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Original article

Use of formaldehyde to increase result quality in urinary cytology

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Abstract

Cytology is often used in screening programs for individuals at high risk of certain diseases, such as urothelial (bladder) tumors and prostate cancer. High-risk individuals may include those with a family history of cancer or other risk factors. While cytology is less commonly used for prostate cancer screening compared to other methods like prostate-specific antigen (PSA) testing, it can still be employed in certain cases to detect abnormal prostate cells. Specimen collection, often through urine or brushing of specific surfaces, doesn't typically require special preparation or invasive procedures, reducing the need for prolonged hospitalization.

Our study aimed to optimize the long-term preservation of urine samples for cytopathological diagnosis by using 10% formaldehyde, ensuring high-quality smear preparation, and assessing the effectiveness of Papanicolaou staining. These objectives are essential for advancing the field of cytopathology and improving the accuracy of diagnostic procedures.

Keywords

Papanicolaou staining, formaldehyde, cytology

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Introduction

Cytology is an ideal method for screening in people at high risk. There are specific programs for screening for urothelial tumours, prostate cancer, etc. Cytology prevents or eliminates prolonged hospitalization of the patient because specimen collection does not always require special preparation. Exfoliative cytology is used to detect lesions of the urothelium as a method of surveillance of the patient. For patients with malignant tumours of the urinary tract, urinary cytopathological examination may contribute to early diagnosis. Urine sampling was done by bladder lavage, spontaneous urine, urethro-pyelo-calyceal lavage, urethral lavage and urinary catheter sampling. The amount of spontaneous urine collected was a minimum of 160 ml.

Processing of urine samples, is done within 2 hours of collection. This duration is recommended to avoid cell cytolysis. The first cells affected by the cytolytic process are malignant cells and thus the incidence of false-negative results increases.

The majority of false-negative results are caused by improper collection or paucity of samples.

False-negative results are those in which cytology does not identify atypical cells, while endoscopic and histopathological examination confirms the presence of tumour. The goal for the atypical urothelial cells (AUC) category is to capture those cases worrisome for high-grade urothelial carcinoma (HGUC) but that fall short of the suspicious for HGUC (SHGUC) category [1].

The causes of false-negative results are diverse, including limited or intermittent exfoliation of atypical cells, abundant exfoliation of cells of no diagnostic significance masking atypical cells and delaying examination of samples. To make a correct diagnosis in these two situations, the immunocytochemical method may be required.

In patients who have recently undergone resection for bladder tumours, false-positive results may occur due to the presence of post-TUR reactive cells, characterised by hyperchromatic nuclei and granular basophilic cytoplasm.

An important role in obtaining quality urine sample results is played by the fixation step, done as soon as possible after collection [2]. Fixation preserves the shape, structure and relationships between cellular constituents in a state very close to the time of collection. The fixation agent commonly used in the preparation of urine samples is 50% alcohol. Alcohol has a rapid denaturing action on proteins and is used especially in smears, allowing good nuclear detail to be obtained.

Another fixative used in fixation is formaldehyde (10% standard solution). This has a slow fixing action by polym-

erizing proteins. Formaldehyde provides good penetration power, does not alter the structure of proteins and is recommended in immunological techniques. Formaldehyde is a strong preservative and can influence the chemical and staining processes of urine samples.

Cytodiagnosis has become one of the most important and effective methods for the early detection of malignant tumours [1]. Accuracy of results is essential for the clinical utility of cytodiagnostics. The processing of the material is complex and involves many steps, from collection to sample processing and interpretation. The most accurate interpretation is performed by the immunocytochemical method.

In the case of smears obtained from urine samples, repeated cytological examinations may detect atypical cells, even if endoscopically or by random biopsy the presence of a tumour is not revealed [1, 3, 4]. However, the result can only be considered false-positive after excluding the possibility of a tumour in the upper urinary tract, prostatic urethra or neighbouring organs. This category of results is very important as it may lead to urological investigations that may be unnecessary or, in their absence, to neglect of an early stage malignancy.

Normal urine contains few cells, most of which are urothelial cells. These cells rarely exfoliate, especially in situations such as physical exertion or endoscopic manoeuvres. They may occur singly or in small groups and have frequent cytolytic forms. Urothelial cells often have single, centrally located nuclei with basophilic cytoplasm.

Renal tubular cells are rare in normal urine but common in patients with chronic renal failure or inflammatory renal disease. These cells have central, hyperchromatic nuclei and the cytoplasm may have a cyanophilic or chromophobic appearance.

