Methods of Preparation of Cellular Wax Blocks for Plasmapheresis and Their Diagnostic Evaluation

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Abstract: Plasmapheresis specimens, which are commonly utilized in clinical laboratory diagnostics, contain crucial information for the diagnosis of numerous medical conditions. This article delves into the preparation and diagnostic evaluation of cellular wax blocks derived from plasmapheresis, with a focus on their application in clinical pathology and cytology. Utilizing techniques such as centrifugation and fixation, cellular components are extracted, preserved, and processed for histopathological examination. Immunohistochemistry and molecular diagnostics further enhance diagnostic accuracy, guiding personalized treatment strategies. Despite their advantages, challenges such as contamination and variability in block quality persist. This article presents a comprehensive analysis of cellular wax block techniques, including preparation methods, clinical case studies, and diagnostic interpretation. Moving forward, standardization and refinement of these techniques are essential to optimize diagnostic accuracy and reproducibility, ultimately improving patient outcomes in pathology.

Keywords: Plasmapheresis; Cellular Wax Blocks; Histopathology; Immunohistochemistry; Cytology; Diagnostic Evaluation;

1. Introduction

Plasmapheresis is a common fluid specimen used in clinical laboratory diagnostics and is usually collected from the patients' thoracic, abdominal, or joint cavities. It is usually obtained by the clinician by puncturing the pleura or peritoneum. Plasmapheresis testing is usually used quite a bit in clinical chemistry and histopathology testing. In clinical chemistry testing, a chemistry analyzer is used to detect serum albumin, glucose, pH, and other biochemical tests in plasmapheresis, giving a numerically quantified laboratory result. By testing the above biochemical indicators, the difference in the numbers of the corresponding indicators in different specimens can be determined, and the plasmapheresis can be categorized as exudate or leakage, thus providing a reference for the clinician to analyze the pathogenesis of the patient.

In the diagnosis of histopathology, the extraction of the active cellular components of the plasma lumen fluid can be used as one of the ways to differentiate between cancer and other inflammatory diseases. After centrifugation of the fluid, which is sent for testing, the supernatant is discarded, the cell precipitates are extracted, wrapped in filter paper, and placed in neutral formalin for fixation, and then undergoes the routine pathology specimen processing procedures such as dehydration, embedding, sectioning, staining, etc., and the finally is placed under the microscope for observation whether there are any malignant tumor cells. The prepared cell wax blocks can be serially sectioned for several immunological and molecular assays. Unlike normal liquid specimens, cell wax blocks can be stored and archived for a long period of time, preserving the original specimen material for the future evolution of the assay technology [1]. Sometimes, for further diagnostic purposes, a wax block of cells made from the plasma membrane fluid is further subjected to immunohistochemistry or molecular diagnosis more accurate. Immunohistochemistry allows for the identification of specific protein markers expressed by cancer cells, aiding in subtype classification and prognosis assessment. Molecular diagnostics, such as polymerase chain reaction (PCR) or fluorescence in situ

hybridization (FISH), provide further insights into the genetic alterations present within the tumor cells. The cell wax block technique maximizes the preservation of cancer cells and reduces the rate of missed diagnoses. Shedding tissue fragments is close to histopathological testing in the traditional sense, maximizing the integrity of cell morphology and improving detection rates.

From the perspective of diagnosing a cell wax block, a qualitative diagnosis can be established, and the histopathological assessment of a cell wax block can aid in refining the therapeutic strategy and patient care [2]. Cytological samples typically originate from naturally exfoliated cells within bodily fluids, such as sputum, urine sediment, pleural fluid, ascites, and vaginal smears. Additionally, they may be obtained through pathology or laboratory cytologic tests (TCT), cervical liquid-based cytology, and endoscopic brushing to collect cells from the tumor surface. In the context of cytology diagnostic reports, the terminology employed is of particular significance. The language within the report must be exceedingly precise, often utilizing terms such as "considered," "suspected," "cannot be excluded," arranged in order of diagnostic certainty: consistent > considered > tends to > suggests > suspects > cannot be excluded > not currently seen microscopically (no obvious lesion).

