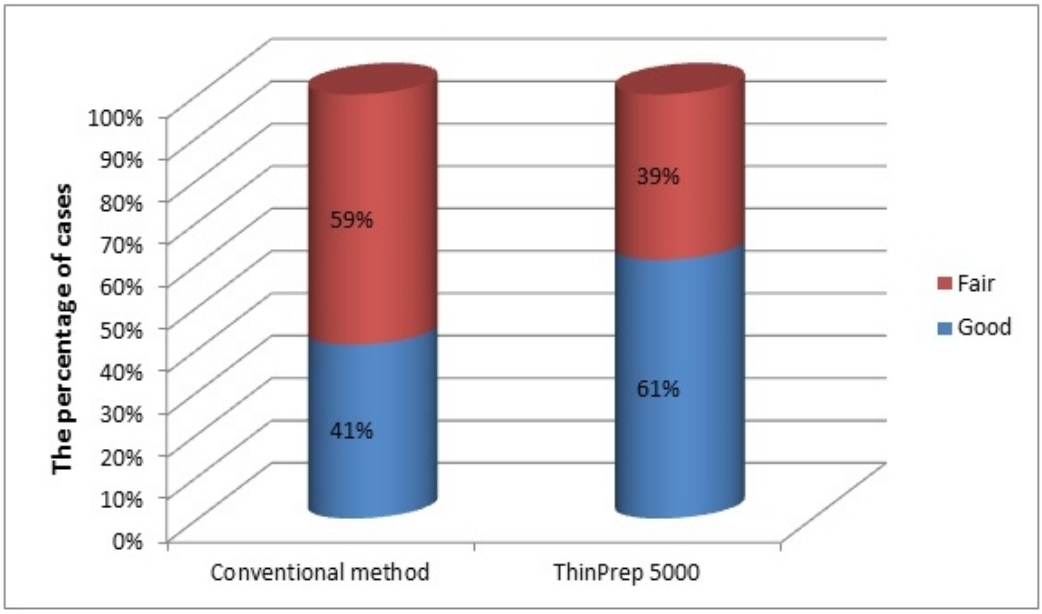

Fig. 7. Cytomorphological difference between conventional and ThinPrep® 5000

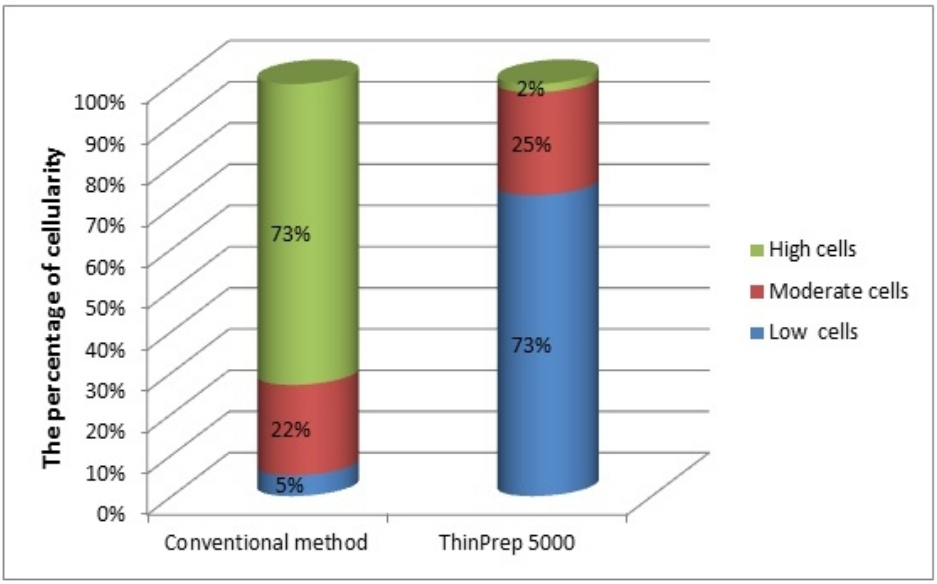

Fig. 8. Cellularity difference between conventional and ThinPrep® 5000

4. DISCUSSION

The finding of this study is in line with other previous studies which collectively reported that liquid-based cytology is better than the conventional method. However, most of these studies used gynaecological samples rather than non- gynaecological samples [4,9-12]. The current study investigates the performance of ThinPrep® 5000 method using pleural and peritoneal samples.

