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RESEARCH ARTICLE

# SUGAR SYRUP AS A CYTOLOGICAL FIXATIVE: A COMPARATIVE STUDY WITH 95% ETHANOL DURING ROUTINE PAPANICOLAOU STAINING

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## Abstract

**Background and Objectives:** Fixation is an initial and important step in cytology for microscopic examination in cytopathological techniques, which ensures the preservation of cell morphology and structures. The routine cytological fixative at Muhimbili National Hospital (MNH), Central Pathology Laboratory (CPL), is 95% ethanol, which causes shrinkage of the cell which disrupts the physical structure of almost any type of membrane, including the plasma membrane, membrane of cell organelles and liposomes. Objective of present study was to assess the effectiveness of 30% sugar syrup as a cytological fixative as compared to 95% Ethanol in Papanicolaou staining procedures in Cytology unit at MNH.
**Methods:** The study design was cross-sectional prospective study conducted at MNH in histopathology laboratory from April to August 2023. Whereby Thirty cytological samples were obtained from different bodily fluids, including pleural (n=12), ascetic (n=10), peritoneal (n=5), and pericardial (n=3). Whereby nuclear staining, cytoplasmic staining and cell morphology were examined under the light microscope at X40 magnification.
**Results:** A paired study of thirty specimens revealed that two fixatives created bad quality in two specimens (6.7%), ethanol alone produced good quality in eight specimens (26.7%), sugar syrup alone produced good quality in two specimens (6.7%), and both fixatives produced good overall staining quality in eighteen specimens (60%). While 30% sugar syrup produced acceptable staining quality in 20 of 30 specimens (66.7%), 95% ethanol demonstrated superior staining quality in 26 of 30 specimens (86.7%).
**Conclusion:** The paired statistical analysis did not reveal a significant disparity between the two fixatives, notwithstanding the greater absolute percentage of satisfactory staining quality observed with 95% ethanol. Consequently, 30% sugar syrup may serve as a viable alternative fixative when 95% ethanol is either unavailable or unsuitable.
**Keywords:** Alternative fixative, cytology, papanicolous stain, pap smear, cytologicalfixative, 30% sugar syrup fixative, 95% ethanol fixative.

## INTRODUCTION

The fixation of the sample is the first step that is taken in any cytological or histological laboratory procedure. Moreover, fixation is the very first and an important part of the processing of tissues for microscopic study[1]. The purpose of fixation is mainly to conserve the tissues in the living state, preventing putrefaction, auto-digestion, increasing the refractive index of the tissue. It is also the process in which the cells become chemically and physically stabilized, along with the preservation of all biochemical and enzymatic processes within the cell, so that the cells would not be affected morphologically or undergo decomposition due to any further treatment[2]. Smear technique is an important tool in the early diagnosis of lesions of a cancerous nature and inflammation. The fixative ensures that the cell is in its natural form within the living system[3]. There are certain reasons, for which the fixation of tissues is done, and they include retaining

the cell in the living form without altering its shape and size during the process, to prevent any autolysis, and to achieve good staining[4]. The use of cytopathology in the current age is considered as a valuable and acceptable diagnostic process[5]. The accuracy of diagnosis depends entirely on sample collection, fixing, staining, examination, and evaluation of the sample, and quality control. All these aspects are important during the diagnostic procedure. For example, performing oral exfoliative cytology is a non-invasive, easy, and painless process, which helps to manage the patient depend on the result from cytopathology laboratory[6]. So, each step on processing the sample must have a quality so as the result which provides to be in good and standard quality and for better diagnosis[7].

Actually, laboratory working focuses on processing patient sample and releasing diagnostic results of the patient to the clinician of a good quality. And 95% ethanol is conventionally used as a fixative for exfoliative cytology but has its own obstacles in which it's subjected to toxic, inflammable, expensive, evaporates easily, and requires license for its procurement, so the Histotechnologist are in constant search of a natural and eco-friendly cytological fixative. It has also been shown to be carcinogenic in some animal models[8]. With all those effect of 95% ethanol on healthy of Histotechnologist, this study has attempted to evaluate the efficacy of sugar syrup as a cytological fixative in cytopathology laboratory as compared to when 95% ethanol is used. Hence, 95% ethanol will be replaced with a bio friendly alternative. In cytopathology laboratory fixation is one of the important steps in the processing the cytology samples such as different body fluids, Fine needle aspiration samples, and different smears.

At MNH the routine fixative is 95% ethanol which causes shrinkage of the cell which disrupt the physical structure of almost any type of membrane, including the plasma membrane, membrane of cell organelles and liposomes, so there is not an alternative fixative at our setting which make them to be limited on using ethanol. Ethanol is much cost on purchasing so this study aims to reduce the cost by using sugar syrup as a cytological fixative in the laboratory[9].

