10.5005/jp-journals-10037-1120

ORIGINAL RESEARCH

Overhead Projector Sheet: An Alternative to Glass Slide in Cytology

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ABSTRACT

Introduction: Oral exfoliative cytology (OEC) is used by pathologists for visualizing cellular and nuclear changes, especially for initial screening. Traditionally, glass slides are used for OEC. As OEC is commonly used as an adjunct to biopsy and for mass screenings, it is necessary to minimize the cost of the procedure.

Aim: The aim of this study is to compare hematoxylin and eosin (H&E) as well as periodic acid–Schiff (PAS) staining quality of cytological smear on glass slide with that of overhead projector sheets (OHP).

Materials and methods: Smears were taken and spread on glass slide and OHP sheet and each of such set was stained using H&E and PAS stains. Scoring was done based on cellular morphology, overall staining, and nuclear details, under 10× and 40× magnifications. Photographs were taken on 1, 7, 21, and 35 days of preparation. All parameters of smear were compared between glass slide and OHP sheet. Descriptive statistics and "quality index" were obtained for glass slide and OHP sheet (quality index = actual score obtained/maximum score possible) and compared.

Results: There was no statistically significant difference between glass slides and OHP sheets for all smear parameters with H&E staining on quality index. With respect to PAS, glass slides had better score for nuclear details, in 40× magnification.

Conclusion: Glass slides are current gold standard for smear preparation. Our study shows glass slides and OHP sheets show no quality difference, with respect to cellular and nuclear morphology. Overhead projector sheets have potential to be used in mass screening programs in resource-constrained settings because of its cost-effectiveness and ease of handling and storage.

Keywords: Exfoliative cytology, Hematoxylin and eosin, Overhead projector sheet, Periodic acid–Schiff, Stain.

How to cite this article: Joseph I, Sudharsan R, Thavarajah R, Umadevi KRM, Ranganathan K. Overhead Projector Sheet: An Alternative to Glass Slide in Cytology. Oral Maxillofac Pathol J 2018;9(1):6-10.

Source of support: Nil

Conflict of interest: None

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INTRODUCTION

Oral exfoliative cytology enables pathologists to visualize the nuclear and cellular morphological changes. Besides serving as an initial screening tool for malignant and potentially malignant oral lesions, it also helps the oral and maxillofacial pathologists to screen for oral vesiculobullous lesions, metabolic and hormonal changes, and other suspicious lesions. Clinically, OEC is used as preliminary screening tool for malignant and potentially malignant oral lesions.[1] In a bid to source a better replacement for glass slides, especially for mass screening procedures, for the very first time, we attempted with the OHP sheets to study the cellular and nuclear morphology in cytological smears. We propose this substitute for glass slides in community-based screening programs and limited-resource settings.

The OEC methodology is an economical, easy, noninvasive technique that is well tolerated by patients. The use of glass slide for this purpose has remained unchanged for many years. Storing glass slides for medical and medicolegal purposes requires space. Also, the introduction of rapid slide screening protocols and machines renders digital format an easier and convenient method for transfer and indefinite storage of data in secured, digital format.

Under this scenario, there is a need for an economical and viable alternative for glass slide. The new material needs to be relatively cheaper, easier to dispose after use with a lower carbon footprint, easily available, and easy to handle and transport in mass screening programs.

Previous literature reports use of cellulose acetate sheet as a substitute for glass slides in histopathology because of its advantages in transportation and storage.[2] Desilverized X-ray sheet was used, which lost its popularity as glass was relatively cheaper material then, while acetate sheets were costlier. With the introduction and advent of mass production, presently, the situation is reversed with respect to cost. Glass is now costlier than OHP sheets and, advancement in material science has

resulted in better quality OHP sheets as they are cheaper, easily sourced, and very cost-effective.

MATERIALS AND METHODS

The present study was conducted to compare the efficacy of glass slides and OHP sheets in cytological smears and to validate the efficacy of OHP as a potential substitute for glass slides.

