



Giemsa Staining Kit

Catalog number 860-006

INDICATIONS AND USE

Intended Use

Ventana Medical Systems' Giemsa Staining Kit is intended for *in vitro* diagnostic (IVD) use as a qualitative histologic stain to differentiate leukocytes in bone marrow and other hematopoietic tissue (lymph nodes) in formalin fixed, paraffin embedded tissue. The stain can also be used to demonstrate some microorganisms, such as *Helicobacter pylori*.

Summary and Explanation

Giemsa Staining Kit is a modification of the original Giemsa stain. A buffered thiazine eosinate solution is used to stain cells differentially with a characteristic blue or pink color. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests. Prescription only.

Principles and Procedures

This kit is optimized for use on the Ventana NexES[®] Special Stains automated slide stainer. The reagents are applied to tissue on microscope slides and mixed over the entire specimen. The staining reaction is based on the differential affinity of cell types for the dyes in the stain. Because of the high degree of dissociation, active molecules (eosin and thiazine dyes) are absorbed by cellular structures very quickly.

MATERIALS AND METHODS

Reagents Provided

The reagent vials are supplied in barcode labeled carriers to insert into the reagent tray of the automated slide stainer. Each kit contains sufficient reagents for 75 tests.

- 1 - 22 ml vial of Giemsa Stain; contains 0.4% modified Giemsa stain in 70% methanol
- 1 - vial insert with sipping straw

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution or titration is required. Further dilution of any of the reagents may result in unsatisfactory staining quality. The user must validate any such change.

Materials and Reagents Needed But Not Provided

1. Neutral buffered formalin (refer to *Theory and Practice of Histotechnology* by Sheehan and Hrapchak)
2. Control tissue
3. Microtome
4. Microscope slides (positively charged)
5. Drying oven capable of maintaining a temperature of 70° C ± 5° C
6. Xylene (histological grade)
7. Alcohol or ethanol (histological grade)
8. Deionized or distilled water
9. Slide holders
10. Chemically clean laboratory glassware
11. Timer
12. Jars
13. Absorbent wipes
14. Synthetic mounting media
15. Cover glass
16. Ventana NexES Special Stains automated slide stainer
17. Ventana Special Stains 10X Wash Solution
18. Ventana Liquid Coverslip[™] solution

Storage and Handling

The Giemsa Staining Kit should be stored at 15 – 30° C. See vial label for proper storage conditions.

Before first use, a vial insert and sipping straw must be placed in the reagent vial.

1. Remove the shipping cap from the vial and place the insert and straw into the vial. The insert and sipping straw should be left in the vial, once the vial has been opened.
2. Place the soft cap into the slot on the reagent holder when the reagent is in use.
3. Use the soft cap to cover the reagent vial when reagent is not in use.

When properly stored, reagents are stable until the expiration date that is printed on the vial label. The user must honor the printed expiration dates. There are no obvious signs to indicate instability of these reagents; therefore, controls should be run simultaneously with unknown specimens. If positive control material shows a decrease in staining it could indicate reagent instability and your local Ventana office should be contacted immediately.

Specimen Collection and Preparation for Analysis

1. Ventana recommends specimen collection and storage be performed according to NCCLS document M29-T2.
2. Fix tissue blocks in 10% neutral buffered formalin.
3. Embed the fixed tissue blocks in paraffin.
4. Cut sections, usually 2 to 4 µm, and pick the sections up on glass slides.
5. Bake the slides for at least 30 minutes at approximately 70° C. Allow to cool.
6. Print appropriate barcode label(s).
7. Apply Giemsa barcode labels to the frosted end of the slides prior to deparaffinization (see NexES Special Stains automated slide stainer Operator's Manual for correct application of labels).
8. Deparaffinize and hydrate slides through xylenes and ethanols to deionized water following recommended procedures within the laboratory. Place tissue slides in Special Stains Wash Solution (1X) until loading the instrument. It is recommended that slides be loaded on the instrument within one hour. If not possible the slides may be left in deionized or distilled water for up to 24 hours as long as the tissue is placed in the refrigerator and not allowed to dry.

WARNING AND PRECAUTIONS

1. The reagents in this kit have been optimally diluted for use on the NexES Special Stains automated slide stainer. These reagents may not be optimal for manual procedures or for use on other instruments. Further dilution may result in inadequate staining. The user must validate any change made to the reagent.
2. Differences in tissue processing and technical procedures may produce significant variability of results necessitating regular performance of controls (see Quality Control).
3. Normal precautions should be observed when handling laboratory reagents. Do not smoke, eat or drink in areas where specimens or reagents are being handled. Never pipette by mouth.
4. Disposal of liquid waste resulting from the use of this kit should be performed in accordance with all local, state and federal laws.
5. These reagents may cause irritation. Avoid contact with eyes and mucous membranes. If reagent contacts these areas, rinse with copious amounts of water.
6. Giemsa Stain contains methanol which is flammable and poison. If swallowed, it may be fatal or cause blindness. Keep away from heat, flame and sparks.
7. Patient specimens and all materials contacting them should be handled as if capable of transmitting infection. Use universal precautions when handling and disposing of specimens.
8. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
9. Consult local or state authorities with regard to recommended method of disposal.

