# May-Grünwald solution

**REF. 320070**

Fixation and differential staining of cellular structures

CE IVD

**IFU016B**

For professional use only.
Read all information carefully before using this device.
IFU content may change, make sure you have the latest version available at my.ral-diagnostics.fr.

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## Intended use

May-Grünwald solution is intended to be used in combination with Giemsa solution for the fixation and the differential staining of cellular structures prior microscopic examination.

If applicable, CellaVision RAL Diagnostics recommends using the associated CellaVision RAL Diagnostics products and cannot guarantee that the expected results will be achieved if used in combination with products of other brands.

## Principle

Staining according to Pappenheim allows differential medullary and blood cells counting. It combines two stains: May-Grünwald and Giemsa stains. These are neutral mixtures with very distinct properties. They are not active in alcoholic medium and only act selectively when released in a buffered aqueous solution. This releasing induces the precipitation of neutral stains. May-Grünwald stains the acidophilic elements and the neutrophilic granulations of the leukocytes. Giemsa stains the cytoplasm of monocytes and lymphocytes as well as the chromatin of the nuclei.

## Device description

**May-Grünwald solution**
Clear dark blue solution

| | |
|---|---|
| REF. 320070-1000 | 1 x 1.0 L |
| REF. 320070-2500 | 1 x 2.5 L |

For a specific batch, refer to the analysis certificate of the batch available at my.ral-diagnostics.fr.

## Storage and use conditions

Storage and use temperature: 15-25°C.
Storage and use conditions: away from light and heat sources.
Bottle shelf life before opening: refer to the expiry date on the label.
Bottle shelf life after opening: refer to the expiry date on the label and if the "period after opening" symbol is present take it into account.


## Active components

**May-Grünwald solution**
May- Grünwald: ca 0.3%

## Hazard classification and safety information

**May-Grünwald solution**

Danger:
H225 - Highly flammable liquid and vapour.
H301+H311+H331 - Toxic if swallowed, in contact with skin or if inhaled.
H370 - Causes damage to organs.
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.
P264 - Wash hands thoroughly after handling.
P280 - Wear protective gloves, protective clothing, eye protection, face protection.
P301+P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor.
P308+P311 - IF exposed or concerned: Call a POISON CENTER or doctor.

| | |
|---|---|
| **CONT** | CH3OH |

## Personnel qualification

All samples and products must be handled by qualified and authorized personnel, using individual or collective protection, in accordance with the national directives in force in the laboratories. Personnel must also be aware of the classification of hazardous materials indicated on the label and the safety data sheet (available at my.ral-diagnostics.fr).

The diagnosis must be conducted by qualified and authorized personnel, in accordance with the procedures in force within the laboratory.

## Specific equipment and reagents required but not provided

Sodium hyposulfite aqueous solution, ethanol, acetone, mounting media, microscope slides, and these following CellaVision RAL Diagnostics devices:
Giemsa R solution REF. 320310
pH=6.8 buffer solution for Hematology REF. 330368
pH=7.0 buffer solution for Hematology REF. 330370
Lugol, PVP-stabilized solution REF. 367400

This equipment may vary depending on the protocol. Refer to the relevant protocol (see the section operating procedure) to ensure that you have the necessary equipment to carry out tests.

## Operating procedure

The equipment used for sample processing must comply with the Instructions for use of the supplier.

**Sample preparation**
The specimen must be treated in accordance with procedures available in the laboratory and promulgated by national authorities.

Manual blood smear: Mix the tube by slow inversion and install a smearing droplet device. Invert the tube and lightly press the drop depositor onto a slide to deposit a small drop of blood (Fig. 1-slide A at step 1).
Using another slide tilted at 45° (Fig. 1- slide B at step 1), spread the blood by capillarity on the short edge (Fig. 1-steps 2 and 3) using a pushing motion (Fig. 1-step 4). A good quality smear does not reach the end of the slide and has a gradual decrease in thickness until the end is feathered. Allow the smear to air dry before fixing or staining.
***Note***:*if you do not have a smearing droplet device, open the tube, and use a pipette to deposit a blood drop.*

