

Liquid-based Cytology for Oral Keratinocytes

Cellular and Molecular study

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INTRODUCTION

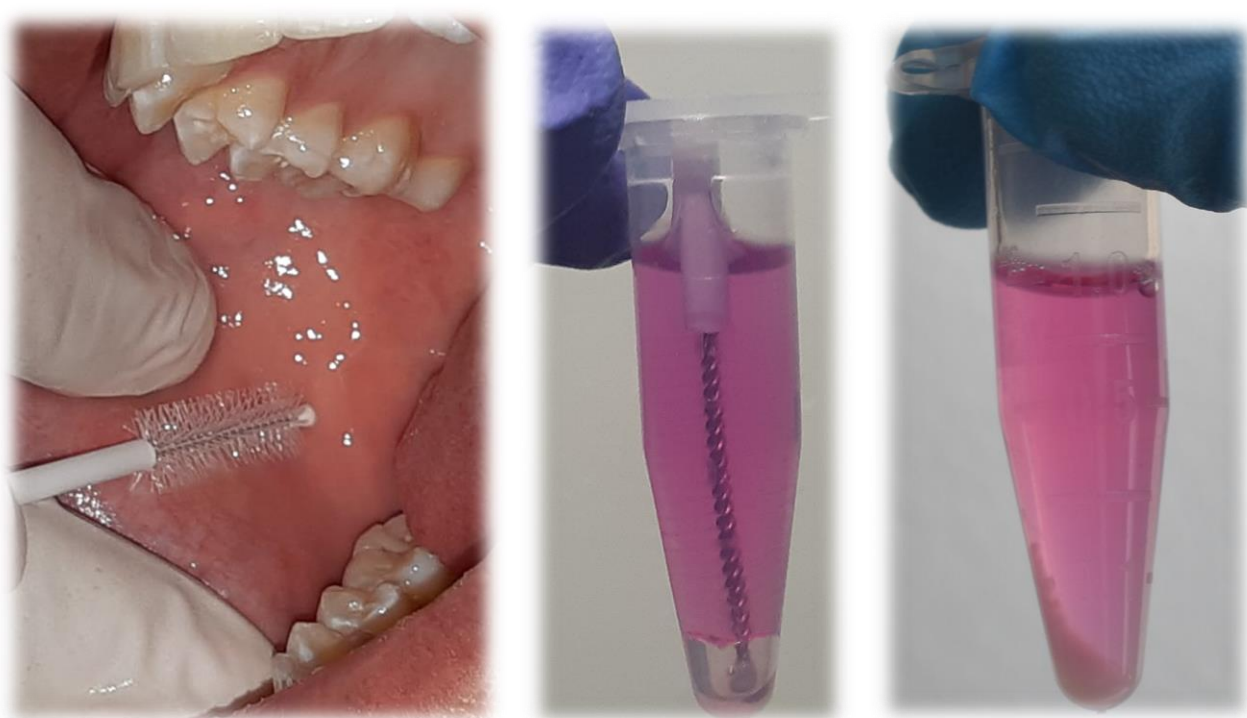
Exfoliative cytology is a simple non-aggressive technique that is well accepted by patients. Nevertheless, it has not been yet utilized as a diagnostic method for epithelial atypia because it requires a specialized pathologist for histology interpretation. This has limited its use widely in both diagnosis and research¹. Fortunately, the recent development of techniques such as immunophenotyping and molecular assays based on RNA², DNA and proteins have allowed the re-emergence of this technique for analysing the malignancy of a tumor³.

AIM

To validate a liquid-based cytology with a conventional cytobrush as a non-invasive method for the isolation and analysis of oral keratinocytes with cellular and molecular assays.

