

MODULE

Histology and Cytology



Notes

23

CYTOLOGY : SPECIMEN PROCESSING & STAINING

23.1 INTRODUCTION

Laboratory sample processing includes steps from the receipt of the specimen in the laboratory to the delivery of a stained slide ready for microscopic examination.

Throughout processing, the identity and integrity of the specimen must be maintained, and the principles of universal precautions followed.



OBJECTIVES

After reading this lesson, you will be able to:

- describe various methods of cytology sample processing
- explain the methods of slide staining
- dispatch labeled slides and forms for cytoscreening.

23.2 SPECIMEN PROCESSING

The laboratory should confirm the identity and integrity of the specimen received. Specimens are accepted only when ordered by physicians or other persons authorized by law. Each sample must have a request completed by the authorized provider prior to processing.

1. Specimen Preparation

(a) Smears

The preparation objective of direct smears is a slide with an evenly and thinly applied cellular specimen that is free of mechanical distortion and

free of drying artifact when the slide is fixed in alcohol. Smears fixed in alcohol (wet fixation) are usually stained by the Papanicolaou method; air-dried smears are usually stained with a Romanowsky stain. Smears preserved with spray fixatives should be soaked in 95% alcohol.

(b) Liquid Specimens

Liquid specimens should be processed according to the manner in which they are submitted. Liquid specimens may be received fresh, with heparin, with preservative (alcohol or other fixative), or with physiologic solution or tissue culture medium. Additional processing should be considered for grossly bloody specimens prior to slide preparation. Blood clots should be removed and processed as a cell block.

Specimens of low cellularity and low volume may be cytocentrifuged directly. High volume specimens are usually concentrated prior to preparation. Centrifugation is frequently used with the re-suspended pellet used for direct smears.

2. Specimen staining

The Papanicolaou stain is recommended for the staining of alcohol fixed cytology slides. Romanowsky stains may also be used for wet fixed slides, but are primarily applied to air-dried smears.

(a) Papanicolaou Stain

The Papanicolaou stain uses a standard nuclear stain, hematoxylin, and two cytoplasmic counterstains, OG-6 and EA. The outcome of this method is crisp nuclear detail and transparency of the cytoplasm, which allows the examiner to clearly visualize cellular morphology. Either a progressive or regressive technique may be used for nuclear staining. Several automatic programmable stainers are available.

(b) Romanowsky Stain

A Romanowsky stain is recommended for air-dried smears. Romanowsky stains, mixtures of eosin and methylene blue, are a family of polychrome stains that produce their effect by the production of azure dyes as a result of demethylation of thiazines and the acidic component eosin. Unlike the Papanicolaou stain they are metachromatic. Most Romanowsky stains used in cytology are aqueous stains as opposed to the methyl alcohol based stains of hematology. Many commercial stains are available, and most consist of a methanol-based fixative, and two dyes which result in differentiation of cytoplasmic and nuclear components. Most Romanowsky stains are rapid and are useful in enhancing pleomorphism, and distinguishing extracellular from intracytoplasmic material.

**Notes**



Notes

3. Dehydration, Clearing and Coverslipping

(a) Dehydration and Clearing

After staining, the sample is dehydrated by a series of increasing concentrations of alcohol followed by rinsing in clearing solutions. The last clearing solution should be colorless and its refractive index should be close to that of the coverslip, slides and mounting medium. Xylene is the most commonly used clearing agent. Xylene clearing must be performed in a well ventilated area or fume hood to limit exposure to xylene fumes. Slides should remain in the clearing agent until coverslipping is performed.

(b) Coverslipping

Mounting medium used to bond the slide and the coverslip should be compatible with the clearing agent, transparent, and have a refractive index similar to the glass slide and the stained specimen. Adequate mounting medium should be applied to protect the cellular material from air-drying and shrinkage, and to prevent fading of the cell sample. The cellular material should be covered by a suitably sized coverslip or covering material of appropriate quality.

Different methods used to coverslip include placing the mounting medium on the coverslip, then inverting the coverslip onto the slide surface, or lowering the slide onto a coverslip containing adequate mounting medium. Glass coverslips, coverfilm and automated coverslippers are available. Ideally, the mounting medium should be allowed to dry before the slides are reviewed to reduce movement of cellular material during the slide examination. Chemical waste collected throughout the staining, dehydration, clearing and coverslipping processes must be disposed of or recycled.

The stained and labeled slide(s) should be matched with its requisition or other laboratory document that displays the same information. The information on the slide must correspond to the information on the requisition or laboratory document.



INTEXT QUESTIONS 23.1

1. Smears fixed in alcohol are stained by method
2. Air dried smears are stained with stain
3. Specimens of low cellularity and low volume may be directly
4. is the most commonly used clearing agent



WHAT HAVE YOU LEARNT

- Laboratory sample processing includes steps from the receipt of the specimen in the laboratory to the delivery of a stained slide ready for microscopic examination.
- Throughout processing, the identity and integrity of the specimen must be maintained, and the principles of universal precautions followed.
- The preparation objective of direct smears is a slide with an evenly and thinly applied cellular specimen that is free of mechanical distortion and free of drying artifact when the slide is fixed in alcohol
- Smears fixed in alcohol (wet fixation) are usually stained by the Papanicolaou method
- Air-dried smears are usually stained with a Romanowsky stain. Smears preserved with spray fixatives should be soaked in 95% alcohol.
- Specimens of low cellularity and low volume may be cytocentrifuged directly. High volume specimens are usually concentrated prior to preparation
- The Papanicolaou stain is recommended for the staining of alcohol fixed cytology slides
- After staining, the sample is dehydrated by a series of increasing concentrations of alcohol followed by rinsing in clearing solutions
- Xylene is the most commonly used clearing agent
- Mounting medium used to bond the slide and the coverslip should be compatible with the clearing agent, transparent, and have a refractive index similar to the glass slide and the stained specimen.



Notes



TERMINAL QUESTIONS

1. Write briefly about objective of smear making.
2. Write about manners of liquid specimen preparation.
3. What are the main staining methods used in cytology
4. Briefly write about the methods of coverslipping.



ANSWERS TO INTEXT QUESTIONS

23.1

1. Papanicolaou
2. Romanowsky
3. Cytocentrifuged
4. Xylene