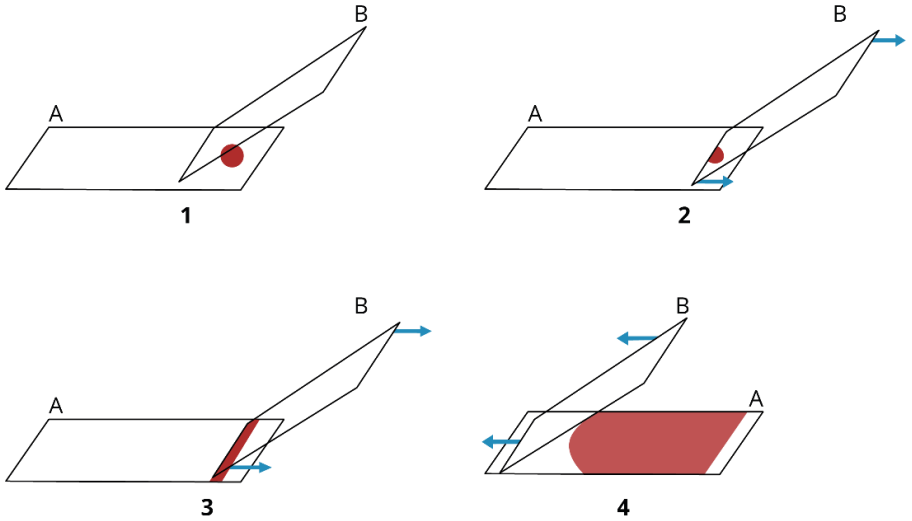



**Figure 1. Schematic representation of performing a blood smear**
A & B: slides, 1–4: steps 1 to 4

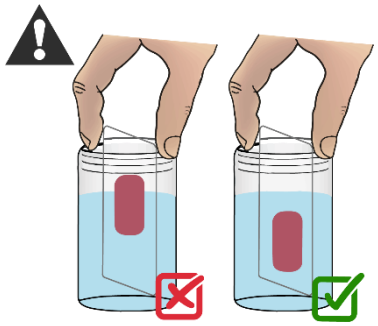
Manual bone marrow smear by crushing method: using a pipette, deposit a small amount of the sample on a microscope slide. Blot up excess blood to keep only shiny lumps. Cover the first slide with a slide. Squeeze and thin the sample by sliding and stretching to the end of the slide. A good quality smear does not reach the end of the slide. Discard the slide used for smearing. Allow the smear to air dry before fixing or staining.

Histological sections: dewax and et hydrate tissues sections in appropriate reagents before staining.

**Reagents and instruments preparation**
If applicable dilute the May-Grünwald solution and the Giemsa solution according to the indications in the protocol section. Transfer the solutions into staining baths as indicated in the protocols below.
Acetic water: 5 drops in 100 mL of distilled water
5% sodium hyposulfite aqueous solution: 5 g of sodium hyposulfite in 100 mL of distilled water

**Protocols**

The staining steps of the protocols indicated below consist of a successive covering of the slides with the different staining reagents or dipping of the slides in the different staining baths. Refer to the title to know which case you are in. The processing time only considers the contact time with the reagents.



***Note:***

*For bath staining method, fill each jar with the appropriate reagent in enough volume to cover the smears. Be sure to cover the entire sample when immersing the slide.*

***Note:***

*For the covering staining method, place the slide on a stand with the fixed smear on top.*

### *Protocol hematological samples - Manual bath staining method - Manual microscopic analysis*

Processing time [hh: mm: ss]: 00: 14 :10

| Steps | Reagent | Time [mm:ss] | Indications |
|---|---|---|---|
| Fix and prestain | May-Grünwald solution | 03:00 | N/A |
| Rinse | Buffer Solution | 01:00 | |
| Stain | 1/20 Giemsa R solution diluted in buffer solution | 10:00 | |
| Rinse | Buffer Solution | 00:10 | Agitate continuously in the bath during the countdown. |
| Dry | N/A | ≥03:00 | N/A |

