STANDARD OPERATING PROCEDURE TEMPLATE · SPECIALTY AND SUBSPECIALTY PROCEDURES

Gram Stain

Governing requirement: 42 CFR 493.1261(a)(2) and 493.1256

Applies to: ☐ Waived ☑ Moderate complexity ☑ High complexity

Laboratory name		**CLIA number**	
Document number		**Version**	
Effective date		**Next review due**	
Author		**Department / section**	
Approved by (laboratory director)		**Director signature**	
Approval date		**Supersedes document**	

Important: Template only. Before use, this document must be reviewed, adapted to your laboratory's instruments, test systems and the manufacturer's instructions, and approved, signed and dated by the laboratory director. Check current federal, state and accreditation requirements. Not legal advice. Sound Lab Consultants is not affiliated with or endorsed by CMS, CDC, FDA, OSHA, CAP, COLA, The Joint Commission, CLSI or any manufacturer.

1. Purpose

To prepare and interpret Gram stains.

2. Scope

Direct specimen and culture smears.

3. Responsibility

- Testing personnel: perform the procedure as written and record QC and patient results.
- Technical supervisor / technical consultant: approves criteria, reviews QC and resolves problems.
- General supervisor (high complexity): provides day-to-day supervision and result review.
- Laboratory director: approves the procedure and is responsible for overall test quality.

4. Definitions

Gram-positive: Retains crystal violet (purple).

Gram-negative: Decolorizes and takes safranin (pink/red).

5. Materials, equipment and records

- Crystal violet, iodine, decolorizer, safranin
- Control slides

6. Procedure

1. Prepare a thin smear; air dry; heat or methanol fix.

2. Crystal violet **[Time]**, rinse; iodine **[Time]**, rinse; decolorize until runoff is clear; rinse; safranin **[Time]**, rinse; dry.

Time	
Time	

Time	

3. Examine under oil; report cells and organisms semi-quantitatively.

4. Check stain with gram-positive and gram-negative control organisms each week of use (493.1261(a)(2)).

5. Call critical Gram stains (e.g. CSF, blood culture) per critical value SOP.

7. Quality control

• Follow the laboratory's QC policy and the manufacturer's instructions; record QC before reporting patient results.

• Stop patient testing and take corrective action when QC or acceptance criteria are not met.

8. Interpretation, reporting and documentation

• Record the activity, date, result and initials on the form or log, and have the supervisor review records **[Review frequency]**.

Review frequency	

• Keep records per the record retention SOP (at least 2 years under 42 CFR 493.1105 unless a longer period applies).

9. Limitations and cautions

• Over-decolorization causes false gram-negative results.

• This is a generic template. Adapt it to your instruments, test systems, manufacturer instructions, state law and accreditation standards before use.

10. References

• eCFR, 42 CFR Part 493 Laboratory Requirements (CLIA)
https://www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493

• CMS, State Operations Manual Appendix C: Survey procedures and interpretive guidelines for laboratories
https://www.cms.gov/regulations-and-guidance/guidance/manuals/downloads/som107ap_c_lab.pdf

• Manufacturer's package insert, operator manual and safety data sheets for each test system in use

• eCFR, 42 CFR 493.1261 https://www.ecfr.gov/current/title-42/section-493.1261

11. Lab-specific notes

Notes and local requirements	

12. Revision history

Version	Date	Description of change	Author	Approved by

13. Annual review log

Procedures and changes must be approved, signed and dated by the current laboratory director before use (42 CFR 493.1251(d)).

Review date	Reviewed by	Signature	Changes needed (Y/N)	Comments

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