METHODS

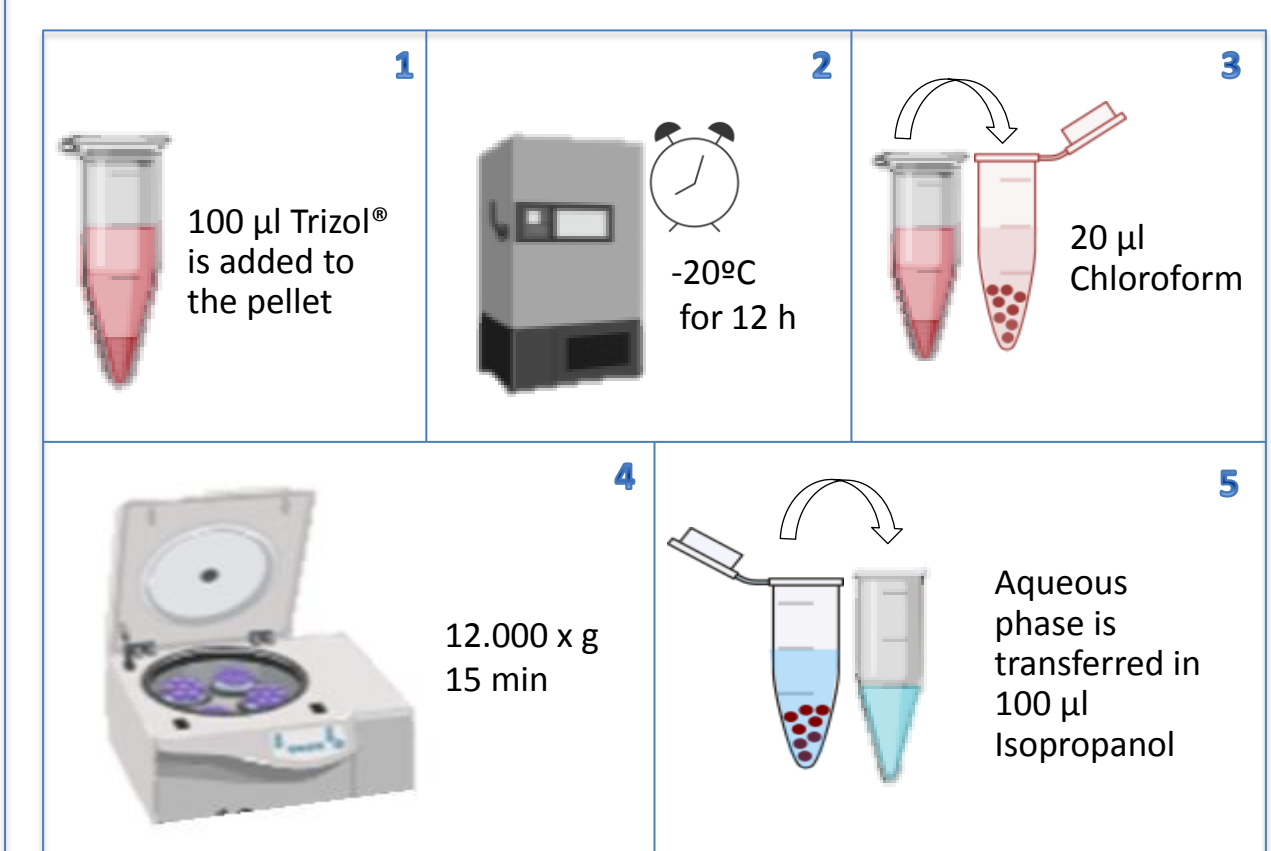
Sample Collection



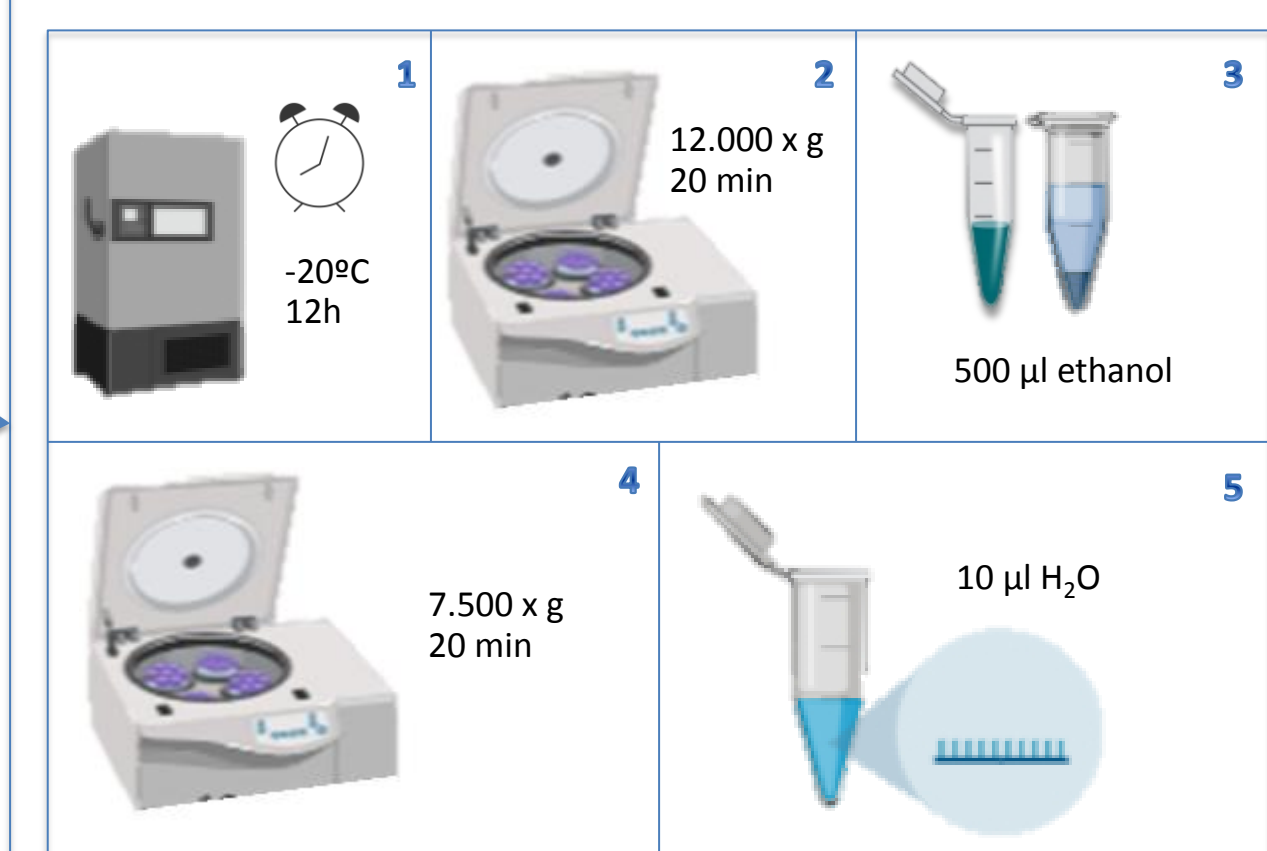
- 30 turns of the cytobrush
- D-MEM treated with DEPC
- Centrifugation at 400 x g to obtain the cell pellet

