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Microscopy

Methylene blue (C.I.52015)

for microscopy Certistain®

For professional use only



In Vitro Diagnostic Medical Device



Intended purpose

This staining dye "Methylene blue (C.I.52015) - for microscopy Certistain®" is used for human-medical cell diagnosis and serves the purpose of the bacteriological investigation of sample material of human origin. It is a dry staining dye that is used to prepare a staining solution, that when used together with other in vitro diagnostic products from our portfolio makes target structures evaluable for diagnostic purposes (by fixing, embedding, staining with the above methylene blue solution, counterstaining, mounting) in human-hematological, clinico-cytological, bacteriological, and histological specimen materials.

Unstained structures are relatively low in contrast and are extremely difficult to distinguish under the light microscope. The images created using the staining solutions help the authorized and qualified investigator to better define the form and structure in such cases. Further tests must be carried out according to recognized, valid methods to reach a definitive diagnosis.

This staining solution, which requires further preparation before it can be used, serves exclusively for overview staining in the differentiation of the target structures of cell and tissue components listed below in the section "Result". For more specific diagnosis, e.g. in hematology, we recommend the use of Cat. No. 101424 May-Grünwald's eosin-methylene blue solution modified.

Principle

The cell wall of acid-fast bacteria (AFB) has a high proportion of wax and lipids and hence absorbs dyes only very slowly. The most efficient staining method is the hot staining according to Ziehl-Neelsen. In this method, carbolfuchsin solution is applied to the specimen and heated. This heating process accelerates the rate at which the fuchsin dye is absorbed and thus also that of the formation of the mycolate-fuchsin complex in the cell wall. Once the acid-fast bacteria have absorbed the fuchsin dye, it is virtually impossible to decolorize them again, even when they are intensively treated with a decolorizing solution such as e.g. hydrochloric acid in ethanol. Accordingly, acid-fast bacteria are termed as acid- and alcohol-fast for staining, and are stained red in the microscopic visualization. Correspondingly, all non-acid-fast microorganisms are counterstained with an appropriate dye. In the present operating instructions is counterstained accordance with methylene blue.

Pretreatment of the specimens with Sputofluor® dissolves the bacteria from the surrounding viscid sputum and cell material.

Sample material

Smears of bacteriological material that have been air-dried, heat-fixed, and pretreated with Sputofluor® like sputum, smears from fine needle aspiration biopsies (FNAB), rinses, imprints, effusions, pus, exsudates, liquid and solid cultures

Sections of formalin fixed, paraffin embedded tissue (3 - 4 µm thick paraffin sections)

Reagents

Cat. No. 115943

Methylene blue (C.I.52015)
for microscopy Certistain®

25 g, 100 g, 1 kg, 5 kg

Color Index No.: 52015

Color Index Name: Basic Blue 9, Solvent blue 8

Also required:

Cat. No. 100327 Hydrochloric acid in ethanol 1 l, 5 l
for microscopyCat. No. 100974 Ethanol denatured with about 1 % 1 l, 2.5 l
methyl ethyl ketone
for analysis EMSURE®Cat. No. 105033 Potassium hydroxide pellets 500 g, 1 kg,
for analysis EMSURE® 5 kgCat. No. 109215 Ziehl-Neelsen carbolfuchsin solution 100 ml, 500 ml,
for microscopy 2.5 l

Alternatively:

Cat. No. 101287 Löffler's methylene blue solution 100 ml, 500 ml,
for microscopy 2.5 l

Instead of the combination of single reagents, the staining kit 1.00497.0001 can be used:

Cat. No. 1.00497.0001

AFB-Color modified

Staining kit for the detection of acid-fast bacteria (AFB) by
hot staining method

1 set

Sample preparation

The sampling must be performed by qualified personnel.

All samples must be treated using state-of-the-art technology.

All samples must be clearly labeled.

Suitable instruments must be used for taking samples and their preparation.

Follow the manufacturer's instructions for application / use.

When using the corresponding auxiliary reagents, the corresponding instructions for use must be observed.

