

Gram Stain for Intestinal Bacteria

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[Abstract] With this protocol you can perform a gram stain in paraffin embedded tissue sections.

Materials and Reagents

1. Tissue sections (paraffin embedded, gained from mice)
2. Paraffin (Engelbrecht, catalog number: 17932)
3. Xylene (VWR International, catalog number: 28975)
4. Ethanol (Sigma-Aldrich, catalog number: 32205)
5. Iodine (Merck KGaA, catalog number: 109261)
6. Safranin (Merck KGaA, catalog number: 109217)
7. Crystal violet (Merck KGaA, catalog number: 109218)
8. Decolorizing agent (Merck KGaA, catalog number: 110218)
9. 100% (v/v) ethanol (see Recipes)
10. 96% (v/v) ethanol (see Recipes)
11. 80% (v/v) ethanol (see Recipes)

Equipment

1. Slide

Procedure

1. Paraffin embedded 5 μ m tissue sections were deparaffinized: 1 h on 58 °C, 5 x 6 min in xylene, 4 x 3 min in 100% (v/v) ethanol, 2 min in 96% (v/v) ethanol, 2 min in 80% (v/v) ethanol and 2 min in aqua dest.
2. Tissue sections were incubated for 1.5 min with crystal violet staining reagent and washed for 30 sec under running tap water.
3. The slide was flooded with Gram's iodine for 3 min followed by a second washing step for 20 sec with tap water.
4. The slide was flooded with decolorizing agent for 20 sec and once again washed for 20 sec under tap water.
5. The counterstain was safranin (1 min) which was followed by a last washing step under tap water.

Representative data

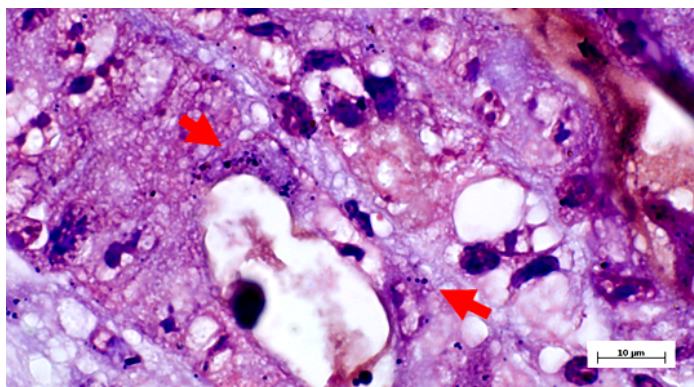


Figure 1. The intestine was stained by gram stain. Gram+ bacteria appear blue and are indicated by a red arrow.

Notes

The staining can also be performed additionally to a previous immune histological staining.

Recipes

1. 100% (v/v) ethanol
Pure ethanol
 2. 96% (v/v) ethanol
96 ml ethanol and 4 ml aqua dest
 3. 80% (v/v) ethanol
80 ml ethanol and 20 ml aqua dest
- Note: The staining solutions are ready to use.*

Acknowledgments

This work was supported by the DFG (ZE872/1-2 individual grant to RZ)

References

1. Schwab, L., Goroncy, L., Palaniyandi, S., Gautam, S., Triantafyllopoulou, A., Mocsai, A., Reichardt, W., Karlsson, F. J., Radhakrishnan, S. V., Hanke, K., Schmitt-Graeff, A., Freudenberg, M., von Loewenich, F. D., Wolf, P., Leonhardt, F., Baxan, N., Pfeifer, D., Schmah, O., Schonle, A., Martin, S. F., Mertelsmann, R., Duyster, J., Finke, J., Prinz, M., Henneke, P., Hacker, H., Hildebrandt, G. C., Hacker, G. and Zeiser, R. (2014). [Neutrophil granulocytes recruited upon translocation of intestinal bacteria enhance graft-versus-host disease via tissue damage.](#) *Nat Med* 20(6): 648-654.