RNA Extraction

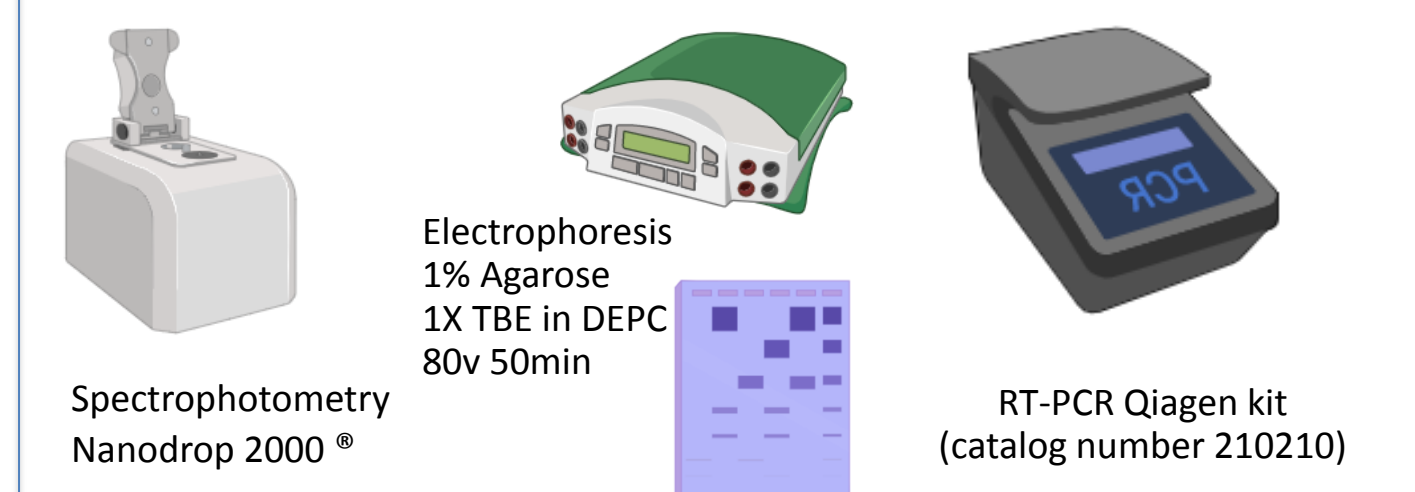
1. Separation Phase



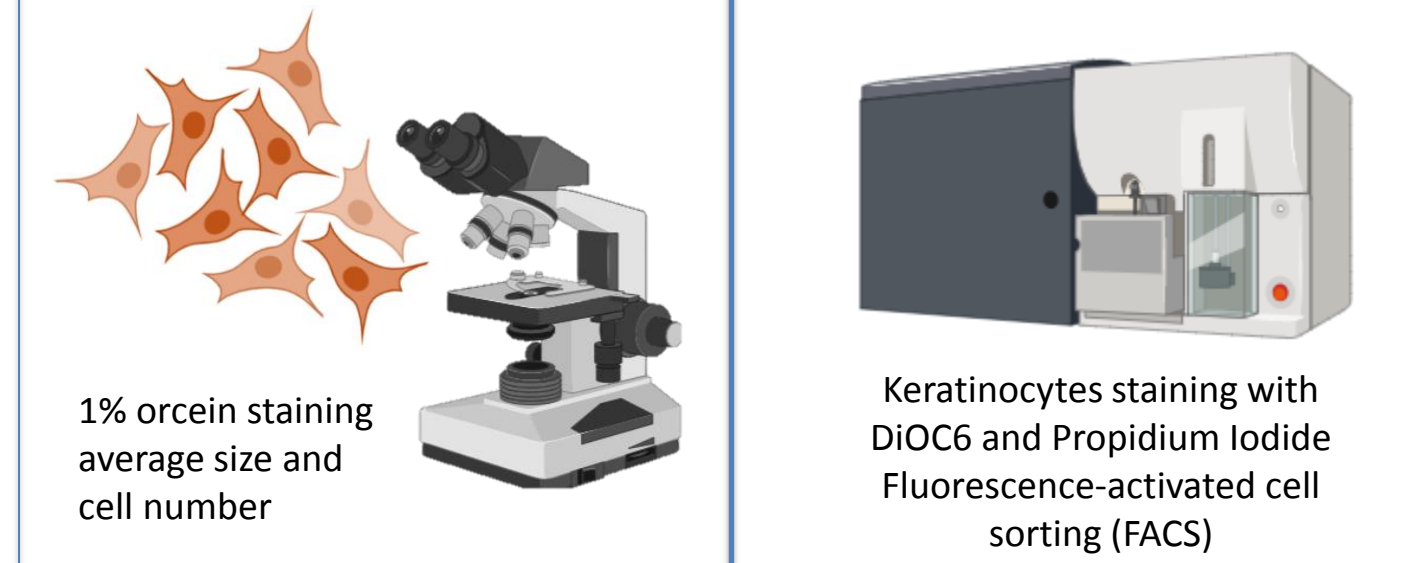
2. Precipitation and Wash Phase



Quantification and PCR



Cell Analysis



RESULTS

1. Isolation of intact intraepithelial keratinocytes

