Ziehl carbolic fuchsin

CE IVD

REF. 320490

Fixation and differential staining of cellular structures

IFU082A-RAL

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

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Intended Use

Ziehl carbolic fuchsin is intended to be used in combination with other staining devices for 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

Ziehl carbolic fuchsin is use for cytological et histological investigation of biologicals sample. The most common uses are Gram-Nicolle staining and Ziehl staining and its variants.
Ziehl carbolic fuchsin solution in combination with Carbolic gentian violet, Lugol, solution and Slow Differentiator or Fast Differentiator allows Gram-Nicolle Staining.
Gram-Nicolle staining is differential staining based on the permeability of the bacterial wall. In this technique, the bacterial wall is not stained but its structure allows classification of Gram-positive or Gram-negative bacteria. Lugol solution allows the formation of an intracellular complex with Carbolic gentian violet. A more important permeability of Gram-negative bacteria wall allows alcohol to eliminate this complex. Gram-negative bacteria can fix Ziehl Carbolic Fuchsin and then appear stained pink.
Gram-positive bacteria, characterized by a less important permeability of wall, are not discolored by alcohol and remain stained violet.
Ziehl-Neelsen staining, and variants allow detection of acid fast and semi-acid-fast micro-organisms.

Ziehl Neelsen staining for histological sections, Ziehl Neelsen methylene blue counterstaining and Ziehl- Armand staining 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). Other bacteria (non-acid-fast) and cell elements are counterstained by Methylene blue

Device description

Ziehl carbolic fuchsin
Clear red violet solution

REF. 320490-0500	1 X 500 mL
REF. 320490-1000	1 X 1.0 L
REF. 320490-2500	1 X 2.5 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 expiry date on label.

Hazard classification and safety information

Ziehl carbolic fuchsin

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 80%

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

Sulfuric acid, absolute ethanol, 90°ethanol, mounting media, microscope slides, and these following RAL Diagnostics devices:
Carbolic gentian violet REF. 320960
Fast differentiator (alcohol / acetone) REF. 361510
Slow differentiator (alcohol-based) REF. 363030
Armand solution REF. 360100
Lugol, PVP-stabilized solution REF. 367400
Lugol solution REF. 367300
Carbolic methylene blue REF. 310100

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 example is for bacterial sample preparation, specimen must treat in accordance with procedures available in the laboratory and promulgated by national authorities.

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
Lugol solution, Lugol, PVP-stabilized solution, Slow differentiator (alcohol-based) or Fast differentiator (alcohol / acetone), are ready to use.
If applicable dilute Ziehl Carbolic Fuchsin and sulfuric acid in distilled water according to the indications in the protocol section.
For staining of histological sections, transfer the solutions into staining baths as indicated in the protocols below.
Alcohol-acid mixture: 1mL of concentrated Hydrochloric acid in 99 mL of 70° ethanol.

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. The information is in the title of the protocols.

For the covering method, place slide on a stand with fixed smear on top.

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

Gram-Nicolle protocol for bacterial smear staining - Manual covering method with Fast differentiator - Manual microscopic analysis

Processing time: 02 min 32 s

Steps	Reagent	Time [mm: ss]	Indications
Stain	Carbolic Gentian Violet	01:00	Can be extended to 5 min
Rinse	Tap water	No	Get rid of reagent and remove the excess
Rinse	Lugol, PVP-stabilized solution	No	A jet of Lugol to remove rinsing water
Stain	Lugol, PVP-stabilized solution	00:30	Can be extended to 1 min
Rinse	Tap water	No	Thoroughly rinse
Discolor	Fast Differentiator	00:02	Can be extend to 5 sec
Rinse	Tap water	No	Quickly
Stain	Ziehl Carbolic Fuchsin 1/10	01:00	No
Rinse	Tap water	No	Quickly
Dry	No	≥03:00	No

Gram-Nicolle protocol for bacterial smear staining - Manual covering method with Slow differentiator - Manual microscopic analysis

Processing time: 02 min 50 s

Steps	Reagent	Time [mm: ss]	Indications
Stain	Carbolic Gentian Violet	01:00	Can be extended to 5 min
Rinse	Tap water	No	Get rid of reagent and remove the excess
Rinse	Lugol, PVP-stabilized solution	No	A jet of Lugol to remove rinsing water
Stain	Lugol, PVP-stabilized solution	00:30	Can be extended to 1 min
Rinse	Tap water	No	Thoroughly rinse
Discolor	Slow Differentiator	00:20	Can be extend to 40 sec
Rinse	Tap water	No	Quickly
Stain	Ziehl Carbolic Fuchsin 1/10	01:00	No
Rinse	Tap water	No	Quickly
Dry	No	≥03:00	No

Protocol for Ziehl- Armand staining- Manual covering method with hot plate - Manual microscopic analysis

Processing time: 11 to 12 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Ziehl Carbolic Fuchsin	No	Place the slide on hot plate fixed smear on top. Cover the smear with the reagent
Heat	No	10: 00	Add Ziehl Carbolic Fuchsin time to time to avoid desiccation
Rinse	Tap water	No	Get rid of the stain and rinse
Stain	Armand solution	01: 00	Can be extend to 2 minutes
Rinse	Tap water	No	Quickly
Dry	No	≥03: 00	No

Protocol for Ziehl- Armand staining- Manual covering method with micro-wave oven - Manual microscopic analysis

