Kit RAL Stainer Gram-Nicolle

REF. 360220-0000

Differential staining of bacteria

CE IVD

IFU054A-RAL

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

Table of contents

Intended Use

Kit RAL Stainer Gram-Nicolle is intended to be used in combination with the RAL Stainer instrument 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

Gram-Nicolle 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 permits 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.

Kit description

Carbolic Gentian Violet
Clear purple solution
REF. 320960-0200 1 X 200 mL

Lugol, PVP-stabilized solution
Clear brown solution
REF. 3674002A0200 1 X 200 mL

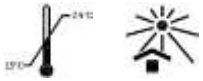
Differentiator
Clear colorless solution
REF. 3615153A200 1 X 200 mL

Ziehl Carbolic Fuchsin 1/10
Clear red solution
REF. 364540-0200 1 X 200 mL

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 opening: refer to the expiry date on the label.
Bottle shelf life after opening: 2 weeks or 500 slides.
Once opened, the duration of use overrules the expiry date.



Hazard classification and safety information

Carbolic Gentian Violet
No labelling applicable

Lugol, PVP-stabilized solution
No labelling applicable

Differentiator

Danger: H225 - Highly flammable liquid and vapour.
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

Ziehl Carbolic Fuchsin 1/10
No labelling applicable

The RFID label used is a passive short-range contactless memory chip (13,56MHz).

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, mild heat source (Bunsen burner or hot plate), chemical fixative (methanol, ethanol, acetic acid, or formalin etc.) and these following RAL Diagnostics devices:
RAL Stainer REF. 405000
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 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
No preparation needed. The solutions are ready to use.

The reagents containers have been designed to be used for slides staining.
Place the 4 bottles labelled 1 to 4 in their respective set position and remove the caps and security rings.

The usual rinsing liquid for staining, validated by the laboratory, can be tap water, demineralized water, etc. Only de-ionized water is deprecated because it can cause a dysfunctionment of the RAL Stainer level sensor.

Protocols
The staining steps of the protocols indicated below consist of a successive dipping of the slides in the different staining baths.

Staining settings, recommended by RAL Diagnostics, are pre-set in the RFID tag.

Protocol for bacterial smear staining – Automated bath method- Manual microscopic analysis

Processing time: 06 min 14

Steps	Reagent	Time [mm: ss]	Indications
Stain	R1 Crystal violet oxalate	00: 45	With agitation
Rinse	Rinsing liquid	00: 45	
Stain	R2 Lugol, PVP-stabilized solution	00: 30	
Rinse	Rinsing liquid	00: 45	
Differentiate	R3 Differentiator	00: 10	
Stain	R4 Safranin	00: 15	
Rinse	Rinsing liquid	00: 04	2 x 2 seconds with agitation
Dry	No	03:00	No

Expected results

Gram-positive Bacteria: violet
Gram-negative Bacteria: pink

If observed results vary from those expected, please contact RAL Diagnostics technical service through your usual supplier for assistance.

Performance

The efficacy of the Kit RAL Stainer Gram-Nicolle reagents was evaluated in a hospital laboratory with 503 clinical samples (CBEU, sputum, blood culture, bronchoalveolar fluid, bile, external ventricular bypass, synovial fluid, ascite fluid).
The sensitivity of the Kit RAL Stainer Gram-Nicolle reagents was evaluated in comparison with the laboratory's manual reference technique: Kit Gram Nicolle. All tests were performed in parallel under the same conditions. Each clinical sample was cultured to determine its status.
The results obtained in this study show that the efficiency of the Kit RAL Stainer Gram Nicolle is equivalent to the Gram-Nicolle manual routine method.

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

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

RAL Diagnostics recommend using a Gram positive and a Gram negative sample 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 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 control procedures should only be performed 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.

Realize a thinner smear as possible to guarantee an optimal fixation, especially in case of sample coming from cultures.

According to the thickness of the smear, it may be necessary to increase the Differentiator time.
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.

The usual rinsing liquid for staining, validated by the laboratory, can be tap water, demineralized water, etc. Only de-ionized water is deprecated because it can cause a dysfunction of the RAL Stainer level sensor.
Decontamination of the rinsing container must be carried out according to the recommendations of the RAL Stainer operating manual.

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
	Authorised Representative in the European Community
	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
	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	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

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Change tracking

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