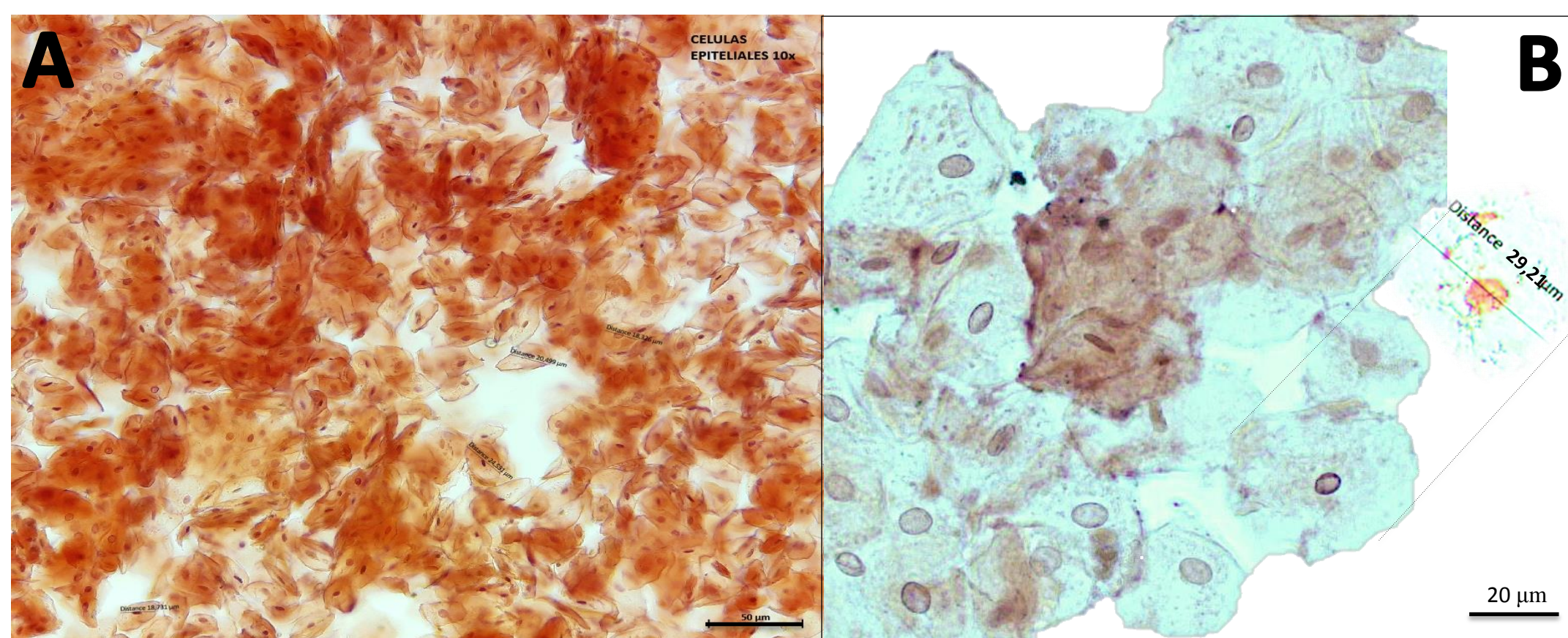


Figure 1. (A) Cellular integrity is observed in staining with 1% Orcein at 100 magnification. (B) Average keratinocyte size of 29.21µm is observed at 200 magnification. Zeiss Axio Imager 2