Processing time: 1 to 12 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Ziehl Carbolic Fuchsin	No	Place the stand in a plastic box Place the slide on the stand fixed smear on top. Cover the smear with the reagent and close the plastic box.
Heat	No	01: 00	Put the plastic box in a micro-wave oven at maximal power
Rinse	Tap water	No	Get rid of the stain and rinse
Stain	Armand solution	00: 30	Can be extend to 1 minute
Rinse	Tap water	No	Quickly
Dry	No	≥03:00	No

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

Processing time: 18 min 30 s

Steps	Reagent	Time [mm: ss]	Indications
Stain	Ziehl Carbolic Fuchsin	No	Place the slide on hot plate fixed smear on top. Cover the smear with the reagent
Heat	No	10: 00	Add Ziehl Carbolic Fuchsin time to time to avoid desiccation
Rinse	Tap water	No	Get rid of the stain and rinse
Discolor	¼ Sulfuric acid solution	03: 00	No
Rinse	Tap water	No	No
Discolor	90° ethanol	05: 00	No
Rinse	Tap water	No	No
Stain	Carbolic methylene blue	00: 30	No
Rinse	Tap water	No	No
Dry	No	≥03: 00	No

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

Processing time: 10 min 30 s

Steps	Reagent	Time [mm: ss]	Indications
Stain	Ziehl Carbolic Fuchsin	No	Place the slide on hot plate fixed smear on top. Cover the smear with the reagent
Heat	No	05: 00	Add Ziehl Carbolic Fuchsin time to time to avoid desiccation
Rinse	Tap water	No	Get rid of the stain and rinse
Discolor	1% Sulfuric acid solution	No	Util the smear gets a pale pink tint
Rinse	Tap water	No	No
Discolor	90° ethanol	05: 00	No
Rinse	Tap water	No	No
Stain	Carbolic methylene blue	00: 30	No
Rinse	Tap water	No	No
Dry	No	≥03: 00	No

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.

Protocol for Ziehl Neelsen staining of histological sections - Manual bath staining method- Manual microscopic analysis

Dewax and et hydrate histological sections in appropriate reagents before staining.

Processing time: 21 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Ziehl Carbolic Fuchsin	10:00	Filter reagent just before use
Rinse	Tap water	No	Well wash
Discolor	Alcohol-acid mixture	No	Util the section gets a pale pink tint
Rinse	Tap water	08:00	Wash carefully
Stain	1% Methylene blue solution in 0,5% acetic acid	03:00	
Rinse	Tap water	No	No
Rinse	Distilled water	No	No
Dehydrate	Successive alcohol baths	No	Until absolute ethanol
Dehydrate	Toluene or xylene	No	2 baths
Mount	Toluene or xylene based mounting media	No	No

Expected results

Bacterial smear for Gram-Nicolle staining
Gram-positive Bacteria: violet
Gram-negative Bacteria: pink

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

Ziehl Neelsen staining for histological sections
Acid-alcohol Fast Bacilli (A.F.B.): red
Background of the preparation: pale green or blue
Nuclei: dark blue or blue green

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 remain responsible for determining the appropriate quality control procedures for their laboratory and for complying with applicable laboratory regulations.
Staining results for each cell type must also be compliant with this manual expected results.

Gram Nicolle staining: RAL Diagnostics recommend using a Gram positive and a Gram negative sample for reagents quality control at reagents renewal, for each staining set or at least for the first staining cycle if a stain is performed multiple times daily.
These slides can be prepared in advance and heat-fixed appropriately for storage.
This control could be done using Gram positive and Gram negative samples from identified patient samples or using a known Gram positive and Gram negative strains (such as *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922). The strains used must be identified, avoid Gram variable species.

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 controls depend on the authorization 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 the description above, do not use it and contact RAL Diagnostics technical service through your usual supplier for assistance.

Procedures 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.
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 Ziehl-Armand, Ziehl Neelsen methylene blue counterstaining and modified Ziehl Neelsen for semi acid-fast micro-organisms staining.

Adding of Polyvinylpyrrolidone (PVP) to Lugol, PVP-stabilized solution helps to avoid Iodine migration and then provides a satisfactory stability of the ready-to-use plastic bottle packaged solutions.

Products 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.

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
EC REP	Authorised Representative in the European Community
CH REP	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
STERILE R	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
CONT	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

CALMETTE A., BOQUET A., NEGRE L. et BRETEY J., *Manuel technique de Microbiologie et Sérologie*, Masson & Cie, 4ème éd., 1948, p. 94-95.

BEAURIN M.C., *Diagnostic des mycobactéries au laboratoire*, Porphyre Afrique, vol. 1, n°1, nov. 1983, p. 22-24.

COLLINS C.H., LYNE P.M., GRANGE J.M., *Microbiological Methods,* Butterworths, 6ème éd., 1989, p. 368-372.

GANTER P., JOLLES G., *Histochimie normale et pathologique et pathologique*, éd GAUTHIER-VILLARS, vol 2, 1970 p. 1435-1436

LANGERON M., *Précis de microscopie*, Masson & Cie, 6ème éd., 1942, p. 553-556.

PACAUD G., *Coloration en mycobactériologie*, Réactifs RAL, 1977, p. 2-4.

PACAUD G., *Les colorations dans la pratique quotidienne en mycobactériologie*, ATEB, Journée Technique Parisienne, mars 1977.

PILET C., BOURDON J.L., TOMA B., MARCHAL N., BALBASTRE C., PERSON J.M., *Bactériologie médicale et vétérinaire*, Systématique bactérienne, éd. Doin, 1987, p. 260-266.

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