This study was helped on assessing the effectiveness of 30% sugar syrup as a cytological fixative in a cytological sample which may lead to have an alternative cytological fixative in our setting. This will help to reduce the cost due to easy preparation of sugar syrup in the laboratory. Due to lack of study done in Tanzania, this study will lay foundation for further study in Tanzania.

## MATERIALS AND METHODS

### Study design

This was a cross-sectional prospective laboratory-based comparative study conducted at the Muhimbili National Hospital (MNH) Histopathology Laboratory from April 2023 to August 2023. The study employed a paired design in which each cytological specimen served as its own control: two smears were prepared from each specimen, with one fixed in 95% ethanol (standard fixative) and the other fixed in 30% sugar syrup (experimental fixative). This design eliminated inter-specimen variability and allowed direct comparison of fixative performance on identical cellular material

### Study population

All cytological specimens received at the Muhimbili National Hospital (MNH) Central Pathology Laboratory (CPL), Cytology Unit during the study period and Specimens referred from various clinical departments within MNH and affiliated healthcare facilities

### Inclusion criteria

Every cytological sample that satisfied sample processing requirements with sufficient cellularity (>10 cells per high-power field on initial smear)

### Exclusion criteria

Every cytological sample that didn't fit the processing requirements (such as samples with inadequate cellularity, extremely bloody samples, or samples exhibiting degeneration).

### Sampling procedures

The study included consecutive cytology specimens that were received at the CPL during the study period and satisfied the inclusion criteria. For the investigation, cytological samples from reception specifically, bodily fluid samples were employed. Among the specimen types were peritoneal fluid (n=5), ascetic fluid (n=10), pleural fluid (n=12), and pericardial fluid (n=3).

### Sample size

The formula for comparing two proportions in paired samples was used to determine the sample size. A minimum of 26 paired samples were needed, based on an anticipated 30% difference in excellent staining quality between fixatives (drawn from pilot data), with 80% power and $\alpha=0.05$. Thirty samples were used to account for any technological issues. Sample size was 30 samples as similar to comparative study which was conducted at Elrazi University of Khartoum, Sudan in 2022[10].

### Variables

Dependent variables were nuclear stain, cytoplasm stain and cell morphology in the smear. Independent variables were 30% sugar syrup and 95% ethanol.

### Smear preparation

Soon after the samples been received and brought tocytology unit. Samples were centrifuge 3500 RPM and supernatant were discarded and the remaining deposits were used for smear preparation Whereby Mayer albumin were applied onto the glass slides and for each sample two smears were made onto glass slides which Mayer albumin was applied.

### Fixation

95% ethanol solution was prepared by dissolving 475 ml of absolute ethanol into 25 ml of distilled water to make 500 ml of 95% ethanol. And 30% Sugar syrup solution was prepared by dissolving 150g of sugar into 500 ml of distilled water to make 500 ml of 30% Sugar syrup solution. And soon after the smears have been made, they were putted in different fixatives namely 95% ethanol solution and 30% Sugar Syrup solution for 30 minutes according to SOP.

**Staining and mounting**

Soon after smears have been fixed, smears were hydrated followed by nuclear stain with hematoxylin, then counter stain with Orange G and then background staining with Eosin Azure ending with dehydration and the slides were mounted and cover slipped using DPX ready to be examined by a light microscope at X40 magnification.

**Data collection methods**

A unique study number was assigned to each smear slides that were prepared and fixed with different fixative 30% Sugar Syrup and 95% ethanol for each. This unique study number was recorded on a data collection sheet. Using the previously mentioned standardized criteria, two anatomical pathologists independently assessed every stained smear while being blind to the fixative used for each slide. To ensure blinding, slide labels were covered using opaque tape to hide the fixative information, and fresh random codes were assigned. Neither pathologist had access to the fixative key during evaluation or participated in the laboratory processing. The 95% ethanol fixed- slides smear' data were collected separately from the 30% sugar syrup-fixed slides smear' data.

**Assessment criteria for staining quality**

Slides were evaluated at X40 magnification using the following standardized criteria. Based on the aforementioned criteria, each parameter received an independent "good" or "poor" grade. A slide was only considered to have "good" overall staining quality if it scored "good" on each of the three separate criteria. In order to reduce subjectivity and guarantee precise categorization for statistical analysis, this binary classification system was selected.

**Investigation tools and validity and reliability problems**

**Investigation tools**

For the slides to be observed, Bright field microscopes were employed in the investigation. The operations of these instruments were done following the MNH histopathology SOP instructions.