2. Viability of the keratinocytes is preserved only in DMEM treated with DEPC

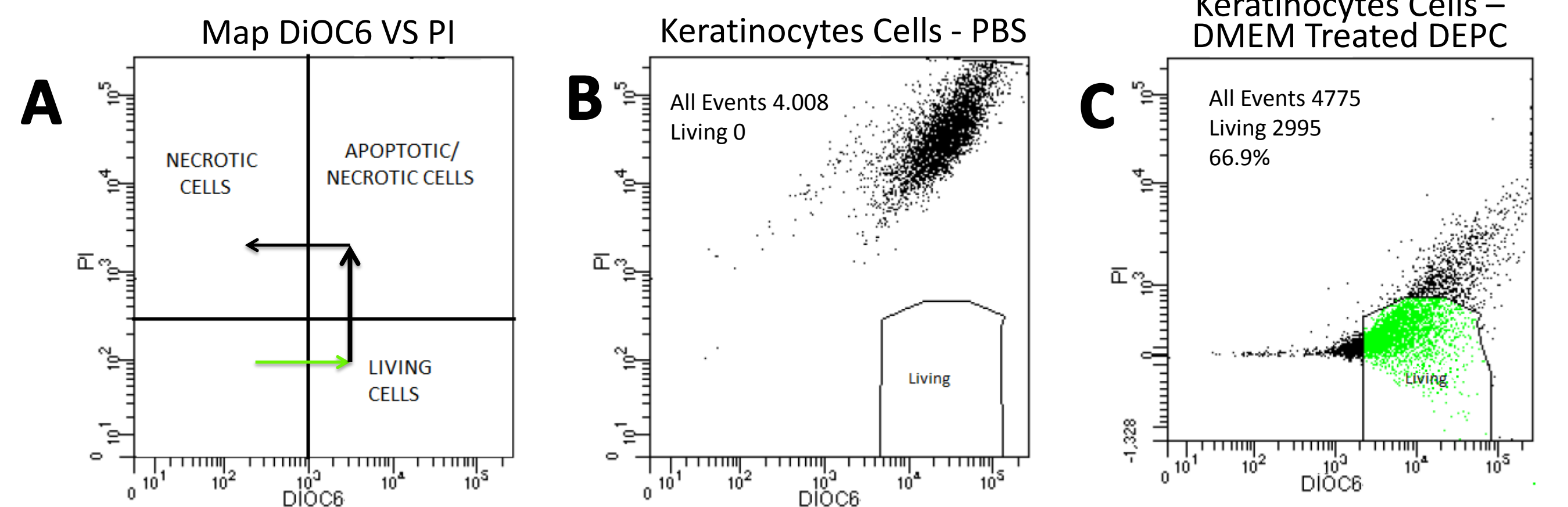


Figure 2. Cell viability assay by FACS. (A) Distribution of the cells according to DiOC6/PI double staining. (B) Cells are collected in PBS. (C) Cells are collected in DMEM treated with DEPC.

3. RNA quantity and quality improves in samples treated with DEPC

RNA Spectrophotometry		DMEM- DEPC	DMEM Without DEPC
n		17	17
Concentration (ng/µL)	Median	116.6	56.3
	Max	259	98.5
	Min	54	23.4
260/280	Median	1.55	1.32
	Max	1.86	1.53
	Min	1.41	1.21
260/230	Median	0.515	0.4
	Max	1.25	0.6
	Min	0.3	0.21

Table 1. RNA quantity and quality analysis. RNA purity was determined by absorbance ratios A260/A280 and A260/A230. The amount of RNA is calculated in ng/µL.

4. RNA integrity is comparable with a cell line control

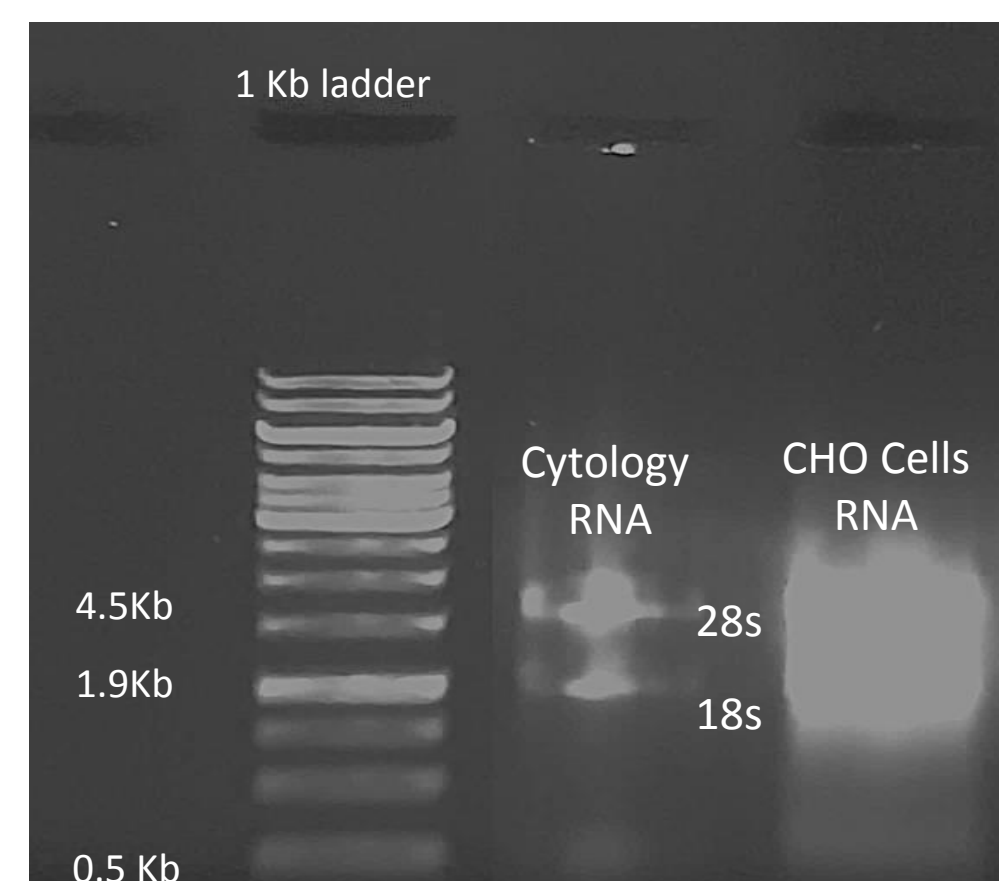


Figure 3. RNA electrophoresis. 400ng of each RNA sample is run in a 1% agarose gel at 80v 30 min.