2. Objective

As required for clinical diagnosis, the plasma effusion sent to Pathology Department for testing will be extracted for cellular components to determine the presence of cancer cells, providing a preliminary result for clinical tumor diagnosis. The purpose of this paper is to describe the technique of cell wax block preparation, the application of cell blocks in immunohistochemistry and the pathological diagnosis of cell wax blocks. Twenty-seven clinical cases were collected and evaluated in relation to the pathologic diagnosis.

3. Method

3.1 Cellular Wax Block Preparation Technology

In most cases, plasmapheresis specimens first require a smear to be placed under a microscope, which is a preliminary testing of the plasmapheresis. If abnormal cells are found, the cells in the plasmapheresis are extracted and cell wax blocks are prepared. To prepare cell wax blocks, the following equipment and materials are required: Centrifugal machine, 50ml conical centrifuges tubes, mixed solution of glacial acetic acid and 10% neutral formalin, fixative, embedding box, forceps, 3x3cm filter paper.

Preparation of cell wax blocks is usually accomplished by the following four steps:

(1) Plasmapheresis specimen resting: The plasmapheresis was allowed to stand for 12 hours and poured into several centrifuge tubes according to the volume delivered.

(2) Centrifugation: centrifuge the liquid in the tube to level, centrifuge at 1500r-2000r/10minutes, collect the cells, discard the supernatant, and use a rubber-tipped burette to aspirate the red sediment in the centrifuge tube, and the flocculent in the liquid.

(3) Placement in embedding box: Place the extracted cells on a 3×3 cm filter paper and wrap the filter paper and place it in the embedding box.

(4) Fixation: the embedding box were fixed in 10% neutral formalin.

In general, research team usually prepare only one cell wax block of serosal effusion based on the cell content extracted from plasmapheresis. Figure 1 is a summary of the preparation of cell wax blocks. Finally, after fixing the wax blocks of the cells, they were put into a dehydrator for dehydration. After dehydration, the cells will be embedded, sectioned, stained, and sent to the microscope for observation.

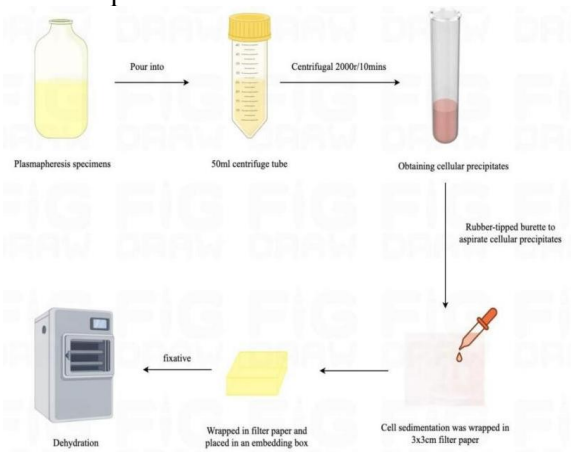

Figure 1. Procedure for the Preparation of Cellular Wax Blocks

3.2 Data Analysis of Clinical Specimen Collection

Our research team collected a total of 28 cases of plasmapheresis from January 1,2024 to April

25, 2024, from Hubei Provincial Hospital of Traditional Chinese Medicine (HBTCM). Twenty-eight specimens were obtained from the Department of Pulmonary Disease (Respiratory Medicine), Department of Oncology and Department of Thoracic Surgery of HBTCM in three different hospitals districts. 90% of the plasma effusions sent to the pathology department for cytologic testing were pleural fluid. As shown in Figure 2 the largest number of plasmaphereses was sent for testing in March, with a total of 13 cases of cellular wax block testing. In addition, the age distribution of patients whose pleural effusion specimens were submitted for assay can be seen from Figure 3 Most of the patients with cases of pleural fluid were aged 65 years and above. Of the 28 cases of plasmapheresis, 17 of them were male patients, and two of them sent pleural fluid multiple times for testing.

