GMS II Staining Kit

REF 860-028

05412749001

IVD Σ 75

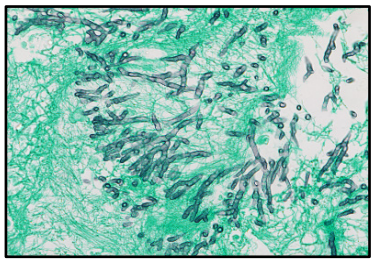

Figure 1. GMS II Staining Kit staining of Aspergillus.

INTENDED USE

GMS II Staining Kit is intended for laboratory use as a qualitative histologic stain to demonstrate polysaccharides in the cell walls of fungal organisms by light microscopy in sections of formalin-fixed, paraffin-embedded (FFPE) tissue stained on the BenchMark Special Stains instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

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

SUMMARY AND EXPLANATION

GMS II Staining Kit is a modification of the Gomori's Methenamine Silver procedure that was modified in 1955 by Robert Grocott, which is often referred to as Grocott Methenamine Silver (GMS) stain.[1]

GMS staining is categorized as an oxidation-reduction silver stain, where oxidation of carbohydrates is followed by the reduction of silver.[2] Fungi are specifically stained by oxidation-reduction silver stains due to their polysaccharide-rich rigid cell walls.[2,3]

GMS staining utilizes a strong oxidizer to oxidize the polysaccharides found in fungal cell walls, resulting in free aldehydes.[1,4] These aldehydes reduce silver nitrate to form metallic silver precipitate and result in black-stained fungal walls.[3]

GMS II Staining Kit is used to aid the pathologist in the diagnosis of infection by fungal organisms.

PRINCIPLE OF THE PROCEDURE

Fungal cell wall polysaccharides are oxidized to aldehyde groups by the GMS II Oxidizer reagent. Chromic acid suppresses weaker background staining of collagen fibers and basement membranes. The GMS II Neutralizer reagent removes excess chromic acid. The GMS II Silver A reagent provides the silver ions. The GMS II Silver B reagent provides the alkaline conditions, which reduce the silver ions to metallic silver. The GMS II Toner reagent contains gold chloride to form a more stable gold complex and remove the yellow tones from the tissue. The GMS II Fixer reagent, with thiosulfate, stops the reaction and removes any unreduced silver from the section. The GMS II Light Green Counterstain is applied to provide a contrasting background.

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 vials are supplied in barcode labeled carriers to insert into the reagent tray of the instrument. Each kit contains sufficient reagent for 75 tests:

One 27 mL vial of GMS II Oxidizer contains approximately 5% chromium trioxide.

One 22 mL vial of GMS II Neutralizer contains approximately 1% sodium metabisulfite.

One 22 mL vial of GMS II Silver A contains approximately 1% silver nitrate.

Two 22 mL vials of GMS II Silver B contains approximately 15% methenamine and approximately 2% sodium borate.

One 22 mL vial of GMS II Toner contains approximately 1% gold chloride.

One 22 mL vial of GMS II Fixer contains approximately 3% sodium thiosulfate.

One 22 mL vial of GMS II Light Green Counterstain contains approximately 1% light green SF yellowish and 1.5% acetic acid.

Eight vial inserts with sipping straws.

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. General purpose laboratory equipment

STORAGE AND STABILITY

GMS II Staining Kit should be stored at 2-8°C. Refrigerated kit components should be brought to room temperature prior to use.

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.

Note: Sealed, unopened GMS II Silver B is stable until the expiration date printed on the vial label. If staining becomes unacceptably dark, use the second sealed and unopened vial of GMS II Silver B. The kit contains one extra vial of GMS II Silver B to allow full use of the kit. Use only one vial of GMS II Silver B at a time.

Controls should be run simultaneously with unknown specimens. Contact your local support representative if positive control material shows an increase or 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.[5]

Perform specimen collection and storage according to CLSI document M29-T2.[6]

Cut sections to the appropriate thickness, approximately 4 µm, and place the sections on positively charged glass slides.

1. Dry the slides.[5]
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. **CAUTION**: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. For customers in the European Economic Area: Contains SVHC Chromium Trioxide. For use as part of an IVD method according to REACH Art. 60.2 and 62.6.
5. Do not use beyond the specified number of tests.
6. 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.
7. 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.[7,8]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.

9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. 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 dialog.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. This product contains gold chloride, hydrochloride, trihydrate; May produce an allergic reaction.
13. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
14. 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 	H290	May be corrosive to metals.
	H302 + H312 + H332	Harmful if swallowed, in contact with skin or if inhaled.
	H314	Causes severe skin burns and eye damage.
	H317	May cause an allergic skin reaction.
	H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
	H335	May cause respiratory irritation.
	H340	May cause genetic defects.
	H350	May cause cancer.
	H360FD	May damage fertility. May damage the unborn child
	H373	May cause damage to organs through prolonged or repeated exposure.
	H410	Very toxic to aquatic life with long lasting effects.
	P201	Obtain special instructions before use.
	P260	Do not breathe mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	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.
	P308 + P313	IF exposed or concerned: Get medical advice/ attention.
	P342 + P311	If experiencing respiratory symptoms: Call a POISON CENTER/ doctor.
	P391	Collect spillage.

