

Methanol as an alternative fixative for cytological smears

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Abstract

Ninety-five percent (95%) ethanol is the standard cytological fixative used in many laboratories. Commercially available ethanol is expensive and not freely available in some institutions. Methanol, a tissue dehydrant, is also known to be a cytological fixative. However its efficacy has not been assessed or documented in the literature.

One hundred and eight consecutive fine needle aspiration biopsies (FNAB) of thyroid performed at the Department of Pathology, Faculty of Medicine, Colombo were included in a study to assess the efficacy of methanol as a cytological fixative. Aspirated material was smeared on at least 2 slides, one fixed in ethanol and the other in methanol, and stained with haematoxylin and eosin (H&E). The 2 smears were separately assessed for preservation of colloid and cells (nuclei and cytoplasm), as determined by the staining quality with the H&E stain. A score was given for each smear and the final scores for ethanol and methanol were statistically compared. The evaporation rates for ethanol and methanol were calculated.

The total score for preservation of colloid was 294/300 (98%) for methanol and 291/300 (97%) for ethanol ($p=0.4$). The total score for preservation of cells (nuclear and cytoplasmic) was 276/279 (98.9%) for methanol and 274/279 (98.2%) for ethanol ($p=0.7$). The evaporation rates per 100ml when the bottles used for fixation were kept closed and open per 24 hours were 1 and 37 for methanol and 0 and 17 for ethanol. Literature search did not show inhalational side effects of methanol in humans under standard laboratory conditions.

We conclude that methanol is as effective as ethanol for fixation of smears and cheaper.

Key words: Methanol, cytology, fixative

INTRODUCTION

Alcohol plays an important role in the processing of histological specimens as a tissue dehydrant, and in cytology as a fixative. Good fixation is necessary for preservation of cellular details, enabling accurate cytological assessment and diagnosis. The routine fixative used is 95% ethanol which is proven to be an efficient one. However, it is expensive, and subject to pilferage due to its addictive properties. Therefore ethanol is not freely available in some clinics and hospitals.

Methylated spirits (methanol) is being used as an alternative, cheap dehydrating agent for histopathological sections in many laboratories including some in Sri Lanka. Toxic effects due to low dose inhalation or skin contamination have not been observed in laboratory settings. Methanol is also being used as a cytological fixative in some laboratories. However its effectiveness has not been evaluated and documented. A literature review did not show

any publications referring to its use as a cytological fixative. We thus undertook a study to assess and document the efficacy of methanol as an alternative fixative for cytological smears.

MATERIALS AND METHODS

One hundred and eight consecutive fine needle aspiration biopsies (FNAB) of thyroid performed at the Department of Pathology, Faculty of Medicine, Colombo were included in the study. Aspirated samples from every patient were smeared on a minimum of two slides; one being fixed in 95% ethanol and the other in commercially available methanol (approximately 99% concentration). The smears were fixed immediately in methanol and ethanol without air drying. After an adequate period of fixation (not less than 15 minutes), the slides were labeled 'A' or 'B' and stained with haematoxylin and eosin (H&E) by the 3rd author.

The first two authors examined the stained slides blindly and allotted a score of 1

(satisfactory), 2 (good) or 3 (very good) for preservation of colloid and cells (cytoplasm and nuclei) separately. The total scores for methanol and ethanol were added for each of the above components and statistically compared. Epi info 6 package was used for statistical comparison.

The preservation of colloid and cells was determined by their appearance in **H&E** stained smears. The colloid was considered to show very good preservation when stained pink or purplish with linear cracks depending on the amount of colloid present. The cells were considered to be well preserved when they showed well stained nuclei with well defined nuclear chromatin and nuclear membranes and intact cytoplasm. Nucleoli and specific features such as nuclear inclusions and grooves were appreciated in relevant lesions. Nuclear and cytoplasmic staining was scored together (as cell preservation) per smear, as preservation did not differ significantly in the 2 components of cells.

The evaporation rates were calculated when lids of containers were kept closed and open for methanol and ethanol separately.

RESULTS

One hundred of the 108 smears were adequate for assessment. All of them had colloid, 93 had cells. The total score for preservation of colloid was 2941300 (98%) for methanol and 2911300 (97%) for ethanol ($p=0.4$). The total score for preservation of cells (nuclei and cytoplasm) was 2761279 (98.9%) for methanol and 2741279 (98.2%) for ethanol ($p=0.7$).

There was no difference in preservation of colloid and cells, and staining quality of cytoplasmic and nuclear details when smears were fixed in either fixative (Figures 1, 2 & 3).