This study showed an even distribution of cells forming monolayer with minimal overlapping. In fact, the principle of ThinPrep® 5000 method allows providing smears with a clear background by removing obscuring materials such as blood, inflammatory cells, proteinaceous and cell debris. Subsequently, cytotechnologists and probably cytopathologists would have to spend less time in screening smears and thus allow more time for other cases. The finding of this study is in line with other previous study which uses respiratory samples [9]. In their study, it was reported that ThinPrep® (Cytyc Corp) reduces slide evaluation time (overall, time saved by ThinPrep® was 2 hours when compared to conventional method) and facilitates optimal use of the experienced cytologist in the final diagnosis. Another study also showed that the assessment for ThinPrep® was approximately half the time required for conventional method [4]. However, it should be kept in mind that with very bloody and inflammatory specimens, some of these cells are retained on the smears [13]. Regarding the cost, there is a total agreement that ThinPrep® chemicals, reagents, equipment, maintenance, preparation time and transportation are more expensive when compared with conventional method [9,14]. However, this can be justified by saving cytologist and cytopathologist screening time.

Another major finding that was seen in this study is the well preservation of cytomorphological features including nuclear details and nuclear - cytoplasmic ratio. Conventional smears showed less preservation of cytomorphological details in comparison with the ThinPrep® method. In addition, cells from pleural and peritoneal fluids can be kept

safely in the PreservCyt® solution for a period of three weeks without changes in the morphology of the cells [8]. Subsequently, additional smears if needed, and/or further investigation or special staining can be carried out using the same sample.

Cytotechnologists who examine the pleural and peritoneal smears should be aware of the method used for preparing smears. Samples for ThinPrep® 5000 system are fixed in PreservCyt® solution and therefore the cells tend to shrink and appeared smaller than should be (as in the conventional method). Subsequently, this change in the morphology picture may slightly make the diagnosis difficult as normal cells may be misinterpreted as abnormal. In addition, the interpretation of neutrophils, lymphocytes and count of microorganisms may give a false assessment of inflammation and infection [15]. It is therefore necessarily to train the cytotechnologists and cytopathologists to screen ThinPrep® smears properly.

In this study, split samples were used to prepare for ThinPrep® system and conventional method. Most of the materials were first smeared onto slides for conventional preparation and then to the ThinPrep® system. This might explain the low cellularity that was found using ThinPrep® 5000 method. In fact, several previous studies reported that the cellularity in ThinPrep® method is not superior to conventional method [16-17].

As a limitation of the study, we should point out the absence of malignancy in all the examined samples, as all the pleural and peritoneal samples showed normal cytological features. Subsequently, the measurement of sensitivity and specificity parameters as a criteria for the assessment of ThinPrep® 5000 method and conventional method was not performed. Thus future investigation should include samples with different diagnostic categories such as benign and malignant. In addition, it is recommended to use direct-to-vial samples, rather than split samples, for ThinPrep® 5000 to yield peritoneal and pleural fluids with superior cytological features [18,19].

Despite the absence of abnormal cases in this study, the results of this study slightly disagree with recently published clinical article [20]. This randomized controlled trial, which involved 89784 Dutch women and used a similar liquid-based cytology system called ThinPrep® (Hologic Corp), indicated that ThinPrep® method did not perform better than conventional method in terms of sensitivity and positive predictive value for the detection of cervical cancer. Similar finding was also reported [21]. In contrast to above clinical studies, Swedish randomized clinical trial, which involved 13484 women, showed that ThinPrep® slides produced a significantly higher yield of histologic high-grade lesions compared with conventional method [22]. Recent similar finding was also reported [23]. In addition, the College of American Pathologists reported no significant difference in performance between ThinPrep® processor and conventional method on various body cavity fluid specimens [24]. This disparity between different clinical trials may indicate the need for further investigations using both liquid-based cytology system and conventional method. The findings of this study support and recommend further trials before considering ThinPrep® method as the method of choice in preparing cytological smears.

5. CONCLUSION

The findings of this study support the use of ThinPrep® 5000 system in the diagnosis of pleural and peritoneal samples.

COMPETING INTERESTS

No financial disclosure information related to this study and we declare that no competing interests exist.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=543&id=32&aid=4785