

Safranin

REF. 361500

Differential staining of bacteria



IFU047A-RAL

For professional use only.

Please read all information carefully before using this device.

Table of contents

Intended Use	1
Principle.....	1
Device description	2
Storage	2
Hazard classification and safety information.....	2
Personnel qualification	2
Specific equipment and reagents required but not provided	2
Operating procedure.....	3
Expected results.....	5
Performance.....	5
User quality Control.....	5
Other products.....	5
Recommendations, notes, and troubleshooting.....	6
Table of symbols and abbreviations	7
Bibliography.....	7
Change tracking	7

Intended Use

Safranin is intended to be used in combination with Crystal violet oxalate, Lugol, PVP-stabilized solution and Slow differentiator (alcohol-based) or Fast differentiator (alcohol / acetone) for differential staining of bacteria 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

Safranin in combination with Crystal violet oxalate, Lugol, PVP-stabilized solution and Slow differentiator (alcohol-based) or Fast differentiator (alcohol / acetone) allow Gram-Hücker staining.

Gram-Hücker staining is a differential staining based on the permeability of the bacterial wall. In this technique, the bacterial wall is not stained but its structure permits classification of Gram-positive or Gram-negative bacteria.

Lugol solution allows the formation of an intracellular complex with Crystal violet oxalate. A more important permeability of Gram-negative bacteria wall allows alcohol to eliminate this complex. Gram-negative bacteria can fix Safranin and then appear stained orangey-pink. Gram-positive bacteria, characterized by a less important permeability of wall, are not discolored by Alcohol and remain stained violet.

Gram-Hücker Staining, which original aim is to differentiate Gram-negative bacteria from Gram-positive ones, is very useful in Mycology and Parasitology (medical and veterinary). Indeed, pathogenic fungi are Gram-positive and this specific property can be very beneficial to detect this kind of agents on samples. Microsporidia spores are Gram-positive as well and can then be detected on smears, e.g. through affixing of duodenal biopsies.

Device description

Safranin

Clear red solution

REF. 361500-1000 1 X 1.0 L

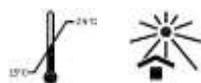
REF. 361500-2500 1 X 2.5 L

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

Safranin

Warning: H226 - Flammable liquid and vapour.

P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.



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

Microscope slides and these following RAL Diagnostics devices:

Crystal violet oxalate REF. 361490,

Lugol, PVP-stabilized solution REF. 367400,

Fast differentiator (alcohol / acetone) REF. 361510 and

Slow differentiator (alcohol-based) REF. 363030

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

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.

Reagents and instruments preparation

No preparation needed. The solutions are ready to use.

Protocols

The staining steps of the protocols indicated below consist of a successive covering of the slides with the different staining reagents.

For the staining steps, place slide on a stand with fixed smear on top.

According to the thickness of the smear the differentiator, it may be necessary to increase the discoloring in the differentiator time.

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, 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	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

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, 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	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

Protocol for smear staining in Mycology and Parasitology with Slow differentiator - Manual covering method - Manual microscopic analysis

Processing time: 01 min 40 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, 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	Water	No	Thoroughly rinse
Discolor	Slow Differentiator	No	No
Rinse	Water	No	Quickly
Stain	Safranin	00:10	Can be extended to 1 min
Rinse	Water	No	Quickly
Dry	No	≥03:00	Quickly

Expected results

Bacterial smear

Gram-positive Bacteria: violet

Gram-negative Bacteria: orangey – pink

Parasitology and Mycology

Pathogenic fungi, Microsporidia spores: violet.

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

The following example is for and bacterial samples.

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.

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.

One can improve the sharpness of the observation with a green light-microscope (a green filter or a yellow one superposed on the blue filter). The contrast depends on the way one carries out the differentiation.

The genus *Campylobacter* is badly colored by R4 Safranin, while the genus *Legionella* remains colorless.

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.

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		Entity distributing the medical advice in the region concerned
	No labelling applicable		CE marking device
			In vitro diagnostic medical device
			Authorized Representative in the European Community
			Authorized 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

CLARK G., *Staining procedures, Williams & Wilkins, 4th éd.*, 1981, p. 377-379.

GENEVA WORLD HEALTH ORGANIZATION, *Manual of basic techniques for a health laboratory*, n°39, 1982, p. 231-234.

VASTEL C.L., *Coloration Gram-Hücker, Le Tech. Biol.*, n°5, 1978, p. 243-245.

WEBER R., BRYAN R.T., OWEN R.L., WILCOX C.M., GORELKIN L., VISVESVARA G.S. and the Enteric Opportunistic Infections Working Group, *Improved light-microscopical detection of Microsporidia spores in stool and duodenal aspirates*, The New England Journal of Medicine, vol. 326, n°3, janv. 1992, p. 161-166.

Change tracking

Date	Version	Changes
05/2022	IFU047A-RAL	IVDR (EU) 2017/746 compliance