The evaporation rates per 100ml when the bottles used for fixation were kept closed and open per 24 hours were 1 and 37 for Methanol and 0 and 17 for Ethanol.

DISCUSSION

This study shows that methanol is as efficacious as ethanol as a fixative for smears. **FNABs** of thyroid were chosen due to several reasons. They are the most common samples received for cytological evaluation in our laboratory and

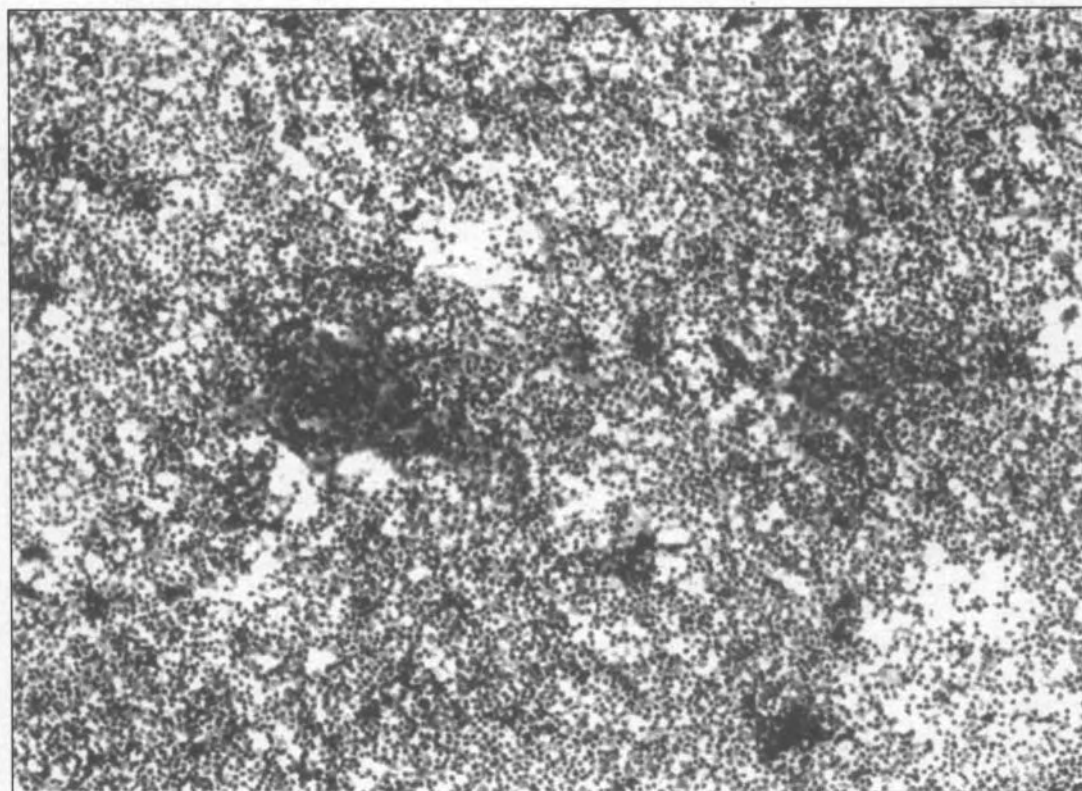


FIG. 1: Overall good preservation of lymphocytes and rare epithelial cells in an aspirate of lymphocytic thyroiditis, with methanol fixation (H&E x 100).

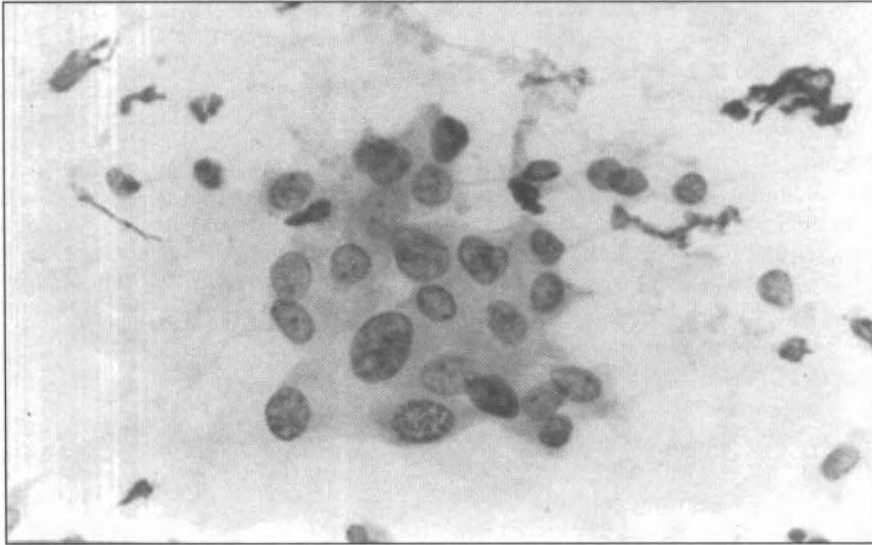


FIG. 2: Very good cell preservation with methanol fixation: well stained chromatin with well delineated nuclear membranes and intact cytoplasm in epithelial cells, showing Hurthle cell change in lymphocytic thyroiditis (H&E \times 400).

