The specimen is now stained by adding to it a few drops of Loeffler's Alkaline Methylene Blue (see formula given before), allow this to stain for three minutes without the addition of heat. The excess of stain is now removed by flowing water from the cover-glass and dried. A cover-slip with a drop of Canada balsam in the centre is prepared and the specimen is everted onto it. The specimen is now ready for the microscopic examination.

The gonococcus will be found to stain a deep blue or black, while the pus-cells and the epithelial cells are less intensely stained (see plate). Other bacteria, such as the staphylococcus and the streptococcus, when present, are also stained, but the staphylococcus is seldom arranged within the bodies of the puscells, and is, therefore, readily differentiated from the gonococcus. By this stain it is not possible to distinguish the gonococcus from other cocci by its peculiar diplococcus (biscuit) formation.

Second Method—Gram Reaction.—Gonococci do not take the "Gram." This means that if these cocci are stained first with an anilin dye and with Lugol's reagent (see below), the resultant stain may be washed from them, from the cells, from many other bacteria, but not from staphylococci and other Gram-positive cocci which, under the microscope, otherwise resemble true gonococci.

Formula Paltauf's Solution.

Anilin oil	3	parts.
Absolute alcohol	7	parts.
Distilled water	90	parts.
Lugol's Iodine	1	part.
Sol'n. Potassium	2	parts.
Distilled water		

This is applied for precisely two minutes.

Shake for two minutes. Filter through moistened paper until filtrate is clear. Add two grammes of Glubler's powdered gentian violet. Set aside for twenty-four hours. Pipette supernatant fluid as required.