Calyceal cells are rare in normal spontaneous urine, but are found in pyelo-calyceal lavages. They are variable in size, with basophilic or chromophobic cytoplasm and may have 2-3 nuclei.

Pavement cells are common in normal urine and originate from the trigonal bladder in females or the anterior urethra in males. They are large cells with small nuclei and rich cytoplasm.

Columnar cells are rarer and come from different areas such as the bladder cap, membranous urethra or prostate. They may have poorly orthorhombic or chromophobe cytoplasm and contain small vacuoles.

Prostate cells are cubic, triangular or round and are rare in urine. They are more commonly found in benign prostatic lesions and have centralised nuclei. Seminal cells are rare and have a different appearance from prostatic cells. Other cell types, such as macrophages, binucleated cells, multinu-

cleated cells, erythrocytes, leukocytes, bacteria and crystals, may be found in the urine depending on the urological condition or operation.

Urinary cytodiagnosis involves examination of smears obtained from samples taken from the urinary tract. In this process, a small lens is used to avoid areas of cell clumping or overlap. To establish a diagnosis, at least 3 major criteria of malignancy are looked for in single cells or in plaques.

In the urinary environment, exfoliated cells tend to have spherical or oval shapes, so their shape is not a major criterion for detecting malignancy [5]. Cytolysis is common and only cells with all well-defined structural elements are considered for interpretation. Sometimes urinary tract infections can complicate cytological diagnosis, as smears may contain numerous urothelial cells that originate from deep layers or regenerating areas following benign lesions such as surgery, bladder lithiasis or chronic cystitis.

Malignant tumors of the urinary tract are mostly transitional cell carcinomas. Exfoliation of atypical cells in spontaneous urine is influenced by the degree of differentiation of the tumor and is more evident in grade 2 (G2) and grade 3 (G3) cases.

Cytodiagnosis has a detection rate of malignant cells in spontaneous urine samples of more than 90% of cases when performed on sequential samples [2].

One of the biggest challenges in urine cytodiagnosis is grade 1 (G1) transitional cell carcinomas. Particularly troublesome is the low sensitivity in detecting low-grade non-invasive lesions [1]. The majority of urothelial tumours are well differentiated and the literature indicates true-positive results in these on average above 38% [3].

In the urinary tract lavage method, better detection results of malignant cells are found than in spontaneous urine. The presence and morphology of atypical cells in urinalysis is not correlated with the histopathological form of the tumour or the degree of invasion. Urinary cytology is indicated for prostate carcinoma and transitional cell carcinoma.

Our research aimed to: test the use of 10% formaldehyde in the long-term preservation of the cellularity of the urine sample in order to perform the smear and/or cytoblot under optimal conditions for cytopathological diagnosis, ii) perform smears by the method of placing sediment on glass slide using a seeding loop; iii) investigate the quality of Papanicolaou staining obtained after using 10% formaldehyde.

Preserving cellularity is crucial for maintaining the quality of the sample and ensuring that a sufficient number of cells are available for cytopathological analysis. The first objective of this study was to investigate whether 10% formaldehyde is effective for preserving the cellularity of urine samples over

an extended period. The second objective involves performing smears using the sediment from urine samples. This likely includes collecting the sediment and applying it to glass slides using a seeding loop or similar technique.

Finally, the third objective is to evaluate the quality and accurate diagnostic information of Papanicolaou staining achieved after using 10% formaldehyde for preservation.

Material and methods

During 2016 - 2019, a study was conducted on a group of 30 urine samples analyzed in the Pathological Anatomy Laboratory of the Clinical Hospital "Prof. Dr. Th. Burghele" and SC. OncoTeam Diagnostic, Bucharest, which were preserved with formaldehyde.

Thirty freshly collected urine samples were received in the laboratory.

