

Lugol solution

REF. 367300

Multi-application microscopy reagent



IFU087A-RAL

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

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Intended Use

Lugol solution 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

Lugol solution in combination with different staining devices allow histo-cytological and bacterial staining.

Lugol solution in combination with Crystal violet oxalate, Slow Differentiator or Fast Differentiator and Safranin allow Gram-Hücker staining or Gram-Nicolle Staining in combination with Carbollic gentian violet, Slow Differentiator or Fast Differentiator and Ziehl Carbollic Fuchsin.

Gram-Hücker and Gram-Nicolle staining are differential staining based on the permeability of the bacterial wall. In these techniques, 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 Crystal violet oxalate or Carbollic gentian violet. A more important permeability of Gram-negative bacteria wall allows alcohol to eliminate this complex. Gram-negative bacteria can fix Safranin or Ziehl Carbollic Fuchsin and then appear stained orangey-pink by Safranin or pink by Ziehl Carbollic Fuchsin. Gram-positive bacteria, characterized by a less important permeability of wall, are not discolored by Alcohol and remain stained violet.

The histo-cytology applications include Gram Weigert staining, Fontana Masson staining, and Mann-Dominici staining with erythrosine- toluidine blue.

Device description

Lugol solution

Clear brown solution

REF. 367300-1000

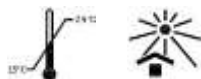
1 X 1.0 L

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

Lugol solution

No labelling applicable

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

Sodium hyposulfite aqueous solution, iodine, absolute ethanol, aniline, ammonia, mounting media, acetic acid, toluene, aluminum sulfate, crystal of thymol, 10% silver nitrate aqueous solution, concentrated ammonia, 0.1 % Gold chloride aqueous solution and microscope slides and these following RAL Diagnostics devices:

SUREFIX REF: 336000-0050

Crystal violet oxalate REF. 361490

Fast differentiator (alcohol / acetone) REF. 361510

Slow differentiator (alcohol-based) REF. 363030

Safranin REF. 361500

Carbolic gentian violet REF. 320960

Ziehl Carbolic Fuchsin 1/10 REF. 364540

Ziehl Carbolic Fuchsin REF. 320490

Mayer Haemalum REF. 320550

Eosin, 1% in aqueous solution REF. 312740

Erythrosin B REF. 350150

Orange G REF. 315370

Toluidine blue, pure grade REF. 361590

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 a 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.

Histological sections: dewax and et hydrate tissues sections in appropriate reagents before staining.

Reagents and instruments preparation

If applicable dilute Ziehl Carboloc Fuchsin in distilled water according to the indications in the protocol section.

Acetic water: 5 mL in 100 mL of distilled water

3 % sodium hyposulfite aqueous solution: 3 g of sodium hyposulfite in 100 mL of distilled water

5 % sodium hyposulfite aqueous solution: 5 g of sodium hyposulfite in 100 mL of distilled water

Iodined ethanol solution: dissolve 0.5 g of iodine in 100 mL of 80 ° ethanol

Erythrosine B - Orange G Solution: apart dissolve 0,2 g of Erythrosine B in 50ml of distilled water then 1g of Orange G in 50ml of distilled water. Mix both solutions.

Toluidine Blue Solution: dissolve 1g of pure Toluidine Blue in 100ml of distilled water and immediately filter before use.

Ammoniacal silver nitrate solution: add concentrated ammonia drop by drop to 20 mL of a 10% silver nitrate aqueous solution. Steadily stirring, until the precipitate that has formed is completely dissolve again (only some very thin granulations must remain).

Nuclear red solution: dissolve by heating 0.2 g of Nuclear red and 5 g of aluminum sulphate in 100 mL of distilled water. Allow the solution to cool, filter and add a crystal of thymol. Filter before use, get rid of the stain when it starts depositing.

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 and the differentiator type, it may be necessary to increase the discoloring in the differentiator time.