**Assessment of stained slides**

Once all the laboratory procedures had been completed, the stained slides were analyzed by the researcher. The slides were also assessed by senior laboratory staff and anatomical pathologists. Once the assessment was done, the findings were entered on specialized data recording forms. The smear was classified into three categories: nuclear staining, cytoplasmic staining and cell morphology

**Quality Assurance**

All procedures carried out in this experiment have been conducted in the histopathology laboratory at MNH. MNH histopathology laboratory is well equipped to conduct such studies All procedures have followed the SOPs of the histopathology laboratory. All the data collected have been recorded in relevant forms.

**Statistical analysis**

For paired statistical analysis, McNemar's test for dichotomous outcomes was chosen because paired samples were obtained from each specimen, one smear per each fixing reagent. Two-tailed $p$-value < 0.05 was considered statistically significant. Data obtained during the research was compared with data reported by literature sources, rationale for dissimilarities/ similarities was provided. Suggestions on how to apply good practices in the area under research were put forward.

## RESULTS

On staining quality, out of 30 samples fixed by 95% ethanol, 26(96.3%) showed good nuclear stain with a good staining quality, 1(3.7%) showed good nuclear stain with poor staining quality while 3 samples out of 30 samples fixed by 95% ethanol showed poor nuclear stain with poor staining quality ($p$<0.05). Compared to 30% Sugar Syrup whereby 20(90.9%) showed good nuclear stain with a good staining quality, 2(9.1%) showed good nuclear stain with poor staining quality while 8sample showed poor nuclear stain with poor staining quality ($p$<0.05) which was statistical significance. Out of 30 samples fixed by 95% ethanol, 24(100%) showed good cytoplasm stain with good staining quality without having good cytoplasm stain with poor staining quality while 2(33.3%) showed poor cytoplasm stain with good staining quality, 4(66.7%) showed poor cytoplasm stain with poor staining quality ($p$<0.05). Compared to 30% sugar syrup whereby 19(100%) showed good cytoplasm stain with good staining quality without having good cytoplasm stain with poor staining quality while 1(9.1%) showed poor cytoplasm stain with good staining quality, 10(90.9%) showed poor cytoplasm stain with poor staining quality ($p$< 0.05) which was statistical significance. Out of 30 samples fixed by 95% ethanol, 23(92%) showed good cell morphology stain with good staining quality, 2(8%) showed good cell morphology stain with poor staining quality while 3(60%) showed poor cell morphology stain with good staining quality, 2(40%) showed poor cell morphology stain with poor staining quality ($p$>0.05) which was not statistical significance. Compared to 30% sugar syrup whereby 16(100%) showed good cell morphology stain with good staining quality without having good cell morphology stain with poor staining quality while 4(28.6%) showed poor cell morphology stain with good staining quality, 10(71.4%) showed poor cell morphology stain with poor staining quality ($p$<0.05) which was statistical significance.

**Table 1: Paired comparison of overall staining quality (n=30 specimens).**

| | Sugar Syrup Good | Sugar Syrup Poor | Total |
|---|---|---|---|
| Ethanol Good | 18 (60%) | 8 (26.7%) | 26 (86.7%) |
| Ethanol Poor | 2 (6.7%) | 2 (6.7%) | 4 (13.3%) |
| Total | 20 (66.7%) | 10 (33.3%) | 30 (100%) |

**Table 2: Paired comparison of nuclear staining quality (n=30 specimens).**

| | Sugar Syrup Good | Sugar Syrup Poor | Total |
|---|---|---|---|
| Ethanol Good | 20 (66.7%) | 7 (23.3%) | 27 (90.0%) |
| Ethanol Poor | 2 (6.7%) | 1 (3.3%) | 3 (10.0%) |
| Total | 22 (73.3%) | 8 (26.7%) | 30 (100% |

**Table 3: Paired comparison of cytoplasmic staining quality (n=30 specimens).**

| | Sugar Syrup Good | Sugar Syrup Poor | Total |
|---|---|---|---|
| Ethanol Good | 16 (53.3%) | 8 (26.7%) | 24 (80.0%) |
| Ethanol Poor | 3 (10.0%) | 3 (10.0%) | 6 (20.0%) |
| Total | 19 (63.3%) | 11 (36.7%) | 30 (100%) |

**Table 4: Paired comparison of cell morphology quality (n=30 specimens).**

| | Sugar Syrup Good | Sugar Syrup Poor | Total |
|---|---|---|---|
| Ethanol Good | 14 (46.7%) | 11 (36.7%) | 25 (83.3%) |
| Ethanol Poor | 2 (6.7%) | 3 (10.0%) | 5 (16.7%) |
| Total | 16 (53.3%) | 14 (46.7%) | 30 (100%) |