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 protocol in Table 2 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 GMS II Staining Kit on a BenchMark Special Stains instrument.

Staining Procedure	S GMS II
Protocol Step	**Method**
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 75°C for 8 minutes is recommended.
Temperature	The recommended temperature is 55°C. Select a temperature from 50-60°C:* 50°C, lighter silver staining 60°C, darker silver staining
GMS II Silver B	The recommended incubation time is 12 minutes. Select an incubation time from 4 to 16 minutes:* 4 minutes, lighter silver staining 16 minutes, darker silver staining
Optimize Counterstain Intensity (Light Green)	The default is 4 minutes. Select to enable adjustment of incubation time:* 4 minutes, lighter counterstain 16 minutes, darker counterstain

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

Recommended Post-Instrument Processing

1. Rinse slides in two changes of 95% ethanol to remove the leftover solution, followed by three changes of 100% ethanol.
2. Dehydrate slides in three changes of 100% xylene.
3. Coverslip with permanent mounting media.

Compatible with the VENTANA HE 600 system coverslipping protocol. For further instructions, refer to the VENTANA HE 600 system User Guide.

QUALITY CONTROL PROCEDURE

An example of a positive control material would be FFPE human tissue positive for fungal infection, which may be found in lung. 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

GMS II Staining Kit is tested to demonstrate fungal organisms.

- Fungal organisms: grey to black
- Background: green

SPECIFIC LIMITATIONS

Only positively charged microscope slides have been used and validated for this assay.

Silver staining can darken over time following initial use of the GMS II Silver B reagent. At expected levels, darkening should not interfere with staining interpretation.

Brown discoloration of glass slides has been observed with GMS II Staining Kit. At expected levels, discoloration should not interfere with staining interpretation.

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 tissue cases was evaluated. Lung represented the majority of the tested tissue types infected with fungus. Normal lung tissues (not infected with fungus) were used to demonstrate negative staining elements. All evaluated tissue cases (62/62) passed for acceptable staining as shown in Table 3.

Table 3. Sensitivity/Specificity of GMS II Staining Kit was determined by testing the following FFPE normal and diseased tissues.

Tissue	# cases passed / # tested
Lung (normal)	6 / 6
Aspergillus (lung, nasal cavity, maxillary sinus)	12 / 12
Candida (esophagus, stomach)	8 / 8
Blastomyces (lung, bone)	3 / 3
Mucor (lung, nasal cavity, soft tissue, kidney)	4 / 4
Coccidioides (lung)	4 / 4
Cryptococcus (lung)	15 / 15
Pneumocystis (lung)	10 / 10

Precision

Precision of GMS II Staining Kit was determined across multiple runs, days, instruments, and reagent lots using multiple cut slides from multiple tissue types infected with fungus (2 aspergillus (nasal cavity/maxillary sinus), 2 cryptococcus (lung) and 2 pneumocystis (lung)). All acceptance criteria were fully met. Precision studies were performed according to Table 4.

Table 4. Precision slide studies for GMS II Staining 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	54 / 54
Intra Run	same day, same instrument	54 / 54
Lot to lot	3 lots	54 / 54

The results demonstrated no significant difference in staining intensity among the slides.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of GMS II Staining Kit were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended use.

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If staining is inappropriate, contact your local support representative for assistance.
2. If staining becomes unacceptably dark, use the second sealed and unopened vial of GMS II Silver B. The kit contains one extra vial of GMS II Silver B to allow full use of the kit. Use only one vial of GMS II Silver B at a time.
3. Necrotic or autolyzed tissue may exhibit nonspecific staining.
4. If the positive control is negative, tissue may have been improperly collected, fixed, or deparaffinized. Follow the proper procedure for collection, storage, and fixation.
5. 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.
6. 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.
7. If tissue sections wash off the slide, confirm the slides are positively charged.
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. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

REFERENCES

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3. Shalin SC, Ferringer T, Cassarino DS. PAS and GMS Utility in Dermatopathology: Review of the Current Medical Literature. J Cutan Pathol. 2020;47(11):1096-1102.
4. Swisher BL, Chandler FW. Grocott-Gomori Methenamine Silver Method for Detecting Fungi: Practical Considerations. Laboratory Medicine. 1982;13(9):568-570.

5. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
6. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
7. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
8. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 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 dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
K	Updates to Material Provided, Warnings and Precautions, and Intellectual Property.

INTELLECTUAL PROPERTY

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