Sputum

The acid-fast bacteria should be pretreated with Sputofluor® to dissolve them from mucus and cellular structures. In this process, the active ingredient hypochlorite dissolves the organic material by oxidation and gently releases the acid-fast bacteria so that they can be processed further.

Reagent preparation: Preparation of Sputofluor® solution 15 %

For preparation of approx. 100 ml solution mix:

Sputofluor®	15 ml
Distilled water	85 ml

Preparing sample material in centrifuge tubes:	
Sample	1 part (min. 2 ml)
Sputofluor® solution (15 % in distilled water)	3 parts
Shake vigorously	10 min
Centrifuge at 3000 - 4800 rpm	20 min
Decant supernatant Prepare smears of the sediment Air-dry	

Punctates, lavages, sediments

After appropriate enrichment measures, smear sample material on the slide and allow to air-dry.

Histological sections

Deparaffinize sections in the conventional manner and rehydrate in a descending alcohol series. Pretreatment with Sputofluor® is not necessary for specimens fixed with formalin.

Fixation

Specimens are fixed over a Bunsen burner flame (2 - 3 times, taking care to avoid excessive heating).

The specimens can also be fixed by heating at 100 - 110 °C in a drying cabinet or on a heating plate for 20 min.

Excessive temperatures or prolonged heating may involve a deterioration of the staining performance.

Reagent preparation

Staining can be carried out using either a ready-to-use (Cat. No. 101287) or a separately prepared Löffler's methylene blue solution (prepared from Cat. No. 115943).

Ethanol 95 %

For preparation of approx. 100 ml of solution, add:

Ethanol	95 g
Distilled water	5 ml
mix	

Potassium hydroxide solution 0.1 %, aqueous

For preparation of approx. 1000 ml of solution, add:

Potassium hydroxide pellets	1 g
Distilled water	1000 ml
dissolve under stirring	

Löffler’s methylene blue solution

For preparation of approx. 100 ml of solution, add:

Methylene blue (C.I.52015) Certistain®	0.3 g
Ethanol 95 %	30 ml
Potassium hydroxide solution 0.1 %, aqueous	add to 100 ml
mix	

The freshly prepared staining solution should be filtered before use.

Procedure

Staining on the staining rack

The stated times should be adhered to in order to guarantee an optimal staining result.

Slide with fixed smear		
Ziehl-Neelsen carbol-fuchsin solution	cover completely, carefully heat three times from below with the Bunsen burner until steam forms Do not allow the staining solution to boil!	stain for 5 min in total
Tap water	rinse until no further clouds of dye are produced	
Hydrochloric acid in ethanol	cover completely and leave to react	15 - 30 sec*
Tap water	rinse immediately	
Löffler’s methylene blue solution	counterstaining, cover completely and leave to react	30 sec**
Tap water	rinse carefully	
Air-dry (e.g. over night or at 50°C in the drying cabinet)***		

- * depending on thickness of specimen
- ** or 1 min with diluted Löffler’s methylene blue solution (dilution: 1:10 (1+9) with dist. water)
- *** Histological samples are not air-dried, after dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, they can be mounted with water-free mounting agents (e.g. Neo-Mount®, Entellan®, DPX new, or Entellan® new) and a cover glass and can then be stored.

Covering with non-aqueous mounting media (e.g. Neo-Mount®, Entellan®, DPX new, or Entellan® new) and a cover glass is recommended for the storage of bacteriological specimens for several months. For this purpose, the stained specimens must be dried very well. When left unmounted, the stain remains stable for approx. 3 days, covered with immersion oil for just a few hours.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Result

Acid-fast bacteria	red
Background	blue

Evaluation

A positive result means “acid-fast bacteria detected” and a negative result “acid-fast bacteria not detected”. A positive result does not mean that a taxonomic classification by microscopy is possible. If acid-fast bacteria are detected, further analyses must be performed in specially equipped laboratories. The vitality (active, inactive) of the bacteria can also not be determined.

Trouble-shooting

Fixation

A sufficient degree of heat-fixing using a Bunsen burner or in a heating cabinet is essential to prevent the infectious potential of the specimens and further proliferation of the bacteria. Excessive temperatures or prolonged heating may involve a deterioration of the staining performance.