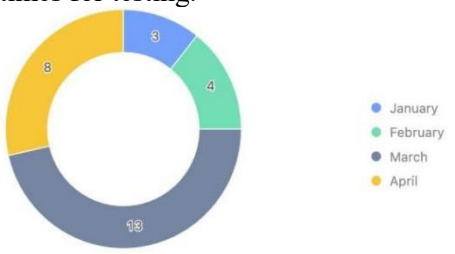

Figure 2. Monthly Distribution of the Number of Cell Wax Cases

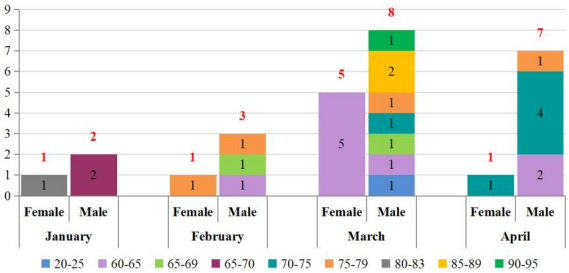

Figure 3. Statistics of Plasmapheresis Cell Wax Blocks Cases from January to April

3.3 Cell wax block preparation technology

3.3.1 Physical and chemical properties of serosal cavity effusion

Serosal effusion can be divided into exudate and transudate based on clinical manifestations and related pathological characteristics. Classifying serosal effusions into two different types based on observable clinical manifestations and pathological features can be available to make diagnostic accuracy and treatment decisions in different medical environment. Comprehensive comprehension of the clinical nuances and pathological underpinnings of serosal effusions is paramount to optimizing patient care paradigms. By elucidating the intricate interplay between clinical presentations and underlying disease mechanisms, clinicians can aptly decipher the etiological landscape, thereby facilitating prompt diagnosis and targeted therapeutic interventions. Moreover, a nuanced understanding of the exudative-transudative paradigm engenders a refined prognostic framework, enabling clinicians to prognosticate disease trajectories and anticipate potential complications.

Transudate is caused by the imbalance of hydrostatic force and colloid osmotic pressure, which causes the patient to generate it in internal spaces such as the chest and abdominal cavity [3]. Transudative effusions, typified by lower protein concentrations, normal leukocyte counts, and a specific gravity less than 1.020, often emanate from systemic conditions such as congestive heart failure or cirrhosis. Conversely, exudative effusions, characterized by heightened protein content, elevated leukocyte counts, and a specific gravity exceeding 1.020, typically arise from clinical diseases such as pneumonia, malignancy, and thromboembolism [4]. This dichotomy underscores the importance of discriminating between exudatives.

Different properties of serosal cavity fluid have different effects on cell wax blocks. The effect of exudate and transudate on the cell wax block is reflected in the following three aspects: the texture of the wax block, tissue staining effect and microscopic morphology. The Table 1 shows the differences between the cell wax blocks prepared by exudate and transudate.

Table 1. The Difference Between Exudate and Transudate in Cell Wax Blocks

	Exudate	Transudate
Texture	The exudate contains high concentrations of protein, cellular debris, and impurities; the high protein content gives the cellular wax mass a dense texture, but cellular debris may introduce impurities in the wax mass.	The lower protein content of the transudate allows the cells to be evenly distributed in the wax block, resulting in a more homogeneous cell structure and density.
staining effect	Exudate contains small amounts of fat and hemoglobin, and denaturation of the	Uniform distribution of cells may lead to aggregation of

	protein or fat may affect the staining. Tends to cause small amounts of impurities on the slide.	cytochromes. May result in darker staining.
microscopic morphology	Exudate contains proteins, cellular debris and inflammatory cells that can interfere with the morphologic structure of the cells needed for diagnosis	The absence of other impurities facilitates the maintenance of cellular morphology.