The samples received were divided into 7 batches, resulting in 210 samples, as follows:

- Batch 1 with 30 freshly collected samples, which were immediately processed;
- Batch 2 with 30 freshly collected samples in which 1 ml of formaldehyde was added to 20 ml of urine sample and left to stand at room temperature above 22° Celsius;
- Batch 3 with 30 freshly collected samples to which 3 ml of formaldehyde was added to 20 ml of urine sample and allowed to stand at room temperature above 22° Celsius;
- Batch 4 with 30 freshly collected samples to which 5 ml of formaldehyde was added to 20 ml of urine sample and allowed to stand at room temperature above 22° Celsius;
- Batch 5 with 30 freshly collected samples to which 7 ml of formaldehyde was added to 20 ml of urine sample and allowed to stand at room temperature above 22° Celsius;
- Batch 6 with 30 freshly collected samples to which 9 ml of formaldehyde was added to 20 ml of urine sample and allowed to stand at room temperature above 22° Celsius;
- Batch 7 with 30 freshly collected samples to which 11 ml formaldehyde was added to 20 ml urine sample and left to stand at room temperature above 22° Celsius.

Macroscopic analysis of the urine samples was conducted, after which they were transferred to disposable tubes for centrifugation at 1000 rpm for 15 minutes using the Hettich EBA 20 centrifuge.

In hypocellular samples, the centrifugation and supernatant removal process was repeated 3-4 times.

After removal of the supernatant from the obtained sediment (1 ml), conventional smears were performed.

In order to decrease cases of unsatisfactory paucicellularity, the smear was performed by placing the sediment on glass slide with disposable seeding loop used in microbiology technique.

Two smears were taken from each sample and fixed by direct drying.

A total of 420 smears were made in 7 batches as follows:

- Batch 1 with 60 smears from the sample received and processed immediately;
- Batch 2 with 60 smears made from the sample preserved by adding 1 ml of formaldehyde to 20 ml of urine sample and processed (after 4, 24 and 48 hours);
- Batch 3 with 60 smears made from the preserved sample by adding 3 ml of formaldehyde to 20 ml of urine sample and processed (after 4, 24 and 48 hours);
- Batch 4 with 60 smears made from the preserved sample by adding 5 ml of formaldehyde to 20 ml of urine sample and processed (after 4, 24 and 48 hours);
- Batch 5 with 60 smears made from the preserved sample by adding 7 ml of formaldehyde to 20 ml of urine sample and processed (after 4, 24 and 48 hours);
- Batch 6 with 60 smears made from the preserved sample by adding 9 ml of formaldehyde to 20 ml of urine sample and processed (after 4, 24 and 48 hours);
- Batch 7 with 60 smears made from the preserved sample by adding 11 ml of formaldehyde to 20 ml of urine sample and processed (after 4, 24 and 48 hours).

From the processed smears (after 4, 24 and 48 hours) one specimen (smear) was taken and kept for fixation by direct drying in the sample receiving room under the influence of formaldehyde vapour, while the other 210 smears were kept for fixation in another room without exposure to formaldehyde vapour.

After direct fixation (drying in formaldehyde vapour-free medium) for up to 12 hours, all 420 smears were stained by the Papanicolaou method.

Upon final examination of the 210 smears exposed in the formaldehyde vapour chamber, the following results were found:

- All 30 of lot 1 had qualitative deficiencies in staining and were unsatisfactory for examination;
- The other 180 smears from lots 2-7, stained and were partially suitable for examination. The staining result was unsatisfactory;
- Batch 4 was the least affected by the action of formaldehyde vapour.

On final examination of the 210 smears stored in the formaldehyde vapour-protected space, the following results were found:

- All 210 smears, had Papanicolaou staining satisfactory for examination;

- The smears in lot 2 had partially lysed cells. Less lysed cells were present on smears processed 4 hours after preservation, and more affected cells were present on smears processed 48 hours after preservation;
- The smears from batch 3, had less lysed cells than those from batch 2. The least lysed cells were on smears processed 4 hours after preservation and the most affected cells were on smears processed 48 hours after preservation;
- The smears from lot 4, showed the most satisfactory staining, with rich, clear and well differentiated cellularity;
- The smears from lots 5-7 had lower cellularity with no lysed cells. The lowest cellularity was present on smears processed 48 hours after preservation.

To preserve the sample until the result was prepared, 10% formaldehyde was added. After examination of the smears, if necessary, since the cells were already fixed, the processing of the material to obtain the cytoblot was proceeded directly. Thus, the cytoblock could be obtained 24 hours earlier than when using alcohol.

Results

In smears obtained by placing the sediment with the seeding loop on the glass slide, a richer cellularity was observed compared to those obtained by smearing the sediment on the glass slide using another slide (Fig 1-2).