Standard glass slides (Blue Star©, India) and commercially available OHP sheets (Oddy®, India) procured from the market were used in this study. A single OHP sheet, cut into the size of the slides, was used for this study. Oral buccal smears were taken from five patients consecutively, irrespective of their demographics and the status of the oral mucosa. Four smears from each patient were taken, two on glass slides and two on OHP sheets and fixed with ethanol. A smaller OHP strip of the same thickness was used as the coverslip with the mountant and other processing materials remaining the same. Each pair of glass slide and OHP sheet was stained using standard, prescribed H&E as well as PAS staining methods. Since the OHP sheet has a potential for sagging during microscopic viewing, a custom-made holder was designed to hold it in place, on the stage of the microscope (Figs 1 and 2).

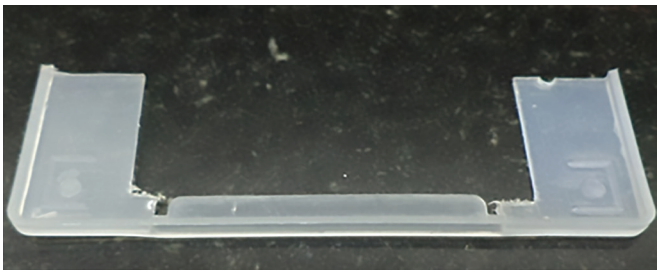


Fig. 1: Custom-made holder

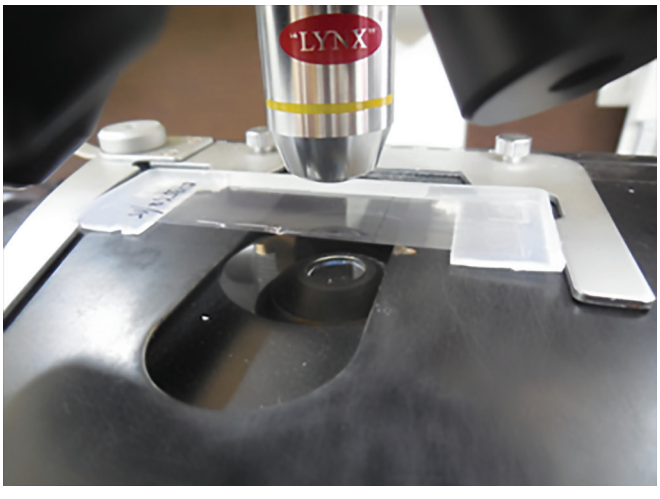


Fig. 2: Overhead projector sheets sheet with the holder on the microscopic stage

After mounting the slides and sheets, they were allowed to dry for viewing. Photographs were taken of the smear under light microscope on both 10× and 40× magnification on the 1st day, 7th day, 21st day, and 35th day. A Microsoft PowerPoint presentation of the photographs without labels was created and was displayed to the observers for scoring. It was a blinded study. Scoring for the slides were done by 12 blinded observers with varying degrees of experience (8 were students and 2 mid-level and 2 senior-level staff members of the Department of Oral Pathology, who were unrelated to this study). Scoring was performed based on the following criteria: Cellular morphology (score 1—not well preserved, score 2—well preserved), overall staining (score 1—inadequate, score 2—adequate), and nuclear details (score 1—not clear, score 2—clear). A "quality index" was obtained for each, glass slide and OHP sheet (quality index = actual score obtained/maximum score possible), and compared.[3]

The following formulae were used for calculation:

Actual score = total score (3 parameters)

Maximum score possible = 3 (parameters) × 2 (maximum score of criteria) × 240 (number of slides)

The score for each parameter was derived as follows:

I. Cellular morphology (Table 1):

(a) 1—not well preserved (glass slide– 10×, H&E) = 36

Number 36 refers to the number of photographs which have obtained the score 1—not well preserved, in turn multiplied by the parameter score: 1

Therefore, the cellular morphology score obtained for 36 (10× glass slides) stained by H&E is 36.

(b) 2—well preserved (glass slide—10×, H&E) = 204

Number 204 refers to the number of photographs which have obtained the score 2—well preserved, in turn multiplied by the parameter score: 2

Therefore, the cellular morphology score obtained for 204 (10× glass slides) stained by H&E is 408.