3.3.2 Technical principle of cell wax block

The principle of a cell wax block involves the preparation of cells for microscopic examination by embedding them in a solid medium, typically paraffin, after fixation and processing. This technique enhances diagnostic accuracy by preserving cellular architecture and allowing for multiple sections to be cut from the same specimen. Cell blocks can be prepared from various cytological samples, including fluids from fine needle aspirations and body cavity effusions. The process typically involves concentrating cells by centrifugation or sedimentation followed by embedding in a medium such as agar, gelatin, or plasma-thrombin. The cells are first fixed, typically with a formalin-based fixative, to preserve cellular structures and proteins. They are then embedded in paraffin to create a block that can be sectioned thinly enough for microscopic examination. This method allows for multiple sections to be cut from a single block, facilitating detailed and repeated analyzes of the same specimen. This method facilitates detailed histological and immunocytochemical analysis and is particularly useful for the evaluation of scant samples or when additional testing such as molecular research is required. Cell block preparation has evolved to include various techniques that improve cell yield and morphological preservation, contributing significantly to the field cytopathology by enabling more comprehensive diagnostic and ancillary studies [5].

(1) The advantages of cell wax block

Cell blocks can provide a more definitive cytopathologic diagnosis compared to smears alone. This enhanced diagnostic capability stems from the ability of cell blocks to encapsulate a larger and more diverse cellular population within a single specimen. They allow for better categorization of tumors, which may not be possible with smears [6]. Unlike smears, which may contain only a small fraction of the cellular material present in a lesion, cell blocks capture a more representative sample, allowing for a comprehensive evaluation of cellular morphology, architecture, and immunohistochemical characteristics. Cell blocks are particularly useful for ancillary testing, including molecular diagnostics, immunocytochemistry, and histochemical staining. This is crucial for personalized medicine and targeted therapies [7].

Modern cell block preparation methods are simple, reproducible, and use routine, safe laboratory chemicals, making them accessible for widespread use in clinical laboratories. This accessibility is instrumental in improving diagnostic accuracy and patient care across diverse healthcare settings. Moreover, their simplicity and reproducibility ensure consistent results, enabling pathologists to confidently interpret findings and make informed clinical decisions. By democratizing access to advanced diagnostic tools, such as cell blocks, healthcare systems can bridge gaps in diagnostic capabilities, particularly in resource-limited regions where access to specialized diagnostic services may be limited. Furthermore, the use of routine laboratory chemicals enhances cost-effectiveness and sustainability, making cell block technology a practical solution for both high-volume reference laboratories and smaller community clinics. As such, the widespread adoption of modern cell block techniques promises to revolutionize cytologic diagnosis and improve patient outcomes on a global scale [6].

(2) The disadvantages of cell wax blocks

Although cell wax blocks have many advantages in histological research and clinical diagnosis, it is conductive to the preservation of specimens, and can be detected in many different items. However, in the preparation method of cell wax blocks, it is easy to produce other in vivo substances such as blood, protein, and chemical reagents in experimental operation to contaminate cell precipitation, thereby interfering with the diagnosis. The cleavage reagents based on acetic acid and alcohol may damage the results of auxiliary tests such as immunohistochemistry and should be avoided [8,9]. The contamination of reagents and other body fluids is one of the major drawbacks, it

may be damaged in the preparation of cell wax blocks, or the quality of the slice is reduced, this can lead to inconsistent results in ancillary testing, which may affect diagnostic accuracy and reliability (Table 2).

The quality of cell blocks can be variable, depending on the technique and the skill of the technician. Poorly prepared cell blocks may not provide adequate material for diagnosis or ancillary testing. Factors such as inadequate fixation, improper tissue processing, or suboptimal embedding can result in fragmented or poorly oriented specimens. Suboptimal embedding techniques may also contribute to inadequate cell block quality, with improper sectioning or inadequate support leading to difficulties in obtaining thin, uniform sections for microscopic examination. Furthermore, variations in technician experience and expertise can significantly impact cell block quality. Technicians with limited training or experience may encounter challenges in mastering the complex techniques involved in cell block preparation, leading to inconsistencies in sample processing and suboptimal results. Additionally, differences in laboratory protocols and equipment can further contribute to variability in cell block quality, highlighting the importance of standardized procedures and quality assurance measures.

The implications of poor cell block quality extend beyond diagnostic limitations to encompass potential delays in patient management and treatment decisions. Inadequate cell blocks may necessitate repeat procedures or additional testing, prolonging the time to diagnosis and potentially delaying the initiation of appropriate therapy. Moreover, suboptimal cell block quality can undermine the reliability of ancillary tests, such as immunohistochemistry or molecular analysis, limiting their utility in guiding patient care.