All samples in batch 1 and all formaldehyde-preserved samples resulted in smears satisfactory for examination if

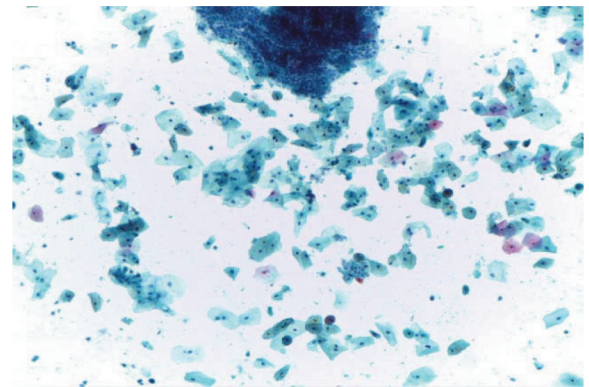


Figure 1. Smear spread with seeding loop

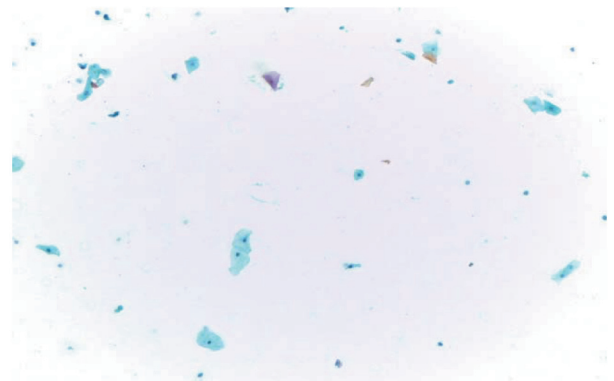


Figure 2. Smear spread with 45-degree angle blade

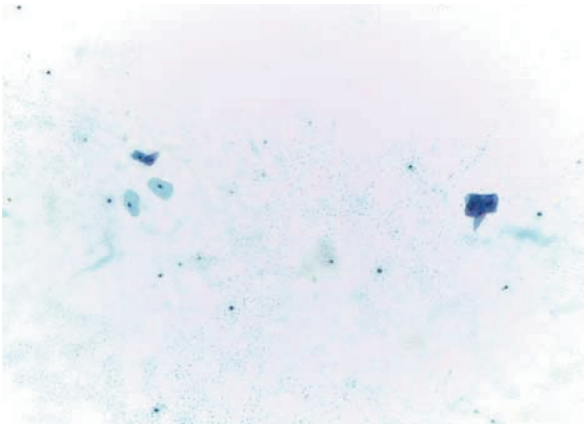


Figure 3. Smear, 1ml formaldehyde to 20 ml urine

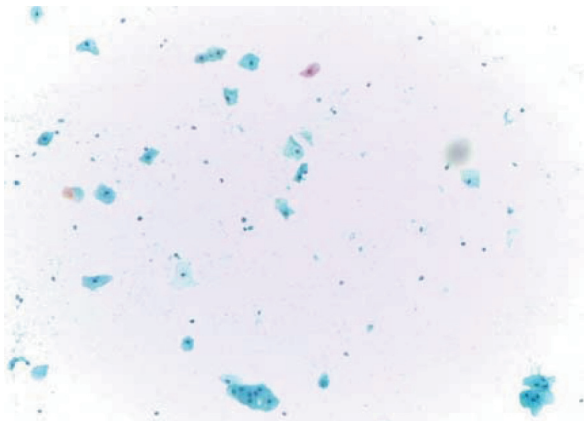


Figure 4. Smear, 3ml formaldehyde to 20 ml urine

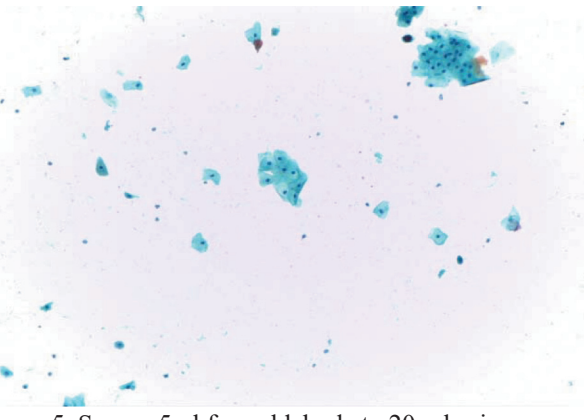


Figure 5. Smear, 5ml formaldehyde to 20 ml urine

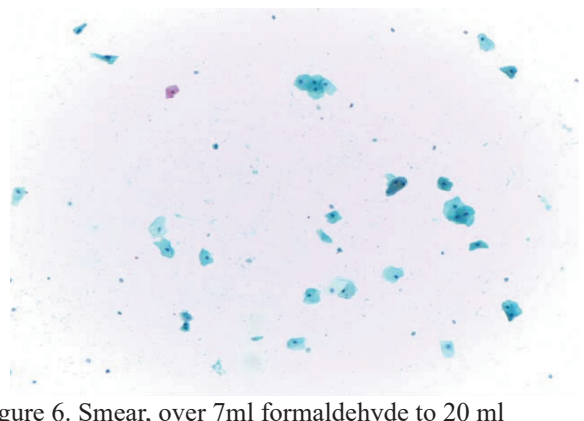


Figure 6. Smear, over 7ml formaldehyde to 20 ml

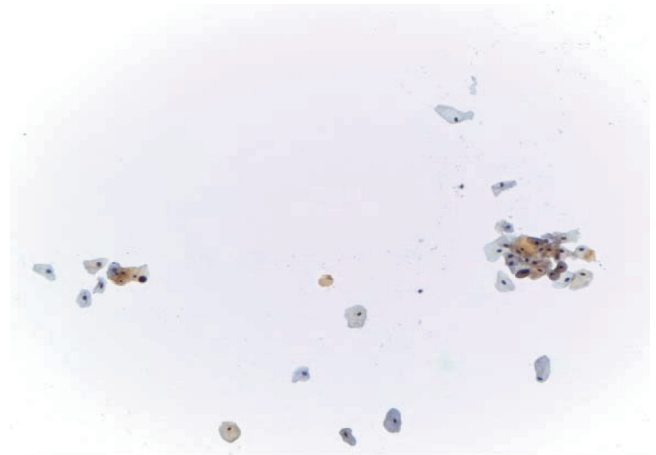


Figure 7. Smear affected by formaldehyde vapour

protected from formaldehyde vapour. The method of preserving the freshly collected urine sample by adding 5 ml of formaldehyde to 20 ml of urine sample gave the best results.

Similar Papanicolaou staining quality standards were found for both smears from samples preserved at receipt and processed after 48 hours and smears from freshly collected samples processed immediately.

Smears obtained from samples preserved with the addition of 1 ml formaldehyde to 20 ml freshly collected urine sample had cells partially lysed for examination (Fig 3). Smears obtained from samples preserved with the addition of 3 ml formaldehyde to 20 ml freshly collected urine sample had less lysed cells compared to smears from batch 2 (Fig 4). Smears obtained from samples preserved with the addition of 5 ml formaldehyde to 20 ml freshly collected urine sample had cells suitable for examination (Fig 5).

Smears obtained from samples preserved with the addition of 7-11 ml formaldehyde to 20ml freshly collected urine sample had lower cellularity compared to those preserved with 5 ml formaldehyde (Fig 6).

