Giemsa R solution

REF. 320310

Fixation and differential staining of cellular structures

CE IVD

IFU020A-RAL

For professional use only.
Please read all information carefully before using this device.

Table of contents

Intended use

Giemsa R solution is intended to be used for the fixation and the differential staining of cellular structures prior microscopic examination.

If applicable, RAL Diagnostics recommends using the associated 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.
Giemsa staining is the most useful panoptic staining method in parasitology namely in the field of tissular and blood protozoa. It can be used in medical and veterinary mycology. Giemsa stain cytoplasmic structures in blue and nuclear structures or other DNA-containing organites (like the kinetoplast of trypanosomatidae) in purple red.

Device description

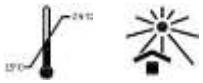
Giemsa R solution
Clear dark blue solution

REF. 320310-0125	1 x 125 mL
REF. 320310-0500	1 x 500 mL
REF. 320310-1000	1 x 1.0 L

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

Storage

Storage temperature: 15-25°C away from light.
Bottle shelf life before and after opening: refer to the expiry date on the label.



Active components

Giemsa R solution
May-Grünwald: ca 0.4 %
Methylene blue - CAS 61-73-4: ca 0.1 %

Hazard classification and safety information

Giemsa R solution

Danger: H225 - Highly flammable liquid and vapour. H301 - Toxic if swallowed. H311 - Toxic in contact with skin. H331 Toxic 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 specimen must be treated in accordance with procedures available in the laboratory and required by national authorities.

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

Methanol, microscope slides, and these following RAL Diagnostics devices:
May-Grünwald solution REF. 320070
pH=6.8 buffer solution for Haematology REF. 330368
pH=7.0 buffer solution for Haematology REF. 330370
pH=7.2 buffer solution for Haematology REF. 330372

This equipment may vary depending on the protocol. Please 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 supplier's instructions for use.

Sample preparation

The following examples are for hematological sample preparations, specimen must treat 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 & 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.

NB: if you do not have a smearing droplet device, open the tube, and use a pipette to deposit a blood drop.

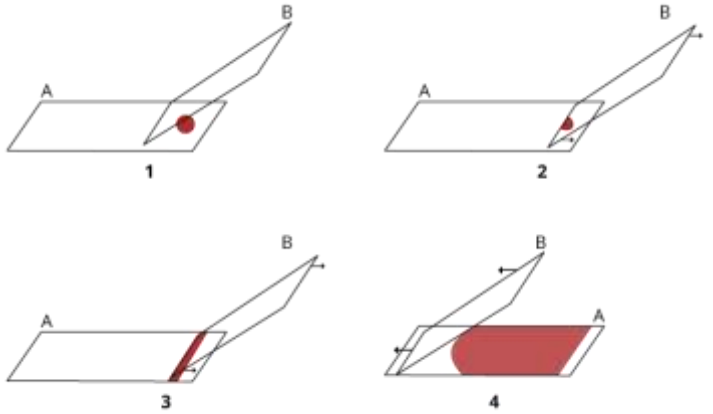


Figure 1. Schematic representation of performing a blood smear
A & B: slides, 1 – 4: steps 1 to 4

Manual bone marrow smear by crushing method: using a pipette deposit, a small amount of the sample on a microscope slide. Blot up blood excess 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.

Reagents and instruments preparation

If applicable dilute the Giemsa solution according to the indications in the protocol section. Transfer the solutions into staining baths as indicated in the protocols below.

Protocols

The staining steps of the protocols indicated below consist of a successive dipping of the slides in the different staining baths.

