Gram staining technique

By

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Gram staining

- ✓ First discovered by Hans Christian Gram in 1884.
- ✓ This is **diffrential staining** technique >>> (gm +ve or gm –ve).
- ✓ Depend on the diffirences between them in ability to retain certain dyes and structure of there C.W .

Gram staining

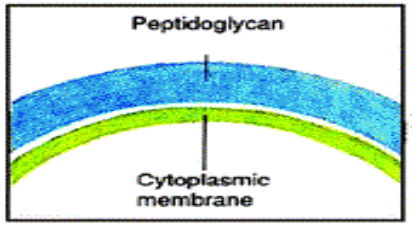
The diffirences between gm +ve & gm –ve:

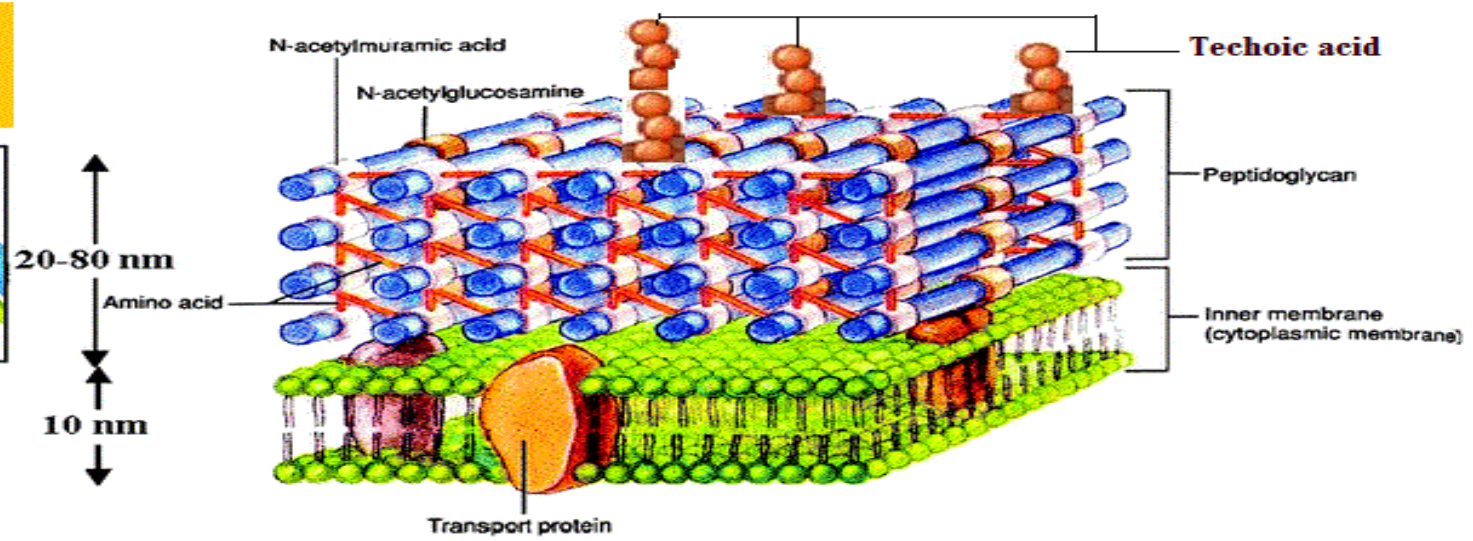
	Gm +ve	Gm -ve
Peptidogycan	Thick (multilayers)	Thin (single or few layers)
Teichoic acid	present	Absent
Outer membrane	Absent	Present
Lipid & lipoprotien	low	High (O.M)
Gram reaction	Retain crystal violet & stain **violet or purple**	Decolorized to accept safranin & stain **red or pink**
Example	Staphylococcus aureus Basillus subtilis	Escherichia coli