It was found practically that the presence of formaldehyde vapour negatively influenced Papanicolaou staining in all smears (Fig 7).

Conclusions

The use of the glass slide sediment placement method using a disposable seeding loop, inspired by the technique used in biochemical analysis, improved the cellularity of smears, decreased the incidence of paucicellular cases and thus the number of false-negative results.

The addition of 5 ml formaldehyde to 20 ml of freshly collected urine allowed good preservation of the urine sample for a period of at least 48 hours from collection to the start of sample processing, without affecting the quality of Papanicolaou staining of the smears obtained.

After the use of formaldehyde, it was possible to obtain Papanicolaou stained smears, qualitatively similar for examination to those obtained using alcohols.

The presence of formaldehyde vapours affects the smears exposed to their action and prevents Papanicolaou staining. This may lead to difficulties in cytopathological diagnosis.

The use of formaldehyde allowed the cytoblock to be obtained more quickly, reducing the immunocytological diagnostic time by one day, compared to the method using alcohol. The use of formaldehyde, which can be purchased at half the price of alcohol, reduced the cost of sample analysis. It is important to follow proper procedures to protect smears from contact with formaldehyde vapour.

In summary, our research aimed to optimize the long-term preservation of urine samples for cytopathological diagnosis by using 10% formaldehyde, ensuring high-quality smear preparation, and assessing the effectiveness of Papanicolaou staining. The results shown in this paper are essential for advancing the field of cytopathology and improving the accuracy of diagnostic procedures.

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