The major study employed paired statistical techniques to directly compare the two fixatives on the same specimens since each of the thirty specimens yielded paired smears (one fixed in ethanol, one in sugar syrup. Overall staining quality revealed that 26 (86.7%) of the 30 samples treated with 95% ethanol had high staining quality, while 4 (13.3%) had poor staining quality. Twenty (66.7%) of the samples treated with 30% sugar syrup had good staining quality, while ten (33.3%) had poor staining quality. Nevertheless, there was no statistically significant difference between the two fixatives for cytoplasmic staining ($p$=0.228), nuclear staining ($p$=0.182), or overall staining quality ($p$=0.109) according to paired analysis using McNemar's test.

## DISCUSSION

The effect of 95% ethanol and 30% sugar syrup on cytological smear preparation by means of fixatives in paired samples was analyzed in the current experiment. In most cases, the use of ethanol for smear fixation contributed to a larger number of smears that had good staining results. Specifically, the percentage of smears that stained well is higher for ethanol (86.7%) than for sugar syrup (66.7%)[10]. It indicates that the use of ethanol as a fixative resulted in a more stable staining result. A statistically significant difference was observed for cell morphology ($p$=0.019), with ethanol demonstrating better preservation.

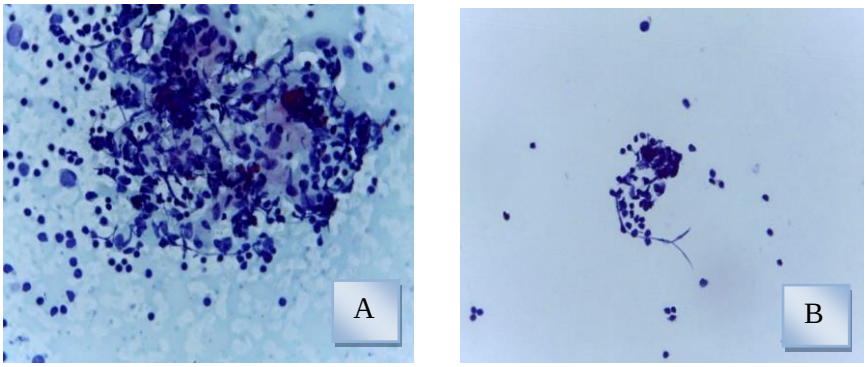


**Figure 1**: **(A): Papanicolou stained smear fixed with 95% ethanol of representing good nuclear stain (X40). (B): Papanicolou stained smear fixed with 30% sugar syrup of representing good nuclear stain (X40).**

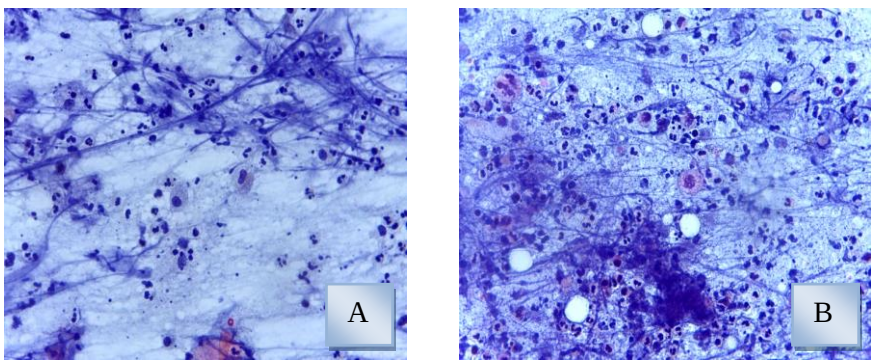


**Figure 2: (A): Papanicolou stained smear fixed with 95% ethanol of representing good cytoplasm and cell morphology staining (X40). (B): Papanicolou stained smear fixed with 30% sugar syrup of representing good cytoplasm and cell morphology staining (X40).**

Nonetheless, although better nuclear and cytoplasmic staining was observed in ethanol smears, there was no significant difference between both fixatives[11]. There was a significant difference between two studied types of samples in regards to cell morphology preservation ($p$=0.019). As opposed to sugar syrup smears, ethanol-fixed smears were characterized by higher rates of preservation of morphology[12]. Consequently, even if the samples have high rates of nuclear and cytoplasmic staining, the alternative cannot be used for cytological analysis due to poor morphology preservation. Some smears with poor morphology were obtained in sugar syrup samples[13]. In summary, according to the results, 30% sugar syrup can be regarded as a cheap local substitute for 95% ethanol.

