

Instructions for Use

Q-SLIDE™ GRAM

Cat. no. Z302	Q-Slide™ Gram	5 slides/box
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INTENDED USE

Hardy Diagnostics Q-Slide™ Gram control slides are used to qualify reagents and the method used in gram stain procedures.

SUMMARY

The gram stain is critical to the identification of organisms in clinical and industrial specimens. Q-Slide™ Gram control slides are used to ensure the quality of reagents used as well as the efficacy of staining procedures. Methanol fixed smears of *Staphylococcus aureus* and *Escherichia coli* serve as the positive and negative controls. The remainder of the slide is marked with a grid to allow for the simultaneous staining of up to four unknown specimens in parallel with the positive and negative control.

The gram stain differentiates bacteria on the basis of their cell wall structure. Gram-positive organisms have a very thick peptidoglycan layer in their cell wall compared to that of gram-negative organisms. Crystal violet stain complexes with the mordant, Gram's iodine and becomes integrated within the peptidoglycan layer. When the smear is decolorized, the crystal violet/iodine complex is washed out of the gram-negative cell wall, but the complex remains integrated within the thicker peptidoglycan layer of the gram-positive organisms. Counterstain is used to give a visible color to the gram-negative cells that were decolorized. Safranin is the most common counterstain used, but carbolfuchsin can be used for faintly staining organisms.

STORAGE AND SHELF LIFE

Upon receipt store at 15-30°C. in the original container. Do not use if there are any signs of deterioration. Protect from moisture and dust.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory

personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

SLIDE PREPARATION

1. Prepare smears of unknown organisms in the empty quadrants on the slides. Avoid making smears that are too thick or too thin, either extreme will make the stain difficult to interpret.
2. Fix the unknown smears using one of the two methods below.

Heat Fixing

After the smear has air dried, pass the slide through a flame or hold next to an incinerator for 5 to 10 seconds. Take care not to overheat the slides. Allow slide to cool prior to staining.

Methanol Fixing

Air dry the smear, and place a few drops of methanol over the smear for 1 minute. Drain off excess without rinsing. Allow to air dry.

STAIN PROCEDURE

Follow the manufacturer's instructions for performing the stain. The following is provided as a reminder.

1. Flood fixed smear with crystal violet stain. Allow to sit for 30 seconds.
2. Drain crystal violet and gently rinse with tap water.*
3. Remove excess water and flood smear with Gram's Iodine. The Gram's Iodine should sit for 30 seconds.
4. Gently rinse with tap water.
5. Decolorize by running the decolorizer over the smear until the runoff becomes clear. The amount of time required for decolorization will vary with the thickness of smear and the type of decolorizer used.
6. Gently rinse away excess decolorizer.*
7. Flood slide with Safranin**, allow stain to set for 30 seconds.
8. Briefly rinse, allow to air dry.

MICROSCOPIC EVALUATION

1. Using a low power objective, locate and focus on the cells.
2. Use the oil immersion lens (100x) to determine gram reaction, cell morphology and arrangement.

* Excessive rinsing in these steps could result in the loss of dye in the gram-positive cell walls.

** Alternatively, basic fuchsin can be used as the counterstain for weakly staining gram-negative organisms.

INTERPRETATION OF RESULTS

If the gram stain procedure has been performed properly and the reagents are fresh, the *Staphylococcus aureus* (ATCC® 25923) in the positive control well should appear as a purple to lavender (gram-positive) cocci. The negative control, *Escherichia coli* ATCC® 25922) should appear as a pink to red (gram-negative) rod.

LIMITATIONS OF THE PROCEDURE

Cultures should be 18-24 hours old for gram staining procedures.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological equipment such as microscopes, inoculating loops, incinerators, gram stain reagents, methanol, etc., are not provided.

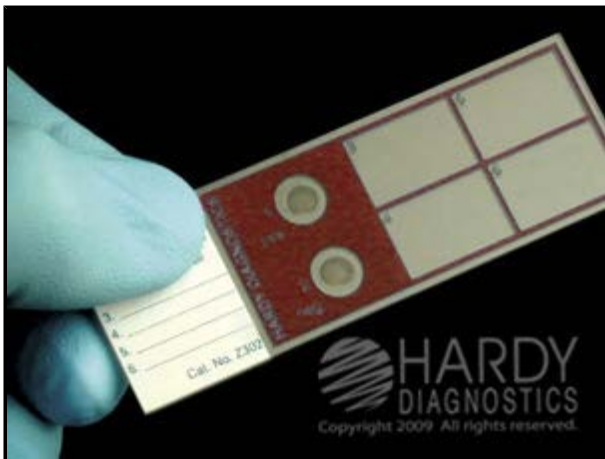
QUALITY CONTROL

Appearance of the positive and negative wells when stained according to standard gram stain procedures:

Test Organisms	Reaction
Positive well:	
<i>Staphylococcus aureus</i> ATCC® 25923	Gram-positive (blue/purple) cocci in pairs or clusters
Negative well:	
<i>Escherichia coli</i> ATCC® 25922	Gram-negative (pink/red) bacilli

PHYSICAL APPEARANCE

Q-Slide™ Gram control slide should have a methanol fixed smear of *Staphylococcus aureus* in the positive control well and *Escherichia coli* in the negative control well.



Q-Slide™ Gram (Cat. no. Z302). Showing the two known QC circles and four test specimen squares.

REFERENCES

1. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

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[Ordering Information](#)

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