Special Stains Van Gieson CS

REF 860-073

09185992001

IVD Σ 50

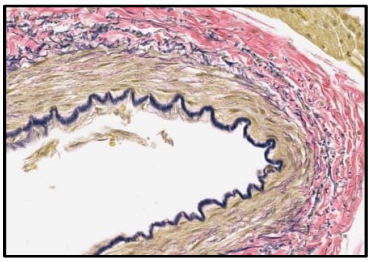

Figure 1. Special Stains Van Gieson CS, used in conjunction with Elastic Stain Core Kit, staining on blood vessel.

INTENDED USE

Special Stains Van Gieson CS used in conjunction with Elastic Stain Core Kit is intended for laboratory use in staining non-target tissue components and providing contrast with target stains in sections of formalin-fixed, paraffin-embedded (FFPE) tissue stained on a BenchMark Special Stains instrument. This reagent is intended as a counterstain in special stains applications.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

The counterstain (CS), Special Stains Van Gieson CS, used in conjunction with the Elastic Stain Core Kit is a modification of the Verhoeff's Van Gieson method that demonstrates elastic fibers within tissues[1]. In 1908 F.H. Verhoeff developed a histochemical stain that could differentiate elastic fibers from collagen and other connective tissue when used in conjunction with Van Gieson's stain.[2] Vascular diseases, both genetic and acquired, are associated with elastic fiber defects that alter arterial function.[3] In these diseases, elastic fibers are degraded or dysfunctional, which can be caused by biochemical modifications, disorganization, or fragmentation.[3]

The elastic staining pattern within blood vessels is used to aid the pathologist in the diagnosis of vascular disease.

PRINCIPLE OF THE PROCEDURE

Verhoeff's Van Gieson method is a regressive stain. Dye binding occurs mainly via Van der Waals forces and hydrogen bonds. Verhoeff's elastic stain binds to elastic fibers with higher affinity than compared to other cell structures. The Ferric Chloride Oxidizer oxidizes the Hematoxylin to hematein. Hematein is a compound that has high affinity for iron together with the second mordant, Lugol's Iodine, to form an iron hematein complex and chelates with the elastic fibers in the tissue. The Differentiator uses lower strength of ferric chloride. This step is used to differentiate the target stain from non-specific binding. The rinsing of the Differentiator removes the loosely bound stain from cell structures other than elastic fibers.

The Special Stains Van Gieson CS stains red blood cells and muscle yellow and collagen pink to provide contrast with the dark purple to black elastic fibers.

This kit is optimized for use on BenchMark Special Stains instruments. The reagents are applied to tissue on microscope slides and mixed over the entire specimen.

MATERIAL PROVIDED

The reagent vial is supplied in a barcode labeled carrier to insert into the reagent tray of the instrument. Each kit contains sufficient reagent for 50 tests:

One 27 mL vial of Special Stains Van Gieson CS contains 0.6% picric acid and 0.02% acid fuchsin.

One vial insert with sipping straw.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration of kit reagents is required. Further dilution of any of the reagents may result in unsatisfactory staining.

The reagents in this kit have been optimally diluted for use on BenchMark Special Stains instruments.

MATERIALS REQUIRED BUT NOT PROVIDED

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. BenchMark Special Stains instrument
4. BenchMark Special Stains Deparaffinization Solution (10X) (Cat. No. 860-036 / 06523102001)
5. BenchMark Special Stains Liquid Coverslip (Cat. No. 860-034 / 06523072001)
6. BenchMark Special Stains Wash II (Cat. No. 860-041 / 08309817001)
7. Elastic Stain Core Kit (Cat. No. 860-047 / 09185984001)
8. General purpose laboratory equipment

STORAGE AND STABILITY

Special Stains Van Gieson CS should be stored at 15-30°C.

When properly stored, unopened reagents are stable to the date indicated on the label. Do not use reagent beyond the expiration date indicated on the kit.

When properly stored, open reagents are stable to the expiration date indicated on the kit.

There are no obvious signs to indicate instability of these reagents; therefore, controls should be run simultaneously with unknown specimens. Contact your local support representative if positive control material shows a decrease in staining as it could indicate reagent instability.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are required for use with this product and BenchMark Special Stains instruments. The recommended tissue fixative is 10% neutral buffered formalin.[4]

Perform specimen collection and storage according to *Histotechnology: A Self Instructional Text*.[4] Cut sections to the appropriate thickness, approximately 3 µm for skin tissue and 4 µm for other tissues, and place the sections in the middle to lower area on positively charged glass slides.