Gram-Hücker 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	Crystal violet oxalate	01:00	No
Rinse	Water	No	Get rid of reagent and remove the excess
Rinse	Lugol solution	No	A jet of Lugol to remove rinsing water
Stain	Lugol solution	00:30	Can be extended to 1 min
Rinse	Water	No	Thoroughly rinse
Discolor	Fast Differentiator	00:02	Can be extend to 5 sec
Rinse	Water	No	Quickly
Stain	Safranin	01:00	No
Rinse	Water	No	Quickly
Dry	No	≥03:00	No

Gram-Hücker 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	Crystal violet oxalate	01:00	No
Rinse	Water	No	Get rid of reagent and remove the excess
Rinse	Lugol solution	No	A jet of Lugol to remove rinsing water
Stain	Lugol solution	00:30	Can be extended to 1 min
Rinse	Water	No	Thoroughly rinse
Discolor	Slow Differentiator	00:20	Can be extend to 40 sec
Rinse	Water	No	Quickly
Stain	Safranin	01:00	No
Rinse	Water	No	Quickly
Dry	No	≥03:00	No

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 Violet Gentian	01:00	Can be extended to 5 min
Rinse	Water	No	Get rid of reagent and remove the excess
Rinse	Lugol solution	No	A jet of Lugol to remove rinsing water
Stain	Lugol solution	00:30	Can be extended to 1 min
Rinse	Water	No	Thoroughly rinse
Discolor	Fast Differentiator	00:02	Can be extend to 5 sec
Rinse	Water	No	Quickly
Stain	1/10 Ziehl Carbolic Fuchsin	01:00	No
Rinse	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 Violet Gentian	01:00	Can be extended to 5 min
Rinse	Water	No	Get rid of reagent and remove the excess
Rinse	Lugol solution	No	A jet of Lugol to remove rinsing water
Stain	Lugol solution	00:30	Can be extended to 1 min
Rinse	Water	No	Thoroughly rinse
Discolor	Slow Differentiator	00:20	Can be extend to 40 sec
Rinse	Water	No	Quickly
Stain	1/10 Ziehl Carbolic Fuchsin	01:00	No
Rinse	Water	No	Quickly
Dry	No	≥03:00	No

Protocol for Gram Weigert staining for histological sections - Manual covering method with Slow differentiator - Manual microscopic analysis

This method applies to cytology and histological sections as well as fixed with chromic fixative (Zenker, Helly) and embedded in paraffin.

Protocol to get rid of mercury precipitates

Mercury precipitates could bother the reading of the preparations, carry out after 95° ethanol bath during the dewaxing

Processing time: 03 min

Steps	Reagent	Time [mm: ss]	Indications
Clean	Iodined Ethanol solution	No	Dip the slide
Rinse	Tap water	No	Quickly
Clean	3% Sodium Hyposulfite solution	03:00	Dip the slide
Rinse	Tap water	No	Rinse well
Rinse	Distilled water	No	No

Staining protocol

Processing time: 17 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Mayer haemalum	03:00	Can be extended to 5 min
Rinse	Running water	03:00	Let it in a bath of running water. Can be extended to 5 min.
Differentiate	hydrochloric alcohol or lithium carbonate	No	If necessary to get a clear nuclear staining
Stain	Eosin, 1% in aqueous	01:00	Can be extended to 5 min
Rinse	Running water	No	No
Stain	Carbolic Gentian Violet	05:00	Can be extended to 10 min
Rinse	Water	No	Quickly
Fix dye	Lugol solution	05:00	No
Rinse	Water	No	Quickly Drain on filter paper to get rid of reagent the water excess
Differentiate and dehydrate	50/50 mixture of aniline and xylene or toluene	No	No
Dehydrate	Xylene or toluene	No	Pass slide in
Mount	Xylene or toluene mounting media	No	No

Fontana Masson protocol for tissues sections staining - Manual bath method - Manual microscopic analysis

Processing time: 18h 36 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Lugol solution	10: 00	No
Rinse	Distilled water	15: 00	Deeply wash, can ne extend to 2 hours
Stain	Ammoniacal silver nitrate solution	*	In a closed container and in obscurity
Rinse	Distilled water	No	No
Stain	0.1 % Gold chloride aqueous solution	04:00	
Rinse	Distilled water	No	No
Fix	5% sodium hyposulphite aqueous	02: 00	Thoroughly rinse
Rinse	Water	No	Pass under
Stain	Nuclear red solution	05: 00	No
Differentiate	Ethanol baths	No	Successively
Dehydrate	Toluene or Xylene	No	2 baths
Mount	Mounting media	No	No

* Step of 18 to 24 hours

Mann-Dominici staining with erythrosine protocol for tissues sections staining - Manual bath method - Manual microscopic analysis

Items must be fixed in a chromic fixative (Zenker, Helly).