many others. The site of the lesion is easily palpable and accessible, the yield of material is usually plentiful. Additionally, thyroid smears allow assessment of 2 components, namely colloid and cells (nuclei and cytoplasm) in one smear unlike most others with a yield of cells only.

Preservation of cellular (cytoplasmic and nuclear) details were exceptionally good (nearly

99% of total possible score) with fixation in methanol. Therefore it can be concluded that any smear in which preservation of cellular details is necessary can be adequately assessed with fixation in methanol. Methanol preserves nuclear and cytoplasmic details and colloid as much as ethanol.

Methanol evaporates faster if the containers are kept open, however, under standard

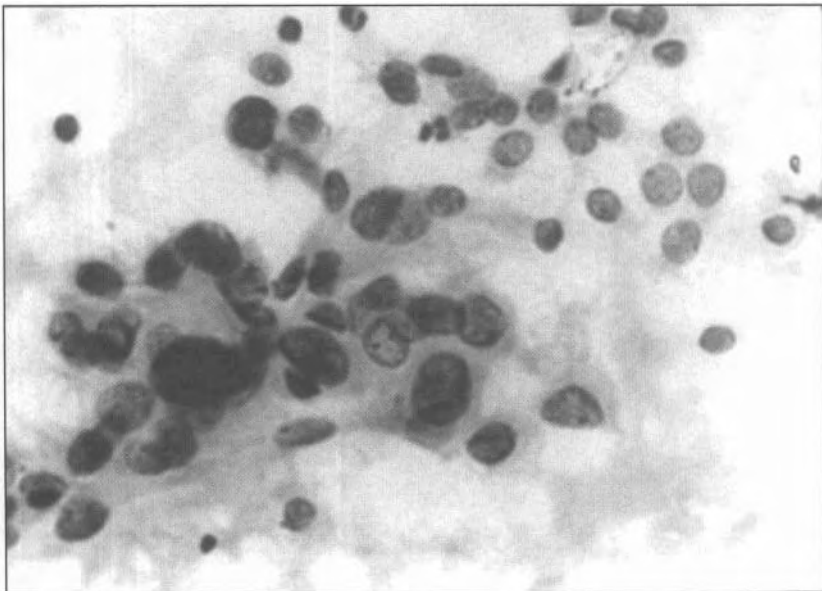


FIG. 3: The smears of aspirates of the case shown in Fig. 2, fixed in ethanol showing similar nuclear features (H&E \times 400).

laboratory procedures, bottles except when in immediate use should be kept tightly closed.

If methanol is used in adequately ventilated laboratories equipped with exhaust fans, inhalation of vapour from the small containers used for fixation is minimal. Kavet & Nauss showed that accumulation of formate (the toxic metabolite of methanol) will not challenge the metabolic capacity of the folate pathway at low levels of exposure to methanol in humans.² Medinsky & Dorman subjected human volunteers to low doses of methanol and proved that blood formate levels did not rise above endogenous concentrations, concluding that humans may not be at added risk of neurotoxic effects resulting from exposure to low levels of methanol.³ Studies in non human primates show similar results.^{4,5} Studies in rodents however, show that high doses can cause foetal defects.^{6,7}

Methanol is also well absorbed through intact skin, but no step in the performance of the FNAB or fixation or staining of slides necessitates skin contact with methanol. Therefore, unless ingested or deliberately inhaled, it is safe for use in the laboratory. The problem of pilferage does not exist for methanol. However, when methanol is used adequate warning should be given to laboratory staff of its ingestional side effects. On the other hand, methanol is used for many other laboratory procedures and is the routine tissue dehydrant used in some histology laboratories. Commercially available methanol is much cheaper than ethanol in many countries; in Sri Lanka it is 3 to 9 times cheaper.

We conclude that methanol is as effective as ethanol for fixation of smears. We recommend that if ethanol is unavailable due to any reason, methanol can be used as an effective substitute. If laboratory costs are high and economizing is necessary, methanol is a cheaper alternative cytological fixative to use.

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