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Immunocytochemical Stability of Antigens in Cytological Samples Stored in In-house Liquid-Based Medium

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Abstract : The decision for immunocytochemistry (ICC) is usually made in the basis of the findings in Giemsa- and/or Papanicolaou- smears. More demanding diagnostic cases require preparation of additional cytological preparations. Therefore, it is convenient to suspend cytological samples in a liquid based medium (LBM) that preserve antigen and morphological properties. However, the duration of these properties being preserved in the medium is usually unknown. Eventually, cell morphology becomes impaired and altered, as well as antigen properties may be lost or become diffused. In this study, the influence of cytological sample storage length in in-house liquid based medium on antigen properties and cell morphology is evaluated. The question is how long the cytological samples in this medium can be stored so that the results of immunocytochemical reactions are still reliable and can be safely used in routine cytopathological diagnostics. The stability of 6 ICC markers that are most frequently used in everyday routine work were tested; Cytokeratin AE1/AE3, Calretinin, Epithelial specific antigen Ep-CAM (MOC-31), CD 45, Oestrogen receptor (ER), and Melanoma triple cocktail were tested on methanol fixed cytospins prepared from fresh fine needle aspiration biopsies, effusion samples, and disintegrated lymph nodes suspended in in-house cell medium. Cytospins were prepared on the day of the sampling as well as on the second, fourth, fifth, and eight day after sample collection. Next, they were fixed in methanol and immunocytochemically stained. Finally, the percentage of positive stained cells, reaction intensity, counterstaining, and cell morphology were assessed using two assessment methods: the internal assessment and the UK NEQAS ICC scheme assessment. Results show that the antigen properties for Cytokeratin AE1/AE3, MOC-31, CD 45, ER, and Melanoma triple cocktail were preserved even after 8 days of storage in in-house LBM, while the antigen properties for Calretinin remained unchanged only for 4 days. The key parameters for assessing detection of antigen are the proportion of cells with a positive reaction and intensity of staining. Well preserved cell morphology is highly important for reliable interpretation of ICC reaction. Therefore, it would be valuable to perform a similar analysis for other ICC markers to determine the duration in which the antigen and morphological properties are preserved in LBM.

Keywords : cytology samples, cytospins, immunocytochemistry, liquid-based cytology
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