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

Processing time: 14 min 10 s

Steps	Reagent	Time [mm:ss]	Indications
Fix and pre-stain	May-Grünwald solution	03:00	No
Rinse	Buffer Solution	01:00	
Stain	1/20 Giemsa solution diluted in buffer solution	10:00	
Rinse	Buffer Solution	00:10	Agitate continuously in the bath during the countdown
Dry	No	≥03:00	No

Protocol for histological sections according to Lennert - Manual bath staining method - Manual microscopic analysis

Processing time: 21 min 02 s

Steps	Reagent	Time [mm:ss]	Indications
Stain	Methanol	01:00	Can be extend to 2 minutes
Rinse	Water	00:02	Quickly
Stain	1/10 Giemsa solution diluted in buffer solution	20:00	Check under microscope and repeat differentiation if necessary
Rinse	Water	No	Quickly
Dry	No	≥03:00	No

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: red
Blood parasites cytoplasm: blue

Parasitological and mycological samples
Cytoplasms of host, fungic or parasitic eukaryotic cells: blue to dark blue, depending on the ribosomal richness.
Nuclei: purple red

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

Performance

This medical device is state of the art. Its analytical performance, scientific validity and medical relevance are assessed in the CE marking review.

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: 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 intended test (properly stained and free of precipitate).

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

Other products

For more information, please 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 RAL Diagnostics technical service through your usual supplier for assistance.

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

Quality and reproducibility of staining are obtained by using buffer solution.
Due to complex mode of action of stains, it is essential to set up standard staining conditions to assure a 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.
Quality and reproducibility of staining are obtained using Buffer solution specially formulated for Hematology. Safe for users, Buffer solutions for Haematology are formulated with phosphates, are specially developed for hematology, and allow to guaranty a better products stability and rinsability.

Product stability
Every 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 will remain stable for several days. If it is necessary to store the stained smears for several months or years, 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
LOT	Batch code
SN	Serial number
REF	Catalogue reference
	Date of manufacture
	Use up to
UDI	Unique device identifier
	Manufacturer
	Importer
	Entity distributing the medical advice in the region concerned
CE	CE marking device
IVD	In vitro diagnostic medical device
	Authorised Representative in the European Community
	Authorised Representative in Switzerland
UK CA	Complies with UK guidelines
	Do not use if packaging is damaged
	Keep away from light
	Temperature limit: 15-25°C
	Temperature limit: 15-30°C
	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	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

Bibliography

BESSIS M., *Réinterprétation des frottis sanguins*, éd Masson Springer, 1976, p.9

DATRY A., LECSO G., RICHARD-LENOBLE D. et KOMBILA M., *Coloration rapide des plasmodies et des microfilaires par les colorants solubles dans l'eau, Med. Trop., vol 42*, n°6, nov-déc 1982, p.673-675.

DUHAMEL G., DUHAMEL E., *Cytologie hématologique, Les cellules pathologiques I et II, Coloration au May-Grünwald Giemsa RAL,* Biologiste et Praticien et Réactifs RAL, 1984 et 1989.

Ecole Nationale de Chimie, *Coloration de Pappenheim, Présentation théorique des mécanismes cytochimiques des colorants neutres avec applications techniques détaillées, Journée du technicien biologiste*, mars 1980, p. 1-9.

GENTILHOMME O., TREILLE–RITOUET D., BRYON P-A.,*Cytologie hématologique, Les cellules normales, Coloration au May-Grünwald Giemsa RAL, Réactifs R.A.L,* 1989.

LANGERON M., *Précis de microscopie,* Masson & Cie 6ème éd., 1942, 587-591.

MATHIOT C., *Cytologie en hématologie, quelques aspects de la pathologie.* Biologiste, praticien et Réactifs RAL, 1979

SOCIETE FRANCAISE D'HEMATOLOGIE (SFH), *Guides des bonnes pratiques des ponctions médullaires*, Juin 2003, VI.2

THEML H., *ATLAS de poche d'Hématologie,* Médecine-Sciences Flammarion, p. 19-25, 2000

Changes tracking

Date	Version	Changes
05/2022	IFU020A-RAL	IVDR (EU) 2017/746 compliance

RAL Diagnostics - Site Montesquieu - 33650 Martillac – France
T+33(0)5 57 96 04 04 - F +33 (0)5 57 96 04 55 - ral-diagnostics.fr / cellavision.com