No staining of acid-fast bacteria

The critical step of this staining process is the decolorizing step, which can be influenced by the thickness of the specimen smear. In addition, a fresh solution of hydrochloric acid in ethanol is highly reactive, meaning that the result should be evaluated with caution. The incubation times stated in this protocol should be kept accurately in the decolorizing step, since otherwise false-negative results may ensue.

Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory. The freshly prepared staining solution should be filtered before use. Remove surplus immersion oil before filing.

Diagnostics

Diagnoses are to be made only by authorized and qualified personnel. Valid nomenclatures must be used. This method can be supplementarily used in human diagnostics. Further tests must be selected and implemented according to recognized methods. Suitable controls should be conducted with each application in order to avoid an incorrect result.

Storage

Store Methylene blue (C.I.52015) - for microscopy Certistain® at +5 °C to +30 °C.

Shelf-life

Methylene blue (C.I.52015) - for microscopy Certistain® can be used until the stated expiry date. After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +5 °C to +30 °C. The bottles must be kept tightly closed at all times.

Additional instructions

For professional use only. In order to avoid errors, the application must be carried out by qualified personnel only. National guidelines for work safety and quality assurance must be followed. Microscopes equipped according to the standard must be used.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines. Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. Information on disposal can be obtained under the Quick Link “Hints for Disposal of Microscopy Products” at www.microscopy-products.com. Within the EU the currently applicable REGULATION (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 applies.

Auxiliary reagents

Cat. No.	100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No.	100497	AFB-Color modified Staining kit for the detection of acid-fast bacteria (AFB) by hot staining method	1 set
Cat. No.	100579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No.	100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No.	101287	Löffler’s methylene blue solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No.	103699	Immersion oil Type N acc. to ISO 8036 for microscopy	100-ml dropping bottle
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No.	105033	Potassium hydroxide pellets for analysis EMSURE®	500 g, 1 kg, 5 kg
Cat. No.	105387	Leishman’s eosin methylene blue solution modified for microscopy	500 ml
Cat. No.	107960	Entellan® rapid mounting medium for microscopy	500 ml
Cat. No.	107961	Entellan® new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
Cat. No.	108000	Sputofluol® for microscopy	1 l
Cat. No.	108298	Xylene (isomeric mixture) for histology	4 l
Cat. No.	109016	Neo-Mount® anhydrous mounting medium for microscopy	100-ml dropping bottle, 500 ml
Cat. No.	109215	Ziehl-Neelsen carbolfuchsin solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No.	109843	Neo-Clear® (xylene substitute) for microscopy	5 l

Hazard classification

Cat. No. 115943

Please observe the hazard classification printed on the label and the information given in the safety data sheet.

The safety data sheet is available on the website and on request.

Main components of the product

Cat. No. 115943

C.I. 52015

$C_{16}H_{18}ClN_3S \times 2 - 3 H_2O$

M = 319.86 g/mol (x 3 H₂O)

Other IVD products

Cat. No. 101352	May-Grünwald's eosin methylene blue for microscopy	25 g, 100 g
Cat. No. 101424	May-Grünwald's eosine-methylene blue solution modified for microscopy	100 ml, 500 ml, 1 l, 2,5 l
Cat. No. 109204	Giemsa's azur eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No. 109468	Buffer tablets pH 7.2 for preparing buffer solution acc. to WEISE for staining of blood smears	100 tabs
Cat. No. 111374	Buffer tablets pH 6.8 for preparing buffer solution acc. to WEISE for the staining of blood smears	100 tabs

General remark

If during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national authority.

Literature

1. Romeis - Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2015, Springer Spektrum, 19. Auflage
2. Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 6th Edition
3. Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for Use in Biology and Medicine, 10th Edition, (ed. Horobin, R.W. and Kiernan, J.A). Bios, 2002
4. Laboratory Manual of Histochemistry, Linda L. Vacca, 1985, Raven Press
5. Staining Procedures, George Clark, 1981, Williams&Wilkins, fourth Edition



Consult instructions for use



Manufacturer



Catalog number



Batch code



Caution, consult accompanying documents



Use by
YYYY-MM-DD



Temperature
limitation

Status: 2021-Jul-21

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