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Quick-Hot-Gram-Cromotropo QHGC lugol solution (Agudelo-Lopez SP and Montoya-Palacio MN, 2004)

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Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

Abstract

Lugol's solution is an aqueous solution which contains iodine (I_2) and potassium iodide (KI) in a ratio of 1:2

Lugol's iodine solution is used for Gram staining and can be used as an antiseptic and disinfectant for emergency disinfection of drinking water, and as a reagent for starch detection in routine laboratory and other medical tests. In the standard Gram procedure the aniline dyes in the cell tissue of microorganisms form a red dye-iodine complex when exposed to iodine. In Gram-positive microorganisms the dye-iodine complex cannot subsequently be dissolved from the cells by decolorizing agents such as alcohol or acetone. The cells remain dark blue in color. In Gram-negative microorganisms the dye-iodine complex is dissolved and the cell turns red as a result of counterstaining.

Lugol's solution is required to form the dye - iodine complex of Gram-positive bacteria. Stock solutions of I_2- KI in water are unstable. Iodine can be lost by evaporation and that can influence the result of the staining as well as affecting the working conditions in that area. Stainless steel parts of staining instruments can also be affected by the iodine in the Lugol's solution. If the concentration of iodine in the Lugol's solution decreases the bacterial smears may become more susceptible to decolourization. Therefore, I_2- KI solutions should always be stored in completely closed bottles.

The problem of iodine loss can be overcome by using polyvinylpyrrolidine (PVP) in the I_2- KI solution. PVP forms a complex with iodine. This complex is more stable and has a longer shelf life. The results of the material treated with Lugol's solution stabilized with PVP will give identical results with regard to differentiation of Gram-positive and Gram negative bacteria compared to the standard Lugol's solution.

Both Lugol's solutions are available - the standard type and the stabilized type. Suitable protocols are available on the internet or on demand.

Materials

Cristal violate

	Cristal violete	0.5 g
	Oxalate	0.8g
	Metanol	20ml
	Destilated water	100 ml

1 Homogenize the sample well with a wooden toothpick in the container that contains it

2 Make a grade spread of approximately 2 cm long by 2 cm wide, sliding the stick impregnated with fecal matter onto a new degreased, dry, or used but clean and scratch-free slide.

3 Remove excess stool with a clean, chopped wooden toothpick and repeat this procedure with another clean chopped toothpick, until the spread is as thin as possible and with the least amount of lumps of stool

4 Let to dry at room temperature

5 Fix the plate in absolute methanol for 5 min 5m

6 Let it dry at room temperature to proceed with staining or store it in a box wrapped in absorbent paper until staining.

7 Cover the spread with Crystal violet for 1 min 1m

8 Wash under running water to remove excess dye

9 Cover with an acid alcohol solution for 3 min until discolored 3m

10 Go through 95% ethanol for 1 min 1m

11 Visualize under a microscope with immersion oil at 100X

12 Permanent fixation use xylene for 1 min 1m

13 Let to dry and cover with a cover slide and Entellan®