5. cDNA can be amplified by RT-PCR

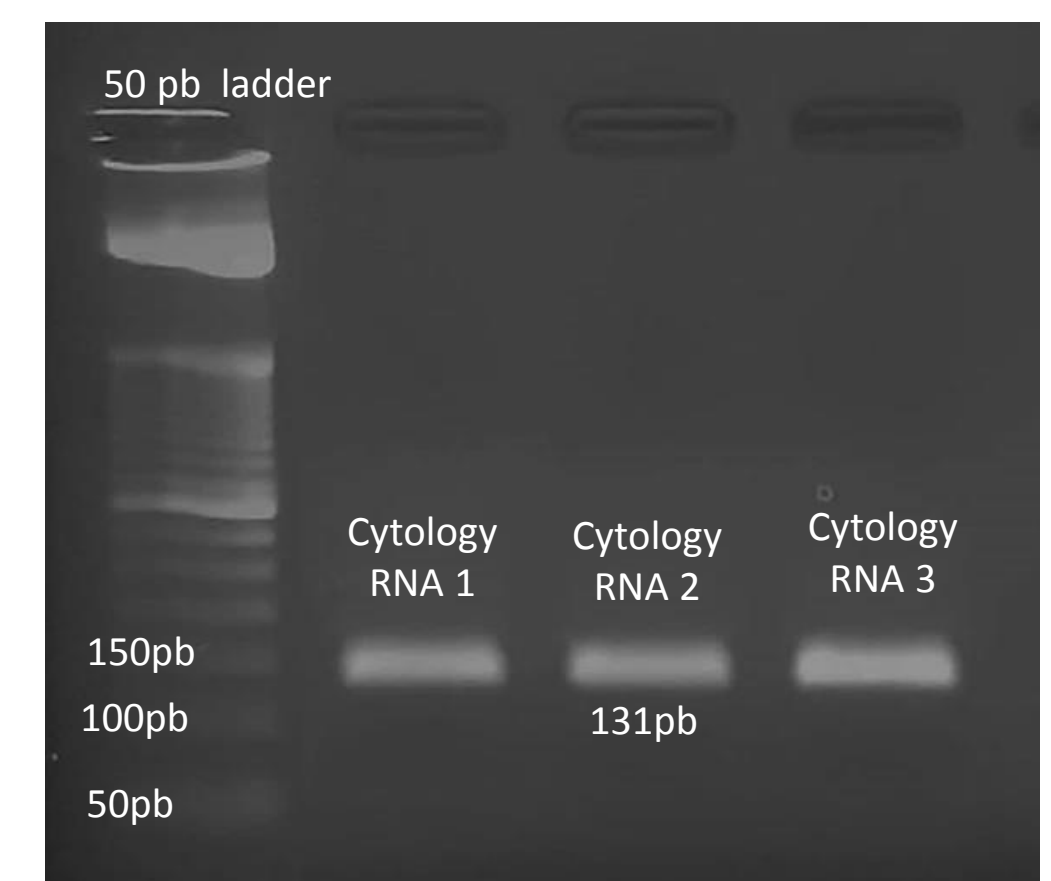


Figure 4. PCR product analysis. GAPDH amplification by RT-PCR is observed in a 1.5% agarose at 80v 55 min.

CONCLUSIONS

Liquid-based cytology, performed with a conventional cytobrush and DMEM treated with DEPC, is a reproducible technique that allows the retrieval of intact intraepithelial keratinocytes and the extraction of good quality RNA suitable for molecular analyses such as PCR. Although this test is proposed as a complementary non-invasive method to the clinical and traditional histological evaluation for the cellular and molecular study of oral mucosa pathologies; it requires further study.

REFERENCES

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2. Oliveira Alves MG, Pérez-Sayáns M, Padín-Iruegas ME, Reboiras-López MD, Suarez-Peñaranda JM, López-López R, et al. Comparison of RNA extraction methods for molecular analysis of oral cytology. Acta Stomatol Croat. 2016;50(2):108–15.
3. Roe CJ, Hanley KZ. Updates in Cervical Cytology: The 90-Year-Long Journey from Battle Creek to Today. Surg Pathol Clin. 2018;11(3):589–599

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