INSTRUCTIONS FOR USE

Step by Step Procedure

Refer to the NexES Special Stains automated slide stainer Operator's Manual for more detailed instructions.

1. Load reagents and slides onto the instrument.
2. Perform the staining run according to the instructions in the manual. Quantities of reagent to be applied are preprogrammed.
3. When the run is complete, remove the slides from the instrument, drain the slides and load into the slide holder.
4. Remove the residual Liquid Coverslip solution by rinsing the slides in three changes of 95% alcohol.
5. Using routine laboratory methods dehydrate through alcohol, clear in xylene, and apply mounting medium and cover glass.

Quality Control Procedures

An example of a positive control material would be formalin fixed, paraffin embedded human tissue such as bone marrow, lymph node, or spleen. Control tissue should be fresh autopsy, biopsy, or surgical specimen prepared or fixed as soon as possible in a manner identical to test sections. Such tissues should monitor all steps of the analysis, from tissue preparation through staining.

Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue processing. For this stain, a control block with two types of tissue may be desirable to ensure all relevant structures are stained. The cellular components of other tissue elements may serve as the negative control.

The control tissue must be tested with each run of the Giemsa Staining Kit.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in formulating a specific diagnosis of patient samples.

If the positive tissue components fail to demonstrate positive staining, results with the test specimens should be considered invalid. If the negative components demonstrate positive staining, results with patient specimens should also be considered invalid.

Unexplained discrepancies in control results should be referred to the local Ventana office immediately. If quality control results do not meet specifications, patient results are invalid. The cause must be identified and corrected, and the patient samples repeated.

LIMITATIONS

General Limitations

1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
3. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
4. Ventana provides stains and reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.

Stability

Stained slides mounted using synthetic mounting media are stable when stored appropriately.

SUMMARY OF EXPECTED RESULTS

1. The Giemsa Staining Kit is tested upon manufacture to show that it stains leukocytes purple, erythrocytes pink and mast cells and many other cell types characteristically blue. Nuclei stain a reddish purple. The pathogenic gram negative spiral bacteria, *H. pylori*, stains blue.
2. Sensitivity of Giemsa Staining Kit is dependent upon the type of fixative, degree of fixation, and thickness of tissue section. Variations in these parameters can have a significant impact on the quality of staining and may result in inappropriate results.
3. Intra run reproducibility of Giemsa Staining Kit was determined by staining 3 slides of the same tissue. The slides were evaluated for staining with pass or fail criteria (comparable to reference). The results demonstrated no significant difference in staining intensity among slides.
4. Inter run reproducibility of staining with Giemsa Staining Kit was determined in 5 runs by staining 3 slides per run. The slides were evaluated for staining with pass or fail criteria (comparable to reference). The results demonstrated no significant difference in staining intensity among slides.

TROUBLESHOOTING

1. Extending exposure time in alcohol dehydration bath will enhance differentiation. One to two minutes in each 95% ETOH bath is a recommended starting point. When fixatives other than neutral buffered formalin are used, red blood cells may appear to stain grayish green.
2. Improved results may be obtained by incubating decalcified tissue in 1X wash buffer for 20 minutes prior to loading.
3. Section thickness may affect quality and intensity of staining. If the staining is inappropriate, call your local Ventana office for assistance.
4. Necrotic or autolyzed tissue may exhibit nonspecific staining.
5. If the positive control does not stain appropriately, check to ensure the slide has the correct barcode label and it is applied correctly. If the label is correct, but no staining or unexpected staining occurs, contact your local Ventana office.

REFERENCES

Lillie RD, Editor. H.J. Conn's Biological Stains, 9th ed. Lippincott Williams and Wilkins Company, Baltimore, 1977.

Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology, 2nd Edition. C.V. Mosby Company, St. Louis, 1980, p 190.

NCCLS documents can be obtained from NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA19087-1898, or through the web site www.nccls.org.

Carson FL. Histotechnology: A Self Instructional Text, 2nd Edition. ASCP Press, Chicago, 1996.

Mikel UV. AFIP Advanced Laboratory Methods in Histology and Pathology. American Registry of Pathology 207-231,1994.

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Covered by the following patents: U.S. Pat. Nos. 6045759, 6192945B1, 6416713B1 and foreign counterparts. US and foreign patent applications pending.

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