Gram staining

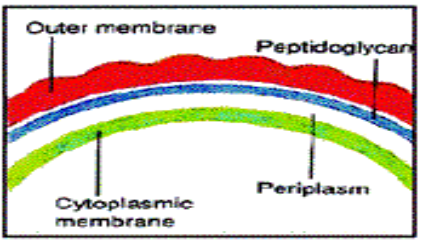
Bacterial C.W structure:

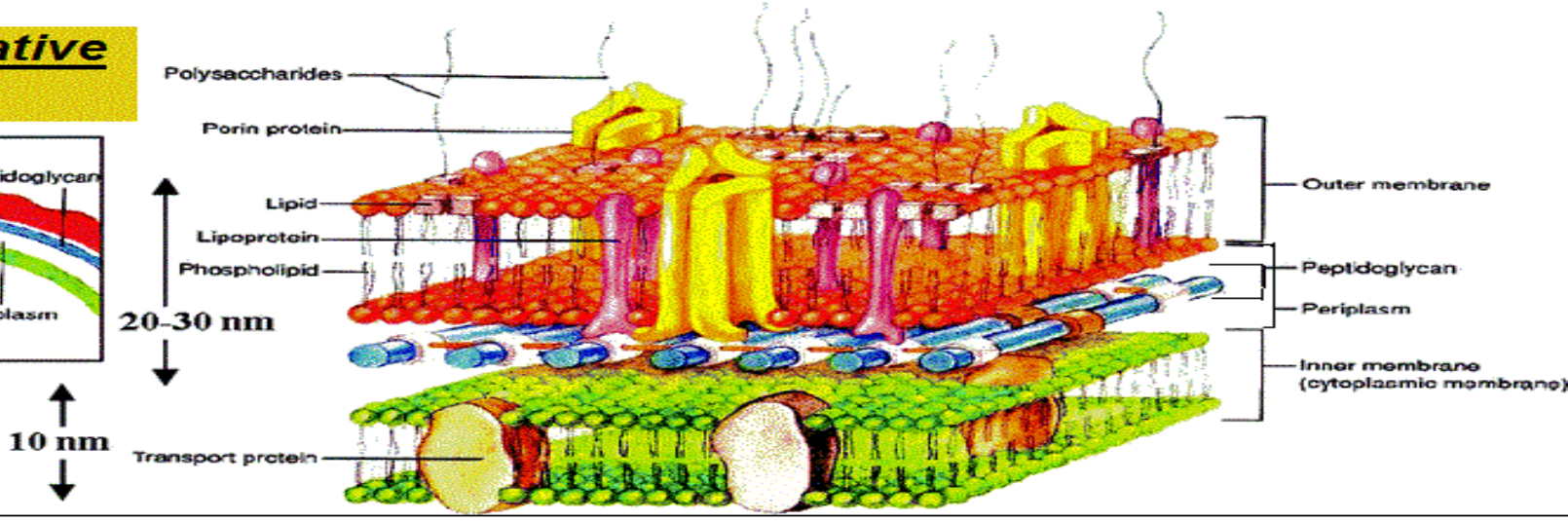
Gram positive (+ve)





Gram negative (-ve)





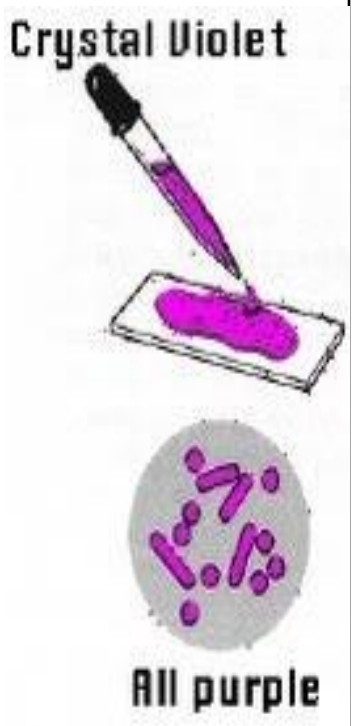
Gram staining

This technique consists of the following steps:

- ✓***Primary stain (crystal violet)>>>>*** basic dye stains all cells violet or purple color.
- ✓***Mordant (gram's iodine)>>>>*** combines with crystal violet in cell & forms crystal violet - iodine complex (CV-I).
- ✓***Decolorizing agent (ethyl alc. or ethyl alc.-acetone)>>>>*** decolorizes primary stain of some bacteria but others ramain unaffected.
- ✓***Secondary stain or counter stain (safranin)>>>*** basic dye stains the decolorized cells red.