Table 2. The Summary of Pros & Cons about Cell Wax Block

Pros	Cons
Cell wax blocks can be tested for multiple auxiliary project	Vulnerable to contamination by chemical reagents and blood products
Cell wax blocks can be stored for a long time	No suitable standard for evaluating cell wax blocks in pathological field.
In terms of diagnosis, it is conducive to the screening of tumors	The liquid specimens were centrifuged without cell precipitation or tiny cell precipitation.

4. The Diagnosis of Cell Wax Block

4.1 Composition of Cytological Report Content

(1) Microscopically, acute and chronic inflammatory cells and a few mesothelial cells were seen in the blood background. No malignant cells were seen.

(2) The cellulose exudates examined showed red blood cells, chronic inflammatory cells and a few mesothelial cells, but no malignant cells.

(3) (Centrifuge cell block in pleural fluid) The group of abnormal cells was seen in the tissue, and the possibility of malignant tumor was high.

Supplementary immunohistochemistry: (pleural fluid centrifugation cell block) found a small amount of heterosexual epithelium, combined with morphology and immunohistochemistry, prone to adenocarcinoma, suggested to check the digestive system and other sites.

immunohistochemistry: CD68(-), CDX-2(-), Syn(-), CgA(-), NSE(-), CK7(+), CK20(+), CK8(+), CK19(+), TTF-1(-), NapsinA(-), E-Ca(+).

(4) Microscopically, malignant tumor cells tend to be adenocarcinoma, and immunohistochemical consultation is recommended.

Supplementary immunohistochemistry, Microscopically, a large number of malignant tumor cells tend to adenocarcinoma. Conjugated morphology and immunohistochemistry are considered to be of ovarian origin.

Immunohistochemistry: CR (-), HBME-1 (-), CK19 (+), CA125 (+), PAX2 (+), CK(+), CK7 (+), CK20 (-), P53 (95% +), Ki 67 (about 45% +) supported the above diagnosis.

(5) (Thyroid puncture cytology) A large number of thyroid epithelial cells. A tight arrangement with nuclear sulcus and nuclear pseudoinclusions. A papillary thyroid carcinoma was considered.

(6) Thin Layer Liquid-Based Cytology: is an improved method for collecting, making, and reading exfoliated cells of cervical cancer after aiming at the shortcomings of traditional Pap smears. It is usually combined with HPV detection for cervical cancer screening and belongs to the first step of " three-step diagnosis of cervical cancer", has the highest detection rate of cervical cancer, and is known as the gold

standard in the United States.

4.2 Interpretation of TCT Report Contents

4.2.1 Normal TCT result

As shown in Figure 4, no intraepithelial lesions, or malignant cells (NILM) were observed. Cervix cells are normal, and no special treatment is required. squamoa epithelium.

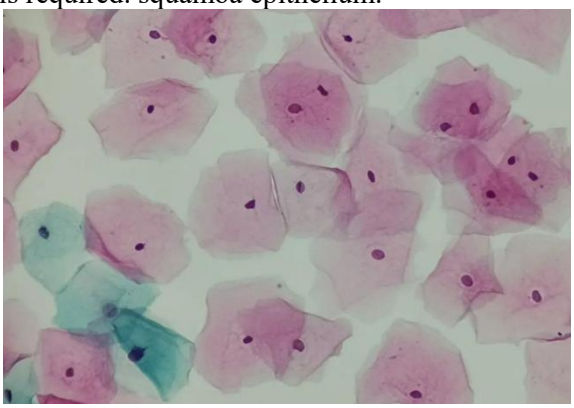

Figure 4. TCT Results without Malignant Cells.

4.2.2 Inflammation (including mild, moderate, and severe inflammation)

Interpretation may have cervix inflammation, basic can rule out malignant lesions. Treatment Suggest to the hospital for further examination, the doctor will make treatment according to the type of inflammation matory. Figure 5 shows TCT images under inflammatory conditions.