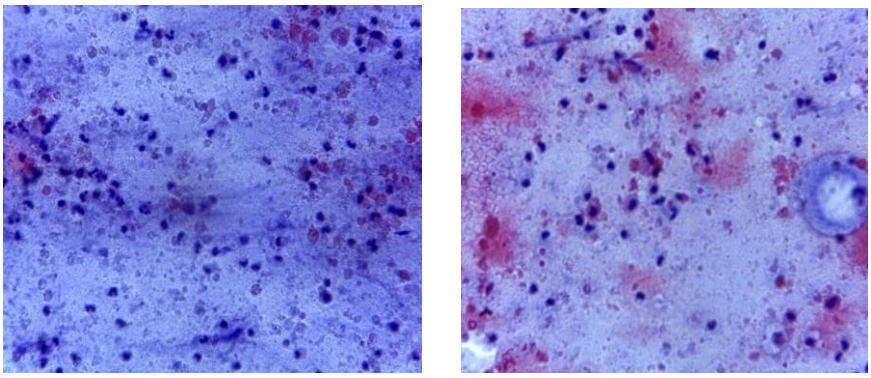

**Figure 3: (A): Papanicolou -stained smear fixed with 95% ethanol of representing poor cytoplasm and cell morphology staining (X40). (B): Papanicolou -stained smear fixed with 30% Sugar Syrup of representing poor cytoplasm and cell morphology staining (X40).**

Although some results did not match expectations, in most cases, the fixative appeared efficient in relation to staining quality. Meanwhile, it can be recommended to prefer ethanol fixation due to its better results in regard to morphology.

**Limitations of the study**

This was a single centre study with a relatively small sample size (though adequately powered). Specimen types were limited to body fluids; results may differ for other cytological specimens such as fine needle aspirates, cervical smears, or oral exfoliative cytology. The scoring system, though standardized and blinded, remains subjective. Long-term storage effects on stained slides were not assessed, nor was preservation of nucleic acids for potential molecular studies. Additionally, we did not evaluate different concentrations of sugar syrup or varying fixation times to optimize performance. The study was conducted at a single national hospital, which may not represent all laboratory settings in Tanzania.

## CONCLUSSIONS AND RECOMMENDATIONS

It was found that the best fixative used for preparing cytological smears was 95% ethanol, which gave the best overall staining of specimens as well as best preservation of the morphology of cells. Although 30% sugar syrup also gave satisfactory staining of nuclei and cytoplasm in most of the specimens studied, it was less effective in preserving the cellular morphology of cells, hence making it unreliable for accurate cytology. However, ethanol still remains the best fixative for conducting cytological examinations of the specimens, although sugar syrup may be considered a cheaper and more readily available alternative when ethanol is not available.

**Preferential use of 95% ethanol as fixative**: Given that 95% ethanol showed superior staining quality compared to 30% sugar syrup, it is recommended to continue using 95% ethanol as the standard fixative for cytological samples whenever possible. Its efficacy in preserving cellular structures and providing good staining quality makes it a reliable choice for routine cytological preparations.

**Consideration of sugar syrup as an alternative fixative:** While 95% ethanol is recommended as the primary fixative, the study shown that 30% sugar syrup can be considered as an alternative fixative. In cases where ethanol is not available or suitable for specific samples, sugar syrup can be used as an acceptable substitute, especially considering its accessibility and lower cost compared to ethanol. It's important to note that the study's conclusions were based on the specific parameters and samples studied. Therefore, before implementing any fixative as a routine practice, further validation and testing in different contexts and with various types of cytological specimens should be conducted to ensure its reliability and effectiveness. Regular monitoring of staining quality is essential to ensure consistent and reliable results. Periodic assessment and quality control measures should be in place to detect any variations in staining performance, especially when using alternative fixatives like sugar syrup. Additional research can be conducted to compare the efficacy of different fixatives beyond ethanol and sugar syrup. Exploring other fixatives, including novel solutions or natural substances, could lead to the discovery of more effective and affordable options for cytological fixation and staining.

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## AUTHOR'S CONTRIBUTIONS

**Ussi HU:** writing original draft, formal analysis, conceptualization, data organization. **Mdee JT:** formal analysis, investigation. **Said SS:** conceptualisation, data curation. **Salum TT:** supervision, critical review. **Makweza C:** formal analysis, critical review. **Samu GC:** methodology. **Suleiman SM:** data curation, supervision. Final manuscript was checked and approved by all authors.

## DATA AVAILABILITY

The associated author can provide the empirical data used to support the study's conclusions upon request.

## CONFLICT OF INTEREST

None to declare.

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