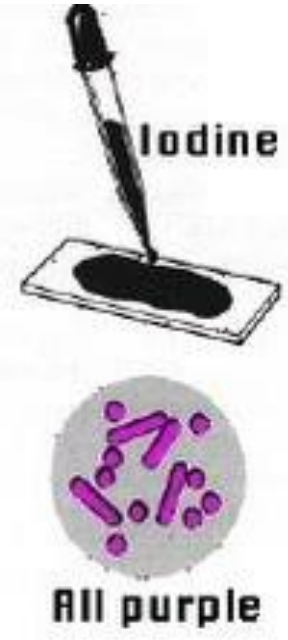
Gram staining

Gram staining technique theory:

	Primary stain (Crystal violet)		
Gm +ve	Violet color	➢Both types of cells stained violet or purple because the dye enters the cytoplasm of both. ➢(Stain >>> carry +ve charge & cells >>> carry –ve charge).	
Gm -ve	Violet color		

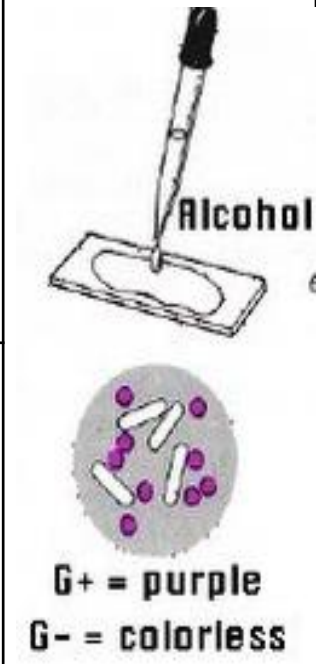
Gram staining

Gram staining technique theory:

	Mordant (Gram's iodine)		
Gm +ve	Violet color	Iodine forms large crystals or large insoluble complex with the dye (crystal violet – iodine complex Or CV-I) that are too large to escape through the C.W.	
Gm -ve	Violet color		

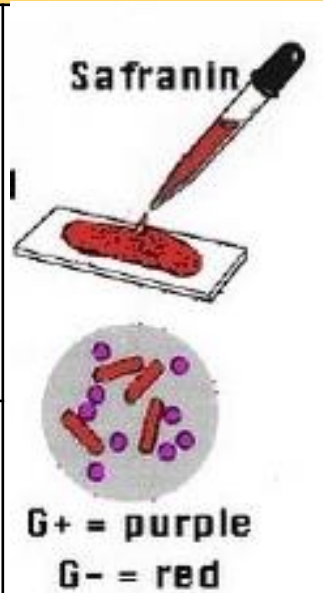
Gram staining

Gram staining technique theory:

	Decolorizing agent (ethyl alc. Or ethyl alc.acetone)		
Gm +ve	**Violet color**	➢Alc. Dehydrates the thick PG >>>> impermieable to CV-I complex. ➢N.B; * Thick PG (multilayers) ** Techoic acid *** No O.M	
Gm -ve	**colorless**	➢Alc. Dissolves the O.M & leaves small holes in thin PG layer>>>> through which CV-I comlex diffuse. ➢N.B; * Thin PG (single layer) ** No techoic acid *** O.M	

Gram staining

Gram staining technique theory:

	Secondary stain or counter stain (Safranin)		
Gm +ve	**Violet color**	Safranin absorbed but masked by the darker violet color previously absorbed by Gm +ve	
Gm -ve	**Red color**	Safranin absorbed so stains previously decolorized cells with red color	

Gram staining

- The critical step in this technique is >>>>>>>>>> ***decolorizing step***???????????