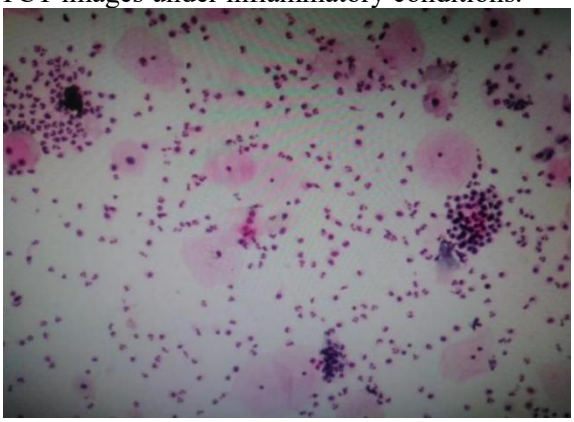

Figure 5. TCT Results in Inflammatory Conditions

4.2.3 Fungal infection, trichomonas infection, actinomycete infection

Unscramble for: vagina normal flora imbalance, or trichomonad and other pathogenic microorganisms' invasion, resulting in infection. Treatment: The doctor may recommend a routine and bacterial vaginosis examination, and then develop a treatment plan based on the results. Figure 6 shows TCT images under microbial infection.

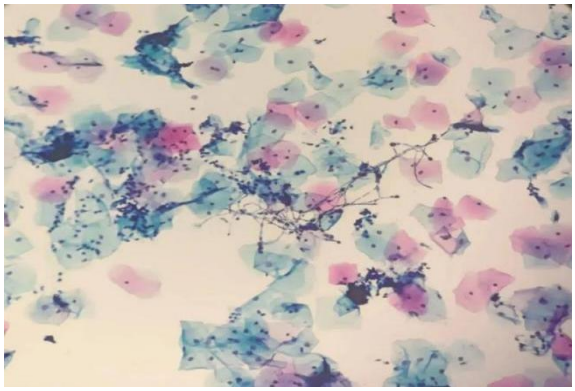

Figure 6. TCT Images Under Microbial Infection

4.2.4 ASC-US

A typical squamous cell of uncertain significance It is interpreted as a sign that there is uncertainty about whether these cells are solution. It is recommended to check "high-risk HPV."

HPV negative with no symptoms (such as irregular vaginal bleeding, intercoastal hemorrhage, bloody leucorrhea, etc.) TCT can be rechecked after one year,HPV negative. But there are symptoms, suggesting that there may be inflammation, can be 3-6 months after anti-inflammatory treatment TCT review if HPV positive. Figure 7 is a TCT image of HPV positive.

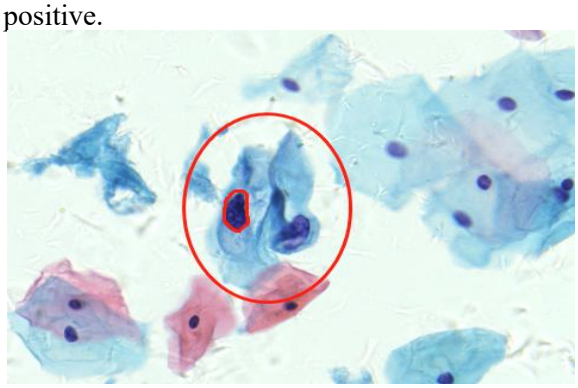

Figure 7. TCT Image of HPV Positive

4.2.5 ASC-H

As shown in Figure 8, a typical squamous cells do not exclude high squamous intraepithelial lesions It is interpreted as having a tendency to have pathological changes, although it is loment it is suggested that "high risk HPV" be examined, colposcopy and cervical biopsy be performed.

4.2.6 LISL,Low-grade squamous intraepithelial lesion

As shown in Figure 9, Deciphered as a possible precancerous lesion, but not too nervous, this stage of the precancerous lesion will mostly recede on its own.

Treatment It is suggested that "high risk HPV"

be examined, colposcopy and cervical biopsy be performed.