1. Dry the slides.[4]
2. Print appropriate barcode label(s).
3. Apply barcode labels to the frosted end of the slides prior to loading the slides onto the instrument (see the instrument User Guide for correct application of labels).

Refer to the Instructions for Use section for the recommended protocol for the BenchMark Special Stains instrument.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
5. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[5,6]
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Avoid microbial contamination of reagents as it may cause incorrect results.
8. For further information on the use of this device, refer to the BenchMark Special Stains instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
9. Consult local and/or state authorities with regard to recommended method of disposal.
10. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
11. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Danger 	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
	P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
	P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
	P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.
	P501	Dispose of contents/ container to an approved waste disposal plant.

INSTRUCTIONS FOR USE

Prepare Reagent Vial

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

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.

Staining Procedure

1. Load reagents and slides onto the instrument.
2. Place the soft cap into the slot on the reagent holder when the reagent is in use.
3. Perform the staining run according to the recommended protocols in Table 2 and Table 3, and the instructions in the User Guide.
4. When the run is complete, remove the slides from the instrument.
5. Use the soft cap to cover the reagent vial when reagent is not in use.
6. After use, store the reagents according to the recommended storage conditions.

Recommended Protocol

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide.

The following procedures allow flexibility to accommodate user preference. This product has been optimized for use with BenchMark Special Stains instruments but the user must validate results obtained with this product.

Table 2. Recommended staining protocol for Special Stains Van Gieson CS, used in conjunction with the Elastic Stain Core Kit, on a BenchMark Special Stains instrument for other tissues as shown in Table 4.

Staining Procedure	S Elastic VVG
Protocol Step	Method
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 70°C for 8 minutes is recommended.
Select Other Tissues or Skin	Select Other Tissues to run the default protocol.
Optimize Staining	Select Optimize Staining to enable adjustment.*
Elastic Stain Intensity	The default is 12 minutes. Select to enable adjustment of staining intensity*: Select an incubation time from 8 to 16 minutes: 8 minutes, lighter staining of elastic fibers 16 minutes, darker staining of elastic fibers
5 Rinse Cycles (optional)	The default is 3 rinse cycles. Select to enable 5 Rinse cycles to decrease background.
Differentiation	The default is 8 minutes. Select to enable adjustment of differentiation*: Select an incubation time from 4 to 12 minutes: 4 minutes, darker staining of elastic fibers 12 minutes, lighter staining of elastic fibers

* To adjust staining preferences, increment the stain incubation time one parameter at a time.

Table 3. Recommended staining protocol for Special Stains Van Gieson CS, used in conjunction with the Elastic Stain Core Kit, on a BenchMark Special Stains instrument for skin tissues as shown in Table 4.

Staining Procedure	S Elastic VVG
Protocol Step	Method
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 70°C for 8 minutes is recommended.
Select Other Tissues or Skin	Select Skin to run the Skin default protocol.
Optimize Staining	Select Optimize Staining to enable adjustment.*
Elastic Stain Intensity	The default is 8 minutes. Select to enable adjustment of staining intensity*: Select an incubation time of 8 or 12 minutes: 8 minutes, lighter staining of elastic fibers 12 minutes, darker staining of elastic fibers
5 Rinse Cycles (optional)	The default is 3 rinse cycles. Select to enable 5 Rinse cycles to decrease background.
Differentiation	The default is 12 minutes. Select to enable adjustment of differentiation*: Select an incubation time of 12 or 16 minutes: 12 minutes, darker staining of elastic fibers 16 minutes, lighter staining of elastic fibers

*To adjust staining preferences, increment the incubation time one parameter at a time.

Recommended Post-Instrument Processing

Slides need to be promptly removed after the end of the run on BenchMark Special Stains instrument.

1. Drain off the leftover solution.
2. Rinse slides in two changes of 95% ethanol for 5 to 10 seconds each with gentle agitation, followed by three changes of 100% ethanol for 5 to 10 seconds each with gentle agitation.
3. Clear slides in three changes of 100% xylene for 5 to 10 seconds each with gentle agitation.
4. Coverslip with permanent mounting media.

Compatible with the VENTANA HE 600 system coverslipping protocol. For further information, refer to the Specific Limitations section and the VENTANA HE 600 system User Guide.

QUALITY CONTROL PROCEDURE

An example of a positive control material would be FFPE human tissue with elastic fibers such as aorta, artery, kidney, lung or skin. 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. The cellular components of other tissue elements may serve as the negative control.

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control.

