Carbolic methylene blue

CE IVD

REF.310100

Counter-stain solution for Ziehl-Neelsen technique

IFU083A

For professional use only.
Please 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

Carbolic methylene blue in combination other staining devices is intended to be used as a counter-stain solution for Ziehl-Neelsen staining variations 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

Carbolic methylene blue in combination with Ziehl carbolic fuchsin allows detection of mycobacteria and acid fast and semi-acid-fast micro-organisms.
Ziehl Neelsen methylene blue counterstaining rechnique allows a detection of mycobacteria or Acid Fast Bacilli (AFB). The characteristic structure of the mycobacteria walls hampers discoloring agent penetration. This property allows AFB to keep Ziehl Carbolic Fuchsin staining after discoloring with acid and alcohol.
Modified Ziehl Neelsen staining for semi-acid-fast micro-organisms allows to detect semi-acid-fast micro-organisms. They are stained in light pink with Ziehl Carbolic Fuchsin and retain the color despite combined action of acid and alcohol. Thereby, *Nocardia genus* (semi-acid-fast) can be rapidly distinguished from *Actinomyces, Actinomadura* and *Streptomyces* (filamentous bacteria related non-semi-acid-fast). For the two staining, other bacteria (non-AFB) and cell elements are counterstained by Methylene blue.

Carbolic methylene blue
Clear blue solution
REF. 310100-1000 1 X 1 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 expiry date on the label.
Bottle shelf life after opening : refer to expiry date on the label and if the "period after opening" symbol is present take it into account.

Hazard classification and safety information

Carbolic methylene blue
Warning:

H226 - Flammable liquid and vapour.
H315 - Causes skin irritation.
H319 - Causes serious eye irritation.
H341 - Suspected of causing genetic defects.
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P264 - Wash hands thoroughly after handling.
P280 - Wear protective gloves, protective clothing, eye protection, face protection.
P308+P313 - IF exposed or concerned: Get medical advice/attention.
P337+P313 - If eye irritation persists: Get medical advice/attention.

CONT	C6H5OH

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

Sulfuric acid, 90°ethanol, hot plate, microscope slides and these following RAL Diagnostics devices:
Ziehl carbolic fuchsin REF. 320490
SUREFIX REF. 336000-0050

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 specimen must be treated in accordance with procedures available in the laboratory and required by national authorities.

The following example is for bacterial sample preparation.

Pre-treatment of sample from liquid culture media: Take around 300 to 400 µL of liquid culture medium (including a few beads if possible) and pour it into an microtube. Centrifuge for 1 min at 10 000 rpm and discard supernatant. Then add 2 to 3 drops of physiological saline to the microtube and vortex or stir with a loop. The sample is now ready to be smeared.

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.

Manual bacterial smear from liquid or solid culture: Apply a drop of SUREFIX on a slide and with a loop place on top of the SUREFIX drop, the preparation from a liquid culture (as described above) or a colony from a solid culture. Mix SUREFIX drop and the sample and made a uniform smear layer. Eventually Leave the smear to air dry before placing the slide on a hot plate for 30 min at a temperature of 80 °C.

Reagents and instruments preparation
Carbolic methylene blue is ready to use.
If applicable dilute sulfuric acid in distilled water according to the indications in the protocol section.

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. Please refer to the title to know which case you are in. For the covering method, place slide on a stand with fixed smear on top. The processing time only considers the dipping time in the reagents.

Protocol for Ziehl Neelsen methylene blue counterstaining - Manual covering method with hot plate - Manual microscopic analysis

Processing time [hh:mm:ss]: 00:18:30

Steps	Reagent	Time [mm: ss]	Indications
Stain	Ziehl Carbolic Fuchsin	NA	Place the slide on hot plate fixed smear on top. Cover the smear with the reagent
Heat	NA	10: 00	Add Ziehl Carbolic Fuchsin time to time to avoid desiccation
Rinse	Tap water	NA	Get rid of the stain and rinse
Discolor	¼ Sulfuric acid solution	03: 00	NA
Rinse	Tap water	NA	NA
Discolor	90° ethanol	05: 00	NA
Rinse	Tap water	NA	NA
Stain	Carbolic methylene blue	00: 30	NA
Rinse	Tap water	NA	NA
Dry	NA	≥03: 00	NA

Protocol for modified Ziehl Neelsen staining for semi acid-fast micro-organisms - Manual covering method with hot plate - Manual microscopic analysis

Processing time: [hh:mm:ss]: 00:10:30

Steps	Reagent	Time [mm: ss]	Indications
Stain	Ziehl Carbolic Fuchsin	NA	Place the slide on hot plate fixed smear on top. Cover the smear with the reagent
Heat	NA	05: 00	Add Ziehl Carbolic Fuchsin time to time to avoid desiccation
Rinse	Tap water	NA	Get rid of the stain and rinse
Discolor	1% Sulfuric acid solution	NA	Util the smear gets a pale pink tint
Rinse	Tap water	NA	NA
Discolor	90° ethanol	05: 00	NA
Rinse	Tap water	NA	NA
Stain	Carbolic methylene blue	00: 30	NA
Rinse	Tap water	NA	NA
Dry	NA	≥03: 00	NA

Microscopic examination is performed with objectives X100 immersion for Ziehl-Armand, Ziehl Neelsen methylene blue counterstaining and modified Ziehl Neelsen for semi acid-fast micro-organisms staining.

Expected results

Ziehl- Armand staining and Ziehl Neelsen methylene blue counterstaining

Acid-alcohol Fast Bacilli (A.F.B.): pink
Background of the preparation: blue

Modified Ziehl Neelsen staining for semi acid-fast micro-organisms

***Nocardia* filaments:** light pink (semi-acid-fast)
***Actinomyces*, *Actinomadura* and *Streptomyces* filaments**: colorless (non-semi-acid-fast)
Background of the preparation: blue

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.
Staining results for each cell type must also be compliant with this manual expected results.

Ziehl Neelsen and variant staining: RAL Diagnostics recommend using a positive smear and a negative smear from different patient samples at reagents renewal and for the first staining cycle of 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).
Slides can be prepared in advance and heat-fixed appropriately for storage.
This control could be done using a positive patient sample or a dilute suspension of AFB recognized positive (such as *Mycobacterium abscessus* CIP 108541).

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 CellaVision 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.

According to the thickness of the smear, it may be necessary to increase the Ziehl Carbolic Fuchsin staining time.

Ziehl Neelsen methylene blue counterstaining: to realize a screening of specimen samples, it is recommended to use an auramine fluorescence technique before.
The observation of a single bacillus on a given slide is a dubious result and should always lead to a new investigation on another sample.
In all cases, the bacteriologist's report should always refer to the number of fields observed and be consequently reported as “no AFB detected on 200 (or 100) microscopic fields” and not as “negative bacilloscopy”.
Likewise, “positive bacilloscopy” is also a bad answer because it gives no indication of the sputum relative richness in bacilli. The report must always provide quantitative information.

Microscopic examination is performed with objectives X100 immersion for Neelsen methylene blue counterstaining and modified Ziehl Neelsen for semi acid-fast micro-organisms staining.

Product 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 will remain stable for several days.

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

Symbols	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
	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
	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

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Changes tracking

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Legal representatives

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United Kingdom	QAvis UK Ltd, company N° SC679796, 56-66 Frederick Street Edinburgh, EH21LS, United Kingdom
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