### *Protocol hematological samples - Manual covering staining method - Manual microscopic analysis*

Processing time [hh: mm: ss]: 00: 14 :10

| Steps | Reagent | Time [mm:ss] | Indications |
|---|---|---|---|
| Fix and prestain | 1 mL of May-Grünwald solution | 03:00 | Cover the slide |
| Rinse | 1 mL of Buffer Solution | 01:00 | Add carefully and mix without spilling.<br>Get rid of the reagent at the end of countdown. |
| Stain | 1/30 Giemsa R solution diluted in buffer solution | 10:00 | Cover the slide and get rid of the reagent at the end of the countdown. |
| Rinse | Buffer Solution | 00:10 | Quickly.<br>Tap water can be used. |
| Dry | N/A | ≥03:00 | N/A |

### *Protocol for histological sections - Manual bath staining method- Manual microscopic analysis*

Processing time [hh: mm: ss]: 01: 05 :00

| Steps | Reagent | Time [mm: ss] | Indications |
|---|---|---|---|
| Stain | Lugol, PVP-stabilized solution | 05:00 | N/A |
| Stain | 5% sodium hyposulfite aqueous solution | 05:00 | N/A |
| Rinse | Distilled water | N/A | |
| Stain | 1/5 May-Grünwald solution diluted in distilled water | 15:00 | In an autoclave at 37° C |
| Stain | Add Giemsa L solution (3 drops in 2 mL of distilled water | 40:00 | |
| Rinse | Distilled water | N/A | N/A |
| Differentiate | Acetic water | N/A | N/A |
| Rinse | Distilled water | N/A | Then drain the excess onto filter paper |
| Dehydrate | Ethanol/ acetone mixture 50/50 | N/A | N/A |
| Dehydrate | Toluene or xylene | N/A | 2 baths |
| Mount | Toluene or xylene-based mounting media | N/A | N/A |

## Expected results

### *Hematological samples*

**Nuclei/chromatin:** +/- dense purple
**Granulocytes cytoplasm without RNA:** light purplish-pink
**Granulocytes eosinophilic granules:** orangey-pink
**Granulocytes basophilic granules:** dark blue
**Granulocytes neutrophilic granules:** ± deep purple-pink
**Lymphocytes cytoplasm with RNA:** pure blue
**Lymphocytes cytoplasm without RNA:** light blue
**Lymphocytes azurophilic granules:** red
**Monocytes cytoplasm:** purplish-blue
**Erythrocytes:** pinkish-beige to beige-grey
**Platelets chromomere:** purplish-red
**Platelets hyalomere:** bluish
**Blood parasites nucleus (*Plasmodium*):** red
**Blood parasites cytoplasm (*Plasmodium*):** blue

### *Histological sections samples*

**Nuclei/Chromatin:** purple to pink
**Basophilic cytoplasm:** sky to dark blue
**Acidophilic cytoplasm:** light red to pinky
**Polychromatophilic cytoplasm:** greyish or purplish
**Acidophilic leukocyte granulations:** orangey
**Neutrophilic leukocyte granulations:** dirty brown pink
**Basophilic leukocyte granulations:** dark violet
**Azurophilic leukocyte granulations:** purple or purplish
**Basophilic erythrocyte granulations:** cobalt blue

If observed results vary from those expected, contact CellaVision RAL Diagnostics technical service through your usual supplier for assistance.

## Performance

May-Grünwald solution enables cell structure staining and microscopic analysis. As it does not allow the detection of analytes, analytical performance is not applicable to this reagent.
This medical device is based on scientific validity (scientific peer-reviewed literature) and demonstration of clinical performance through experience gained from routine diagnostic testing, as well as the regular evaluation of these performances within the framework of Post Market Performance Follow-up (PMPF), to ensure that it continues to meet the expected performance and safety standards.

To ensure product performance, use clean and dry laboratory equipment.

The laboratory is responsible for notifying the manufacturer and state competent authority of any serious incident relating to the medical device uses.

## User quality control

Users are responsible for determining the appropriate quality control procedures for their laboratory and complying with applicable laboratory regulations.

Hematological sample: CellaVision RAL Diagnostics recommends staining a freshly made blood smear with a normal WBC count and no known abnormal pathology at reagent renewal and for the first staining cycle each day. Slides stained for quality control purposes should be checked to ensure that they are satisfactory for the intended test (properly stained and free of precipitates).

Staining results must also be compliant with this manual expected results.

These quality control procedures should only be performed by qualified personnel.

## Other products

For more information, contact your usual supplier.