Over-decolorization	Under-decolorization
Prolonged exposure	Insufficient exposure
Gm +ve appear as Gm -ve	Gm –ve appear as Gm +ve
Both stained Red	Both stained Violet

- Young culture (less than 24hr) should used>>> older culture lose their Gm staining properities due to C.W changes.

Gram staining

Procedure :

- ✓ Prepare heat fixed smear.
- ✓ Add crystal violet for (.5-1 min)>> then rinse with water.
- ✓ Add gram's iodine for (1-2 min)>> then rinse with water.
- ✓ Add alc-acetone mix. For (20-25 sec)>> then immediately rinse with water.
- ✓ Add safranin for (10-15min)>> then rinse with water .
- ✓ Add oil dps then examine under oil immersion lens.

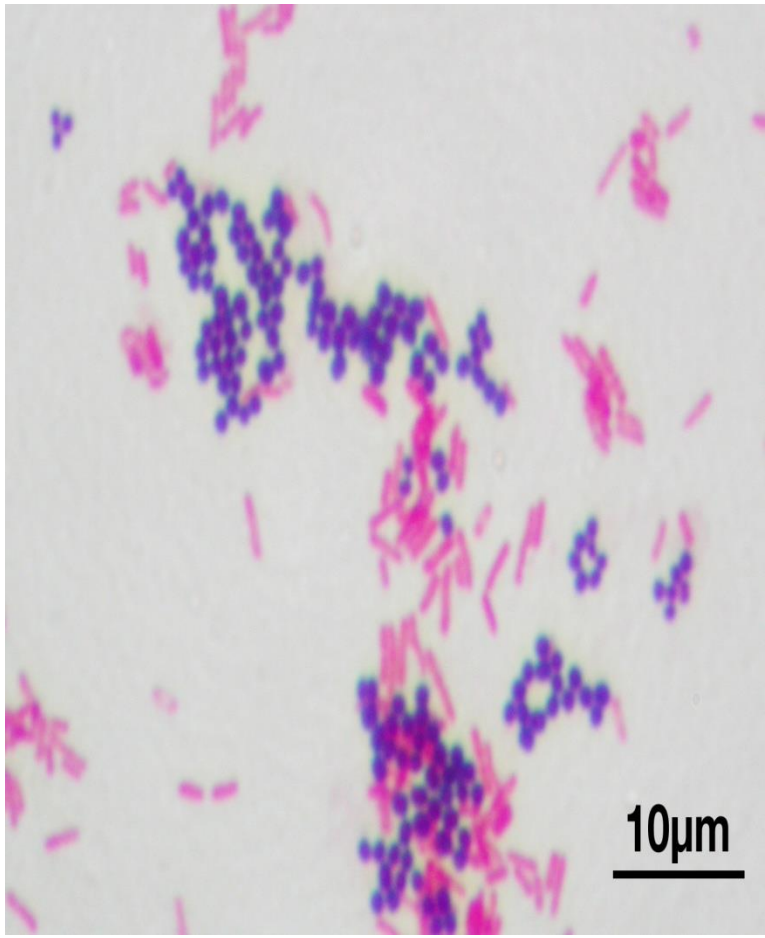
Gram Staining

Results

Gram staining

- I- Staphylococcus aureus & Escherichia coli mix.

	Staph.	E-coli
Shape	cocci	Short rod
Arrangement	Bunch	Single scattered
Gram reaction	Gm +ve	Gm –ve
Color	Violet	Red



Gram staining

- **2- Bacillus subtillus & Escherichia coli mix.**

	Basillus subtillis	E-coli
Shape	Long rod	Short rod
Arrangement	chain	Single scattered
Gram reaction	Gm +ve	Gm –ve
Color	Violet	Red

GOOD LUCK
&
SEE YOU NEXT LAB