Sample kit MCDh

REF. 960200-0000

Fixation and differential staining of cellular structures

CE IVD

IFU007A-RAL

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

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Intended use

MCDh staining 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

The MCDh panoptic staining allows to perform blood cell counting, realized by using successively four reagents: MCDh 1, MCDh 2, MCDh 3 and MCDh 4.
As a variation of May-Grünwal Giemsa staining, MCDh staining also enables a cytological study of fluids (urines, cerebrospinal, articular, pleural, etc.) and solid samples (biopsies, tissues, swabs etc.). The interpretation of smears provides qualitative and quantitative evaluation of the presence of bacteria, leukocytes, and epithelial cells.
MCDh 1, formulated with ethylic alcohol, is a mixture of neutral stains. It allows a smear fixation and prepares the staining, especially the one of hydrosoluble elements such as basophilic granules. Those stains are inactive in alcoholic medium, and only react selectively when released in aqueous MCDh 2 solution. This releasing generates the precipitation of neutral stains, leading to erythrocytes, cytoplasm of some leukocytes like neutrophilic granulocytes, cytoplasm of bacteria as well as eosinophilic granules staining. MCDh 3 is an aqueous solution which stains cytoplasm of monocytes and lymphocytes. MCDh 3 also eases the metachromasia process as it colors azurophilic granules red.
Eventually, MCDh 4 removes excess of stain and participates to differentiation of cellular elements thanks to action of specially selected rinsing agents.
The successive action of MCDh 1, MCDh 2, MCDh 3 and MCDh 4 brings the violet color (typical Romanowsky-Giemsa effect), particularly visible in chromatin, platelets, and neutrophilic granules.

Kit description

MCDh 1
Clear dark blue solution
REF. 313590-0100 1 X 100 mL

MCDh 2
Clear colorless solution
REF. 313570-0100 2 X 100 mL

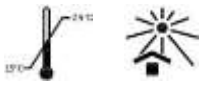
MCDh 3
Clear dark blue solution
REF. 313560-0100 1 X 100 mL

MCDh 4
Clear colorless solution
REF. 313600-0100 1 X 100 mL

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

MCDh 1
May-Grünwald: ca 0.1%
Methylene azure I blue – CAS - 531-55-5: ca 0.05%

MCDh 2
Potassic mono phosphate - CAS 7778-77-0: ca 0.05%
Anhydrous disodic phosphate - CAS 7558-79-4: ca 0.04%

MCDh 3
Methylene blue – CAS - 61-73-4: < 0.25%

MCDh 4
Potassic mono phosphate - CAS 7778-77-0: ca 0.03%
Anhydrous disodic phosphate - CAS 7558-79-4: ca 0.03%

Hazard classification and safety information

MCDh 1

Danger: H225-Highly flammable liquid and vapour.
P210-Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

MCDh 2

Warning: H317-May cause an allergic skin reaction. H412 - Harmful to aquatic life with long lasting effects.
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray. P280 - Wear protective clothing, protective gloves, eye protection. P333+P313 - If skin irritation or rash occurs: Get medical advice/attention. P362+P364 - Take off contaminated clothing and wash it before reuse.

CONT	5-chloro-2-methyl-2H-isothiazol-3-one 2-methyl-2H-isothiazol-3-one

MCDh 3

No labelling applicable

MCDh 4

Warning: H226 - Flammable liquid and vapour. H317 - May cause an allergic skin reaction. H412 - Harmful to aquatic life with long lasting effects.
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. P280 - Wear protective gloves, protective clothing, eye protection. P333+P313 - If skin irritation or rash occurs: Get medical advice/attention. P362+P364 - Take off contaminated clothing and wash it before reuse.

CONT	5-chloro-2-methyl-2H-isothiazol-3-one 2-methyl-2H-isothiazol-3-one

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

Microscope slides and absolute ethanol.

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

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.