## Recommendations, notes, and troubleshooting

**Products appearance**
If the appearance of the products differs from the description above, do not use it and contact CellaVision RAL Diagnostics technical service through your usual supplier for assistance.

**Procedures notes**
To prevent products degradation, comply with the storage and handling recommendations specified in this manual.

Be sure to cover the entire sample when immersing the slide.

Staining quality and reproducibility are obtained by using a buffer solution.
Due to the complex mode of action of stains, it is essential to set up standard staining conditions to ensure perfect staining quality and reproducibility. The use of tap water or water mixing is not recommended because unpredictable variations may occur and alter staining results.

To ensure staining quality and reproducibility, CellaVision RAL Diagnostics recommends the use of a buffer solution specially formulated for Hematology. Safe for the users, Buffer solutions for Hematology are formulated with phosphates, are specially developed for hematology, and guarantee improved product stability and rinsability.

**Products stability**
Every CellaVision RAL Diagnostics product can be used until the expiry date indicated on, in its original packaging if it is still hermetically sealed.

**Staining stability**

Staining quality and reproducibility depend on the correct use of the products. Staining conducted according to these recommendations remain stable for several days. If it is necessary to store the stained smears for several months or years, CellaVision RAL Diagnostics recommended mounting them with a coverslip, using a suitable mounting liquid and storing them in a light and dustproof container.

**Instructions for cleaning and waste disposal**

All biological samples, effluents, and used consumables should be considered potentially hazardous.

To avoid any risk, apply the following instructions: dispose of samples, effluents, and consumables in accordance with laboratory standards and applicable national and local standards and regulations.

Chemical and biological waste must be collected and processed by specialized, registered companies.

## Table of symbols and abbreviations

Depending on the product, you may find the following symbols on the device or the packaging material.

| GHS Pictograms | Interpretation |
|---|---|
|  | Explosive |
|  | Flammable |
|  | Oxidizer |
|  | Compresses gas |
|  | Corrosive |
|  | Toxic |
|  | Harmful |
|  | Health Hazard |
|  | Environmental Hazard |
|  | No labelling applicable |

| SYMBOL | INTERPRETATION |
|---|---|
|  | Batch code |
|  | Serial number |
|  | Catalogue reference |
|  | Date of manufacture |
|  | Use up to |
|  | Unique device identifier |
|  | Manufacturer |
|  | Importer |
|  | Entity distributing the medical advice in the region concerned |
|  | CE marking device |
|  | In vitro diagnostic medical device |
|  | Authorised Representative in the European Community |
|  | Authorised Representative in Switzerland |
|  | Authorised Representative in United Kingdom |
|  | Complies with UK guidelines |
|  | Do not use if packaging is damaged |
|  | Keep away from light<br>Keep away form heat |
|  | Temperature limit: 15-25° |
|  | Temperature limit: 15-30° |
|  | Keep dry |
|  | Box: handling upwards |
|  | Fragile |
|  | Sterilised by irradiation |
|  | Single sterile barrier system with outer protective packaging |
|  | Sterile and radiation-sterilised barrier suit |
|  | Do not reuse |
|  | Do not resterilize |
|  | Contents sufficient for n tests |
|  | Hazardous material contained |
|  | Consult instructions for use |
|  | Use |
|  | After opening, use within XX months |
|  | The product must not be used in conjunction with an automatic colouring machine |
|  | Indicates a medical device that contains potentially carcinogenic, mutagenic or reprotoxic (CMR) substances, or substances classified as endocrine disruptors |

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## Changes tracking

| Date | Version | Changes |
|---|---|---|
| 12/2024 | IFU016B | Update in header and the following paragraphs: Device description,Storage and use conditions, Hazard classification and safety information, Operating procedure, Expected results, Performance and Table of symbols and abbreviations.<br>Add of the covering staining method.<br>Add of the legal representatives.<br>Add of CH-REP and UK-REP symbols. |
| 05/2022 | IFU016A | IVDR (EU) 2017/746 compliance |

## Legal representatives

| Countries | Address |
|---|---|
| UK REP | Qavis UK Ltd, company N° SC679796, 56-66 Frederick Street Edinburgh, EH21LS, United Kingdom |
| CH REP | MedEnvoy Switzerland, Gotthardstrasse 28, 6302 Zug Switzerland |



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