
Overview

Useful For

Identifying microorganisms in normally sterile body fluids

Screening sputum specimens for acceptability for bacterial culture

Guiding initial antimicrobial therapy

Testing Algorithm

See [Infective Endocarditis: Diagnostic Testing for Identification of Microbiological Etiology](#) in Special Instructions.

Special Instructions

- [Infective Endocarditis: Diagnostic Testing for Identification of Microbiological Etiology](#)

Method Name

Conventional Gram Stain Procedure

NY State Available

Yes

Specimen

Specimen Type

Varies

Specimen Required

Sources: Closed/open abscess, lower respiratory, fluid, tissue, or swab

Supplies: Culturette (BBL Culture Swab) (T092)

Container/Tube: Sterile container or culture transport swab (Dacron or rayon swab with aluminum or plastic shaft with either Stuart or Amies liquid medium)

Specimen Volume: Entire collection

Acceptable:

Slides: Prepared microscope slide

Collection Container/Tube: Sterile container or culture transport swab

Submission Container/Tube: Slide container

Collection Instructions: Apply original sample to surface of standard microscope slide using appropriate application method (determined by consistency of specimen type) to assure adequate transfer of specimen onto slide. Allow specimen to dry and then heat-fix the slide. Place in slide container for transport.

Reject Due To

Swab/Other Blood, stool, vaginal/cervical secretions, throat, or nasal specimen

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Refrigerated (preferred)		
	Ambient		

Clinical & Interpretive**Clinical Information**

The Gram stain is a general stain used extensively in microbiology for the preliminary differentiation of microbiological organisms. The Gram stain is one of the simplest, least expensive, and most useful of the rapid methods used to identify and classify bacteria.

The Gram stain is used to provide preliminary information concerning the type of organisms present directly from clinical specimens or from growth on culture plates. This stain is used to identify the presence of microorganisms in normally sterile body fluids (cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid). It is also used to screen sputum specimens to establish acceptability for bacterial culture (<25 squamous epithelial cells per field is considered an acceptable specimen for culture) and may reveal the causative organism in bacterial pneumonia.

Reference Values

No organisms seen or descriptive report of observations.

Interpretation

During the staining process, the crystal violet and iodine form a complex within the heat fixed cell. In gram-negative organisms, this complex is readily washed out by the acetone-alcohol. They appear red because they retain only the

safranin dye (counterstain). Gram-positive organisms retain the crystal violet-iodine complex after decolorization and remain purple.

Cells and Organisms will be reported according to the following tables:

	White Blood Cells	
	Epithelial Cells	
Low Power Field (LPF-10x)	Rare (R)	< or =1
	Few (F)	1-9
	Moderate (O)	10-25
	Many (M)	>25

	Organisms	
	Oil Immersion Field (OIF-100x)	Rare (R)
	Few (F)	1-5
	Moderate (O)	6-30
	Many (M)	>30

Cautions

Over-decolorization may result in the loss of the crystal violet iodine complex from gram-positive organisms and result in a misinterpretation.

Clinical Reference

Atlas R, Snyder J: Reagents, stains, and media: bacteriology. In *Manual of Clinical Microbiology*. Vol 1. 12th edition, Edited by KC Carroll, MA Pfaller. Washington DC, ASM Press, 2019, pp 331-361

Performance

Method Description

The specimen is applied directly to the slide or may be concentrated first by centrifugation or cytocentrifugation and then placed on the slide. The slide is stained with crystal violet, stained with Gram iodine solution, decolorized with acetone-alcohol, counterstained with safranin stain, and blotted dry. The slide is examined using the oil immersion objective on the microscope. (Chan WW: Gram Stain. In *Clinical Microbiology Procedures Handbook*. Vol 1. Fourth edition. Edited by AL Leber. Washington, DC, ASM Press, 2016. Section 3.2.1)

PDF Report

No

Specimen Retention Time

Gram stained slides are retained for 7 days

Performing Laboratory Location

Rochester

Fees & Codes**Test Classification**

This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information

87205

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
GRAM	Gram Stain	664-3

Result ID	Reporting Name	LOINC®
GRAM	Gram Stain	664-3