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## Microscopy

### Löffler's methylene blue solution

for microscopy

For professional use only



In Vitro Diagnostic Medical Device



#### Intended purpose

This "Löffler's methylene blue solution - for microscopy" is used for human-medical cell diagnosis and serves the purpose of the bacteriological and histological investigation of sample material of human origin. It is a ready-to-use staining solution that when used together with other in vitro diagnostic products from our portfolio makes target structures evaluable for diagnostic purposes (acid-fast bacteria (AFB)) by fixing, staining, counterstaining, mounting in bacteriological and histological specimen materials, for example smears of enriched bacterial cultures or histological sections of e.g. the lung.

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.

#### Principle

The cell wall of acid-fast bacteria 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 this instruction for use methylene blue is used for counterstaining. Pretreatment of the specimens with Sputofluol® 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 Sputofluol® 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. 101287  
Löffler's methylene blue solution 100 ml, 500 ml, 2.5 l  
for microscopy

#### Also required:

Cat. No. 109215	Ziehl-Neelsen carbolfuchsin solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No. 108512	AFB-Color carbolfuchsin solution for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	500 ml, 2.5 l
Cat. No. 100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No. 108000	Sputofluol® for microscopy	1 l

#### Alternatively:

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

Cat. No. 1.00497.0001	AFB-Color modified Staining kit for the detection of acid-fast bacteria (AFB) by hot staining method	1 set
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#### Alternatively for histology:

Cat. No. 132450	AFB staining kit for histology for the detection of acid-fast bacteria in histological tissue	1 set
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#### 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 Sputofluol® 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 Sputofluol® solution 15 %

For preparation of approx. 100 ml solution mix:

Sputofluol®	15 ml
Distilled water	85 ml

Preparing sample material in centrifuge tubes:	
Sample	1 part (min. 2 ml)
Sputofluol® 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

Löffler's methylene blue solution should be used to stain histological sections. Compact tissue has the tendency to overstain; for such tissues a dilution of Löffler's methylene blue solution is recommended (dilution: 1:10 (1+9) with distilled water).

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

#### Fixation smear samples

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

The Löffler's methylene blue solution - for microscopy used for staining smears is ready-to-use, dilution of the solution is not necessary and merely produces a deterioration of the staining result and the stability. For histological sections, a dilution of Löffler's methylene blue solution is recommended (1+9 with distilled water).

Procedure

Staining of smear samples

Staining on the staining rack

The stated times should be adhered 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)

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.

Staining of histological samples

Staining in the staining cell

Deparaffinize histological slides in the conventional manner and rehydrate in a descending alcohol series.  
The slides must be immersed and moved briefly about in the solutions, simple immersion alone yields inadequate staining results.  
The slides should be allowed to drip off well after the individual staining steps as a measure to avoid any unnecessary cross-contamination of solutions.

Slide with histological tissue	
AFB-Color carbol fuchsin solution	30 min
Running tap water	45 sec
Hydrochloric acid in ethanol	15 sec
Running tap water	15 sec
Löfflers methylene blue solution*	5 min
Wash with tap water	10 sec
Ethanol 70 %	1 min
Ethanol 70 %	1 min
Ethanol 96 %	1 min
Ethanol 96 %	1 min
Ethanol 100 %	1 min
Ethanol 100 %	1 min
Xylene or Neo-Clear®	5 min
Xylene or Neo-Clear®	5 min
Mount the Neo-Clear®-wet slides with Neo-Mount® or the xylene-wet slides with e. g. Entellan® new and cover glass.	

\* For the counterstaining of histological specimens a dilution of Löffler's methylene blue solution is recommended (dilution: 1:10 (1+9) with distilled water). This working solution can be used for up to 2 weeks.

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 of smear samples

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.

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.  
When using automatic staining systems, please follow the instructions for use supplied by the supplier of the system and software.  
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 the Löffler's methylene blue solution - for microscopy at +15 °C to +25 °C.

Shelf-life

The Löffler's methylene blue solution - for microscopy 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 +15 °C to +25 °C.  
The bottles must be kept tightly closed at all times.

Capacity

approx. 250 stainings / 500 ml

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.  
If necessary use a standard centrifuge suitable for medical diagnostic laboratory.

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](http://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.	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.	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.	108512	AFB-Color carbol fuchsin solution for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	500 ml, 2.5 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
Cat. No.	132450	AFB staining kit for histology for the detection of acid-fast bacteria in histological tissue	1 set

## Hazard classification

Cat. No. 101287

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.

CAUTION! Contains CMR substances. Please observe the corresponding safety instructions given in the safety data sheet.

## Main components of the product

Cat. No. 101287

C.I. 52015	4.2 g/l
C <sub>7</sub> H <sub>5</sub> OH	190 g/l
pH	8.0 - 8.6
1 l =	0.97 kg

## Other IVD products

Cat. No.	105174	Hematoxylin solution modified acc. to Gill III for microscopy	500 ml, 1 l, 2.5 l
Cat. No.	109093	AFB-Fluor Staining kit for fluorescence-microscopic detection of acid-fast bacteria	6x 500 ml
Cat. No.	109204	Giemsa's azur eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No.	109844	Eosin Y-solution 0.5% aqueous for microscopy	1 l, 2.5 l
Cat. No.	111609	Histosec® pastilles solidification point 56-58°C embedding agent for histology	1 kg, 10 kg (4x 2.5 kg), 25 kg
Cat. No.	111885	Gram-Color stain set for the Gram staining method	1 set
Cat. No.	115161	Histosec® pastilles (without DMSO) solidification point 56-58°C embedding agent for histology	10 kg (4x 2.5 kg), 25 kg
Cat. No.	116450	AFB-Color staining kit for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	1 set

## 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: Mulisch, Maria, Welsch, Ulrich, 2015, Springer-Verlag Berlin Heidelberg, 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. Kurzlehrbuch Medizinische Mikrobiologie und Infektiologie, Editor: Uwe Groß, Thieme 2009, 2. Auflage
5. Histological and Histochemical Methods, Theory and practise, J.A. Kiernan, Scion, 5th Edition



Consult instructions for use



Manufacturer



Catalog number



Batch code



Caution, consult accompanying documents



Use by YYYY-MM-DD



Temperature limitation

Status: 2020-Jul-13

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