The quality index for each parameter was derived as follows (Table 2):

For example, cellular morphology (quality index of glass slide with H&E stain, 10×) = cellular morphology score/maximum score possible, i.e., 444/1,440 = 0.31

RESULTS

Cellular morphology, overall staining, and nuclear details of both glass slide and OHP sheet smears (H&E and PAS stains) are shown in Figures 3 to 6 respectively.

The quality index of slide and OHP sheet using H&E stain showed cellular morphology score for 10× of 0.31 and 0.29 respectively, and for 40× of 0.3 and 0.28 respectively. For overall staining and nuclear details, the quality index of

Table 1: Parameters on H&E stain (10×, 40×)

	10×				40×			
Parameters	*Glass slide*	*Score*	*OHP*	*Score*	*Glass slide*	*Score*	*OHP*	*Score*
I Cellular morphology								
1 Not well preserved	36	36	65	65	45	45	81	81
2 Well preserved	204	408	175	350	195	390	159	318
Cellular morphology score		444		415		435		399
II Overall staining								
1 Inadequate	43	43	68	68	45	45	84	84
2 Adequate	197	394	172	344	195	390	156	312
Overall staining score		437		412		435		396
III Nuclear details								
1 Not clear	46	46	75	75	43	43	84	84
2 Clear	194	388	165	330	197	394	156	312
Nuclear details score		434		405		437		396
Actual score obtained		1,315		1,232		1,307		1,191
Maximum score possible		1,440		1,440		1,440		1,440
Quality index		0.91		0.85		0.91		0.83

Table 2: Quality index of parameters based on H&E stain

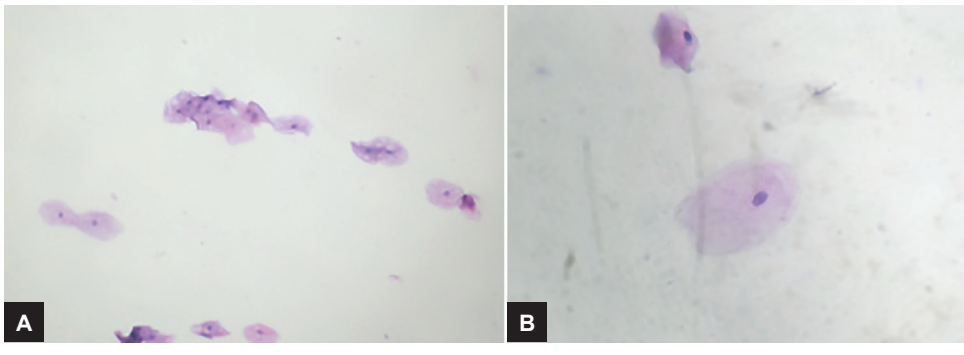
Quality index (H&E stain)				
	Slide		*OHP*	
Parameters	*10×*	*40×*	*10×*	*40×*
Cellular morphology	0.31	0.3	0.29	0.28
Overall staining	0.3	0.3	0.28	0.28
Nuclear details	0.3	0.3	0.28	0.28

slide and OHP sheet under both magnifications (10×, 40×) were similar, namely, 0.3 and 0.28 respectively (Table 3).

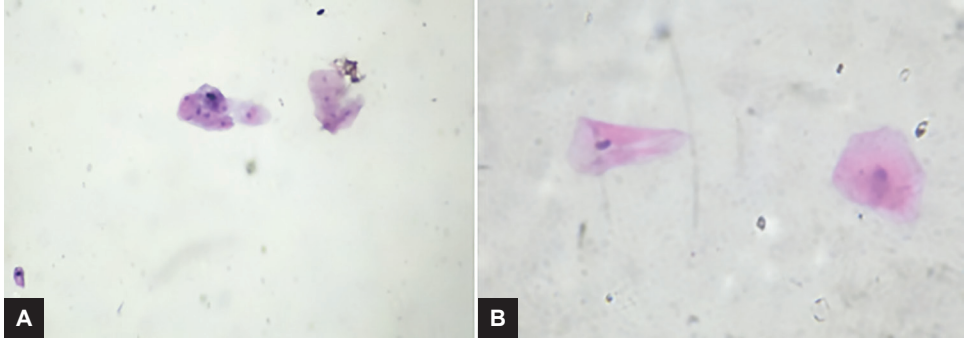
When the quality index of slide and OHP sheet using PAS stain was seen for cellular morphology, 10× showed 0.26 and 0.24, and 40× showed 0.29 and 0.23 respectively. Quality index for overall staining parameter of glass slide and OHP sheet showed for 10× 0.25 and 0.24, for 40× 0.27 and 0.22 respectively. Nuclear details comparing glass slide and OHP sheet showed under 10× 0.25 and 0.24 and under 40× 0.3 and 0.23 respectively (Table 4).