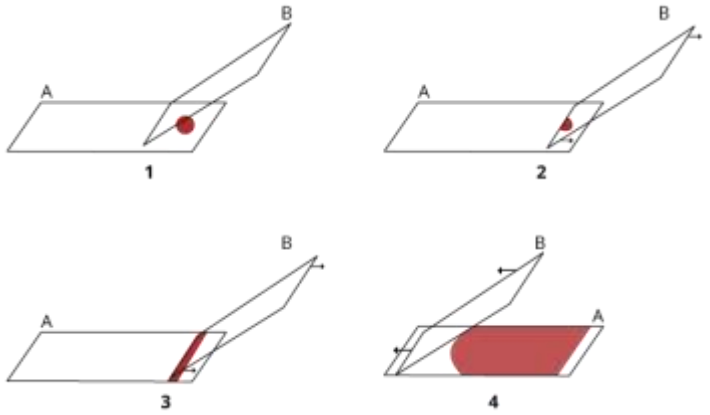


Figure 1. Schematic representation of performing a blood smear
A & B: slides, 1 – 4: steps 1 to 4

Manual bacterial smear: made a thin film of bacteria sample and leave the slide to dry at room temperature. Then bacterial smear can be fixed with mild heat source (Bunsen burner or hot plate) or chemically fixed with chemical fixative (methanol, ethanol, acetic acid, or formalin…)
NB: Never pass through the flame a smear that is not entirely dry, this could cause cracklings and dissemination of bacteria (creation of aerosols).
If necessary, the two fixations can be combined.

Reagents and instruments preparation

No preparation needed. The solutions are ready to use and the reagents containers have been designed to be used for slides staining.

Protocols

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

Protocol for hematological samples – Manual bath staining method - CellaVision® automates analysis

Processing time: 11 min 30

Steps	Reagent	Time [mm: ss]	Indications
Fix and pre-stain	MCDh1	06: 00	Do not agitate
Stain	MCDh2	01: 00	Agitate in the bath 5 to 10 times at the end of countdown
Stain	MCDh2	02: 00	Agitate in the bath 5 to 10 times at the beginning and the end countdown
Stain	MCDh3	00: 30	
Rinse	MCDh4	02: 00	
Dry	No	≥03: 00	No

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

Processing time: 09 min 50

Steps	Reagent	Time [mm: ss]	Indications
Fix and pre-stain	MCDh1	06: 00	Do not agitate
Stain	MCDh2	01: 00	Agitate slowly 3 times in the bath at the end of countdown
Stain	MCDh2	02: 00	
Stain	MCDh3	00: 40	
Rinse	MCDh4	00: 10	Agitate continuously in the bath during countdown
Dry	No	≥03: 00	No

Note: In case of refringence/water artefact phenomena, pre-fix the slides 2min in a bath of absolute ethanol before staining. Directly start the staining after pre-fixation step without drying the slides.

Protocol for cyto-bacterial samples- Manual bath staining method- Manual microscopic analysis

Processing time: 09 min 32

Steps	Reagent	Time [mm: ss]	Indications
Fix and pre-stain	MCDh1	07: 00	Do not agitate
Stain	MCDh2	01: 00	Agitate slowly in the bath at the end of countdown
Stain	MCDh2	01: 00	
Stain	MCDh3	00: 30	
Rinse	MCDh4	00: 02	Agitate continuously in the bath during countdown
Dry	No	≥03: 00	No

Expected results

Hematological samples:
Nuclei / chromatin: +/- dense purple
Granulocytes cytoplasm: light purplish-pink
Granulocytes eosinophilic granules: orangey
Granulocytes basophilic granules: dark blue
Granulocytes neutrophilic granules: +/- deep purple
Lymphocytes cytoplasm with RNA: pure blue
Lymphocytes cytoplasm without RNA: light blue
Lymphocytes azurophilic granules: red
Monocytes cytoplasm: cloudy blue
Erythrocytes: pinkish-beige
Platelets chromomere: purplish-red
Platelets hyalomere: bluish
Blood parasites nucleus: red
Blood parasites cytoplasm: blue

Cyto-bacterial samples
Nuclei / chromatin: +/- dense purple
Cytoplasm: light blue to light purplish- blue
Erythrocytes: pinkish-beige
Bacteria: blue to purplish-pink

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).

Bacterial sample: RAL Diagnostics recommend using a known bacteria sample for reagents quality control at reagents renewal, for each staining cycle or at least for the first staining cycle if a stain is performed multiple times daily.
The result is checked under a microscope, in comparison with the results obtained by the usual technique validated by the laboratory.
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 that describe above in this manual, do not use it and contact RAL Diagnostics technical service for assistance.

Procedures notes
To prevent any degradation of the products, please respect the storage and handling conditions specified in this manual.

In case of refringence/water artefact phenomena, pre-fix the slides 2min in a bath of absolute ethanol before staining.

Products stability
Each RAL Diagnostics product can be used until the expiration date indicated on, in its original packaging and always 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 smear for several months or years, RAL Diagnostics recommends 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

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Changes tracking

Date	Version	Changes
05/2022	IFU007A-RAL	IVDR (EU) 2017/746 compliance

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