The control tissue must be tested with each run.

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 support representative 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.

STAINING INTERPRETATION / EXPECTED RESULTS

Special Stains Van Gieson CS, used in conjunction with the Elastic Stain Core Kit is tested to demonstrate elastic fibers:

- Elastic fibers: dark purple to black
- Collagen: pink to red
- Red blood cells: yellow
- Muscle: yellow to brown

SPECIFIC LIMITATIONS

Only Superfrost® Plus Micro Slides have been used and validated for this assay.

Special Stains Van Gieson CS may not be compatible with all mounting media due to the picric acid.

VENTANA HE 600 system coverslipping may affect stain contrast and/or hue.

VENTANA HE 600 system coverslipping has been validated only for lung tissue.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Analytical sensitivity and specificity for normal and diseased cases was evaluated. All evaluated tissue cases (82/82) passed for acceptable staining as shown in Table 4 and Table 5.

Table 4. Sensitivity/Specificity of Special Stains Van Gieson CS, used in conjunction with the Elastic Stain Core Kit, was determined by testing the following FFPE normal tissues.

Tissue	# cases passed / # tested
Lung	8 / 8
Temporal artery	11 / 11
Colon	8 / 8
Aorta	10 / 10
Heart	8 / 8
Skin	8 / 8
Kidney	5 / 5

Table 5. Sensitivity/Specificity of Special Stains Van Gieson CS, used in conjunction with the Elastic Stain Core Kit, was determined by testing the following FFPE diseased tissues.

Tissue	# cases passed / # tested
Arteritis (Temporal artery)	11 / 11
Adenocarcinoma (Colon)	3 / 3
Carcinoma (Lung)	10 / 10

Precision

Precision of Special Stains Van Gieson CS, used in conjunction with the Elastic Stain Core Kit was determined across multiple runs, days, instruments, and reagent lots using multiple cut slides from two normal lung tissue cases, two normal kidney tissue cases and two normal skin tissue cases. All acceptance criteria were fully met. Precision studies were performed according to Table 6.

Table 6. Precision slide studies for Special Stains Van Gieson CS, used in conjunction with the Elastic Stain Core Kit.

Parameters Tested	# of conditions	# slides passed / # tested
Run to Run	3 runs, same day	54 / 54
Day to Day	5 days	90 / 90
Instrument to Instrument	3 instruments	53 / 54
Intra Run	same day, same instrument	54 / 54
Lot to lot	3 lots	54 / 54

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If staining is inappropriate, contact your local support representative for assistance.
2. Necrotic or autolyzed tissue may exhibit nonspecific staining.
3. If the positive control is negative, tissue may have been improperly collected, fixed, or deparaffinized. Follow the proper procedure for collection, storage, and fixation.
4. If the positive control is negative, check that the slide has the proper barcode label. If the slide is labeled properly, check the other positive controls from the same run to determine if the controls were properly stained.
5. If excessive background staining occurs: incomplete paraffin removal could cause staining artifacts or no staining. If all paraffin is not removed from the slide, repeat the staining run using the extended deparaffinization option, if available.
6. If tissue sections wash off the slide, confirm the slides are positively charged.
7. Section placement on the slide may affect quality of staining. If inconsistent staining occurs, check that the tissue section is not placed close to the barcode label.

8. Extended stay of the slides on the instrument after run completion may affect quality and intensity of the staining. If the staining is inappropriate, remove slides promptly at the end of the run and proceed to post-instrument processing.
9. Insufficient rinsing time during post-instrument processing may affect stain quality. Refer to Recommended Post-Instrument processing section for details or contact your local support representative for assistance.
10. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

REFERENCES

1. Piccinin M, Schwartz J. Histology, Verhoeff Stain. In Treasure Island, FL: StatPearls; 2020.
2. Kazlouskaya V, Malhotra S, Lambe J, Idriss MH, Elston D, Andres C. The utility of elastic Verhoeff-Van Gieson staining in dermatopathology. J Cutan Pathol. 2013;40(2):211-225.
3. Cocciolone AJ, Hawes JZ, Staiculescu MC, Johnson EO, Murshed M, Wagenseil JE. Elastin, arterial mechanics, and cardiovascular disease. Am J Physiol Heart Circ Physiol. 2018;315(2):H189-H205.
4. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
5. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
6. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information):

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
B	Updated to the current template. Updates to the Storage and Stability, Specimen Preparation, Warnings and Precautions, References, Symbols, and Intellectual Property sections.

INTELLECTUAL PROPERTY

For USA: Rx only

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