DISCUSSION

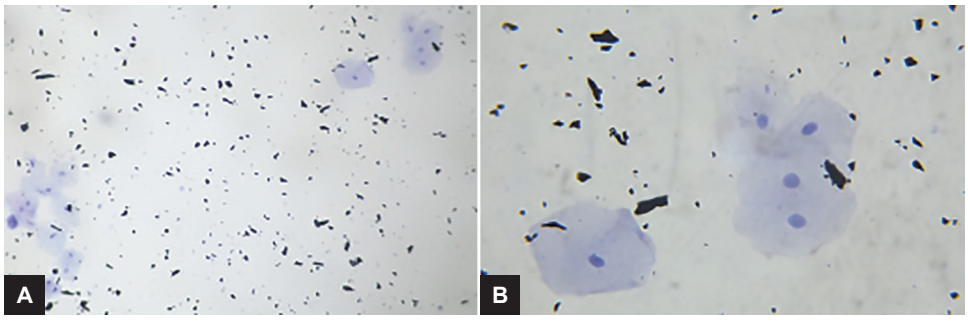
The purpose of this work was to compare the utility of glass slide and OHP sheet for using H&E and PAS



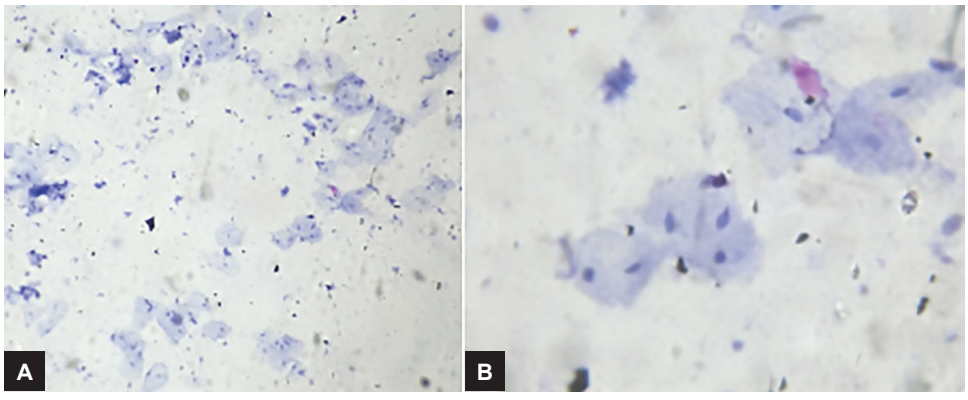
Figs 3A and B: Cytological image in glass slide [H&E stain, ×100 (A) and ×400 (B) respectively]



Figs 4A and B: Cytological image in OHP sheet [H&E stain, ×100 (A) and ×400 (B) respectively]



Figs 5A and B: Cytological image in glass slide [PAS stain, ×100 (A) and ×400 (B) respectively]



Figs 6A and B: Cytological image in OHP sheet [PAS stain, ×100 (A) and ×400 (B) respectively]

staining methods and to assess the usefulness of OHP sheet in cytology.

For H&E stain, the results of glass slide and OHP sheet under 10× and 40× magnifications, under all the parameters, cellular morphology, overall staining, and nuclear details, were alike (Table 3). In PAS stain, the results were compared for both glass slide and OHP sheet under 10× and 40× magnifications, and all the parameters were alike except nuclear details under 40× magnification. The nuclear details were smudgy for the OHP sheet (Fig. 6B) compared with the glass slide (Fig. 5B).