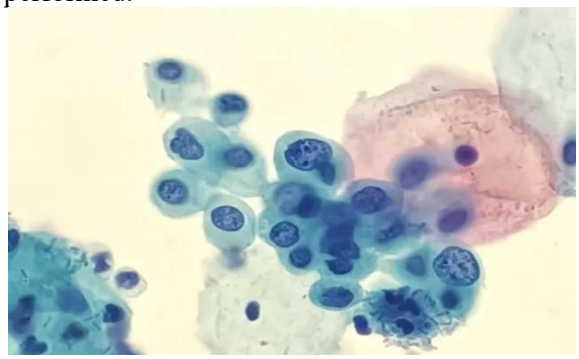

Figure 8. The Image of ASC-H

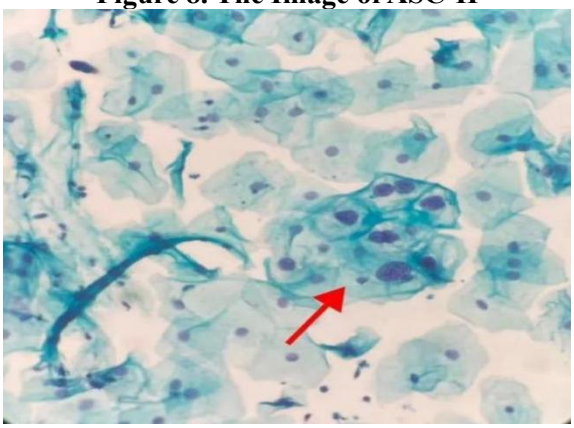

Figure 9. LISH, Low-Grade Squamous Intraepithelial Lesion

4.2.7 HSIL: high squamous intraepithelial lesions

As shown in Figure 10, it is high squamous intraepithelial. Result interpretation: suspicious precancerous lesion cells, need to be further diagnosed and treated, otherwise the possibility of developing cancer is greater.

Treatment It is suggested that "high-risk HPV" be examined, colposcopy plus cervical biopsy should be performed as soon as possible, and the lesion should be excised according to the degree of lesion (leap circumcision or cold knife conization).

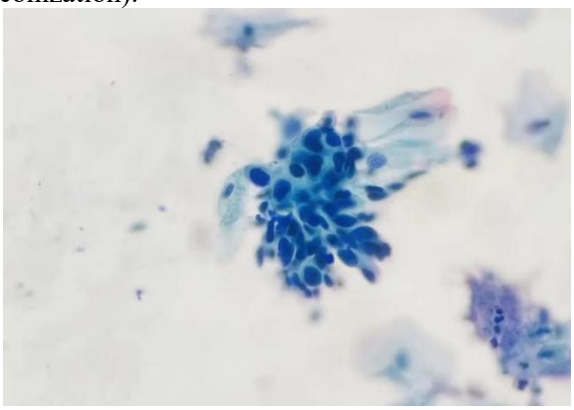

Figure 10. HSIL

5. Conclusion

In conclusion, the preparation of cellular wax blocks from plasmapheresis specimens and their diagnostic evaluation plays a crucial role in clinical pathology and cytology. These techniques enable the extraction and preservation of cellular components from various body fluids, allowing for detailed histopathological diagnose and ancillary testing. Through our study, our research team have demonstrated malignant tumors and inflammatory diseases. The utilization of immunohistochemistry and molecular diagnostics on cell wax blocks enhances the accuracy and reliability of pathological diagnoses, facilitating personalized treatment strategies and patient care. Despite the advantages offered by cell wax blocks, such as long-term storage and compatibility with multiple auxiliary tests, there are challenges and limitations to be addressed. Contamination by reagents and blood products, variability in block quality, and the lack of standardized evaluation criteria are among the issues that need attention. Moving forward, continued research and refinement of cell wax block techniques are warranted to optimize diagnostic accuracy and reproducibility. Standardization of protocols and quality assurance measures will be essential to ensure consistent and reliable results across different laboratory settings.

Overall, the integration of cellular wax block preparation and diagnostic evaluation into routine clinical practice holds great promise for advancing cytological diagnosis and improving patient outcomes in the field of pathology.

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