Protocol to get rid of mercury precipitates

Mercury precipitates could bother the reading of the preparations, carry out after 95°-alcohol bath during the dewaxing

Processing time: 06 min

Steps	Reagent	Time [mm: ss]	Indications
Clean	Iodined Ethanol solution	03:00	Dip the slide
Rinse	Water	No	Briefly
Clean	3% Sodium Hyposulfite solution	03:00	Dip the slide
Rinse	Tap water	No	Rinse well
Rinse	Distilled water	No	No

Staining protocol

Processing time: 33 min 02 s

Steps	Reagent	Time [mm: ss]	Indications
Stain	Lugol solution	05: 00	No
Fix	3% Sodium Hyposulfite solution	05: 00	No
Rinse	Water	No	No
Rinse	05.% acetic acid aqueous solution	01: 00	No
Stain	Erythrosine B - Orange G solution	15: 00	The tint should be strong pink *
Rinse	05.% acetic acid aqueous solution	05: 00	No
Stain	Toluidine blue solution	02: 00	Use a freshly filtered solution Can be extend to 3 min
Rinse	Distilled water	No	Rapidly
Rinse	05.% acetic acid aqueous solution	No	Rapidly, drain excess **
Rinse	Absolute ethanol	00: 02	Dip at once and suddenly and shake. Can be extend to 3 sec
Differentiate	1/3 toluene in Absolute ethanol	No	Transfer to the next bath when the pink color of erythrosine comes up***
Dehydrate	Pure toluene	No	Successively
Mount	Mounting media	No	No

*Do not overstain as Toluidine blue could no stain basophilic cytoplasm anymore.

**Wipe very carefully both faces of the slide and spin-dry very quickly with a filter paper without allowing the section to dry.

***Examine under microscope if differentiation is inadequate repeat this step.

Expected results

Bacterial smear for Gram-Hücker staining

Gram-positive Bacteria: violet

Gram-negative Bacteria: orangey – pink

Bacterial smear for Gram-Nicolle staining

Gram-positive Bacteria: violet

Gram-negative Bacteria: pink

Gram Weigert staining

Nuclei: blue to blackish blue

Bacteria Wall (Pneumocystis carinii, mycosis): violet

Fontana Masson

Reducing substances and pigment (melanin, lipofuscins, Dubin-Johnson disease, bilirubin): black

Mann-Dominici Staining with erythrosine - Toluidine blue

Chromatin nuclei: dark blue

Chromatin nucleolus: red

Basophilic cytoplasm: stronger blue

Acidophilic cytoplasm: pink to red

Neutrophil granulations of leukocytes: pinkish

Eosinophil granulations of leukocytes: bright orangey red

Basophilic granulations of leukocytes: dark blue

Erythrocytes: orangey yellow

Collagen: pale pink

Elastic fibers: bright pink

Bacteria (above all Gram-positive ones): intense blue

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 use of the medical device.

User quality Control

Bacteria Gram 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.

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

Histological sections staining

RAL Diagnostics recommends user quality control at reagent renewal and for the first staining 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).

These quality controls depend on the authorization by qualified personnel.

Users remain responsible for determining the appropriate quality control procedures for their laboratory and for complying with applicable laboratory regulations.

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.

The rinsing liquid for staining can be distilled, demineralized, or tap water.

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.

Histological sections staining

RAL Diagnostics recommends mounting the stained slides with a coverslip using a suitable mounting liquid and to store 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	SYMBOL	INTERPRETATION
	Explosive		Batch code
	Flammable		Serial number
	Oxidizer		Catalogue reference
	Compressed gas		Date of manufacture
	Corrosive		Use up to
	Toxic		Unique device identifier
	Harmful		Manufacturer
	Health Hazard		Importer
	Environmental Hazard		Entry distributing the medical advice in the region concerned
	No labelling applicable		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 re-sterilize
			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

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Change tracking

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