The quality index scores of OHP sheet and glass slide for PAS stain (40×) were 0.23 and 0.3 respectively (Table 4), showing the comparatively superior quality of glass slide over OHP sheet. In this case, the staining factor should also be taken into consideration owing to the smudgy appearance of the nuclei lacking the nuclear details.

The effect of reagents and stains did not impact the cellulose OHP sheets and they did not show any change in its staining properties when stored at room temperature for a period of 2 years. This makes archival of OHP sheets feasible for future reference and for academic purpose.

Table 3: Parameters on PAS stain (10×, 40×)

	10×				*40×*			
Parameters	*Glass slide*	*Score*	*OHP*	*Score*	*Glass slide*	*Score*	*OHP*	*Score*
I Cellular morphology								
1 Not well preserved	110	110	135	136	64	64	150	150
2 Well preserved	130	260	105	210	176	352	90	180
Cellular morphology score		370		346		416		330
II Overall staining								
1 Inadequate	117	117	137	137	93	93	167	167
2 Adequate	123	246	103	206	147	294	73	146
Overall staining score		363		343		387		313
III Nuclear details								
1 Not clear	112	112	137	137	51	51	147	147
2 Clear	128	256	103	206	189	378	93	186
Nuclear details score		368		343		429		333
Actual score obtained		1,101		1,032		1,232		976
Maximum score possible		1,440		1,440		1,440		1,440
Quality index		0.76		0.71		0.85		0.68

Table 4: Quality index of parameters based on PAS stain

Quality index (PAS stain)				
	Slide		*OHP*	
Parameters	*10×*	*40×*	*10×*	*40×*
Cellular morphology	0.26	0.29	0.24	0.23
Overall staining	0.25	0.27	0.24	0.22
Nuclear details	0.25	0.3	0.24	0.23

Additionally, when used in mass screening purposes, this cost-effective substitute saves precious resources such that only suspicious cases are studied further.

The main disadvantages of using OHP sheet would be the accumulation of dust particles and scratch formation because of low hardness, when used without proper care. Even the less transparent property of the OHP sheet, which was used as a coverslip, did not interfere with microscopic examination of the section. Their advantages include cost-effectiveness, ease of storage, and convenience of transport, since they are light, and unlike glass, need no protective packing.[2] Referral of OHP sheets (histopathology and cytopathology) to other pathologists will be convenient as they do not require special safety package in transportation as required in glass slides.

The use of OHP sheets in hematology for higher magnifications (100×) can also prove to be useful, as Karnauchow[2] stated in his observation that there was no difficulty in viewing sections with the oil-immersion objective.

The major advantage arises from saving on the carbon footprint. 1 kilogram of recycled glass is loaded with 1.4 kg CO_2e. With every glass slide weighing at least 6 gm, on an average, each slide produces 8.38 gm of CO_2e (http://www.greenrationbook.org.uk/resources/footprints-glass/). While commercial OHP is made up of Type I polyester and has 6 kg CO_2e (http://www.timeforchange.org/plastic-bags-and-plastic-bottles-CO_2-emissions), typically each A4 size sheet giving about 15 slide-sized sheets, with each A4 sheet weighing about 6 to 8 gm depending on thickness, produces less than 2.4 gm of CO_2e. Hence, use of the OHP sheet instead of glass slides saves the environment, promotes ease of handling, while saving precious resources. In conclusion, OHP sheets can be a helping hand for pathologists in mass screening programs in resource-poor settings because of its cost-effectiveness, environment-friendliness, and easy transportation. Although the formation of dust particles and scratches is quite inevitable in mass screening, the diagnosis of cytological smears is not hampered in any way and only the cases that require further study may be prescribed the traditional way.

ACKNOWLEDGMENTS

Authors sincerely would like to thank all the postgraduate students and staff of the Department of Oral and Maxillofacial Pathology, Ragas Dental College & Hospital, Chennai, Tamil Nadu, India for participating and extending their support in this study.

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