N is for Negative Staining

A-Z of Staining - a series of articles where we share a little extra information about stains, staining techniques and some of the interesting chemicals associated.

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In conventional histological procedures, cells and tissues are stained with dyes whilst the background usually remains unstained. In negative staining however, the cells are unstained but are made visible against a dark background. This method generally involves staining a liquid sample in order to study the morphological shape, size and arrangement of cells that may be difficult or too delicate to stain. Microorganisms and spermatozoa are examples of cells that can be observed in this way since their cellular morphology remains intact. One of the advantages of negative staining is the absence of heat fixation during the procedure, thereby preventing shrinkage of the cells. Negative staining methods make use of acid dyes such as India ink and nigrosin (a mixture of black synthetic dyes). Since these dyes are negatively charged, they are repelled by the negative charge of bacterial surfaces and do not

penetrate the cells. Consequently, the background rather than the cells are stained (Figure 1). Although any negatively charged stain can be used, nigrosin is recommended because it gives a more even background than India ink. However, other acid dyes such as methyl blue and Congo red have also been used in negative staining procedures.

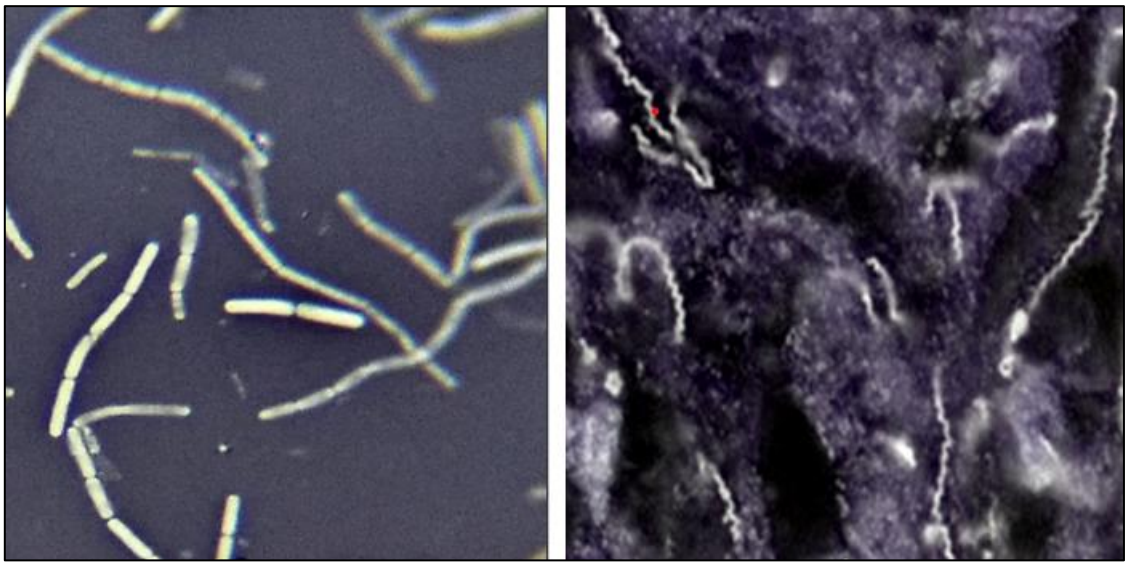

Figure 1. Negative staining showing chains of bacilli (left) and spiral-shaped spirochaetes (right) against a dark background

Certain bacteria, particularly those that are highly pathogenic, have specialized capsules that lie outside the cell walls. These differ from those that form on the surface of other bacteria as they consist of organized layers of polysaccharides that are difficult to remove. They protect the bacteria from the action of phagocytic cells such as macrophages and help them attach to surfaces. Consequently, they play a major role in the virulence of highly pathogenic organisms by enhancing the ability of certain bacteria to cause diseases such as anthrax and meningitis. Since bacterial capsules are non-ionic, neither acid nor basic stains will adhere to their surfaces. The capsules are best visualized by staining the background with an acid dye such as India ink and to stain the cell itself with a basic dye (such as crystal violet). Acid dyes can also be added directly to body fluids such as cerebrospinal fluid for detecting the capsule of other microorganisms such as Cryptococcus neoformans. This encapsulated yeast fungus is commonly found in bird droppings and remains a significant pathogen, particularly in patients infected with the human immunodeficiency virus (HIV). Since capsules do not stain, results show a dark background with dark cells surrounded by unstained capsules (Figure 2).

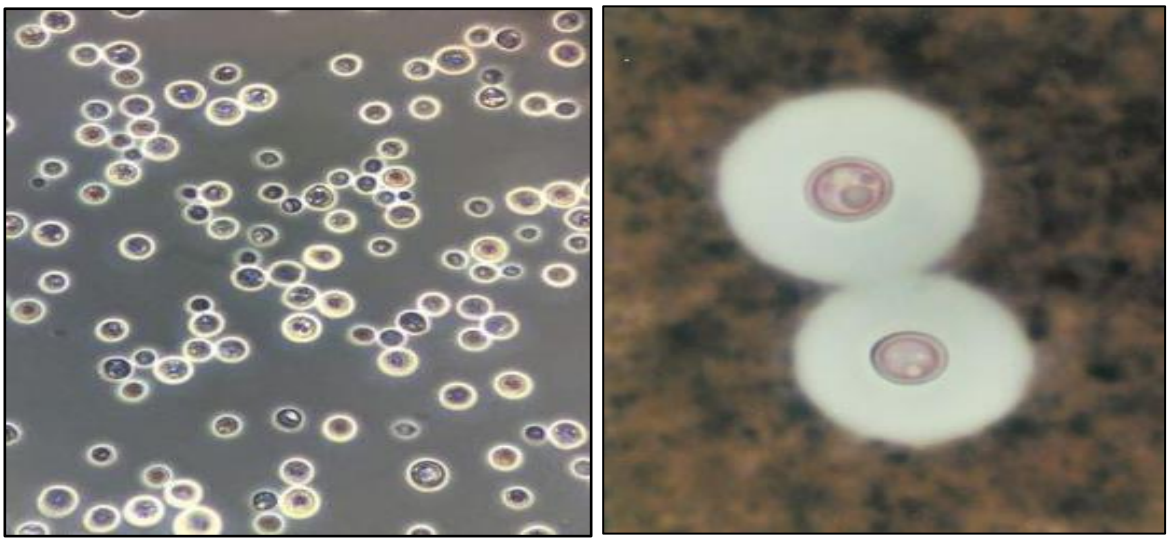

Figure 2. Unstained capsules of yeast cells (left) and Cryptococcus neoformans (right)

In andrology, studies of male infertility utilizes the acid dye nigrosin in combination with the acid dye eosin for estimating the percentage of live and non-living spermatozoa in samples of semen. The eosin-nigrosin stain is the most common method for morphological analysis of sperm and is recommended by the World Health Organization for human semen analysis. When stained, the nigrosin provides recognition of both viable and non-viable spermatozoa by producing a dark background on which the sperm show up as lightly coloured bodies. As the eosin is taken up by dead sperm, they appear pink whereas live sperm appear pale in colour (Figure 3).

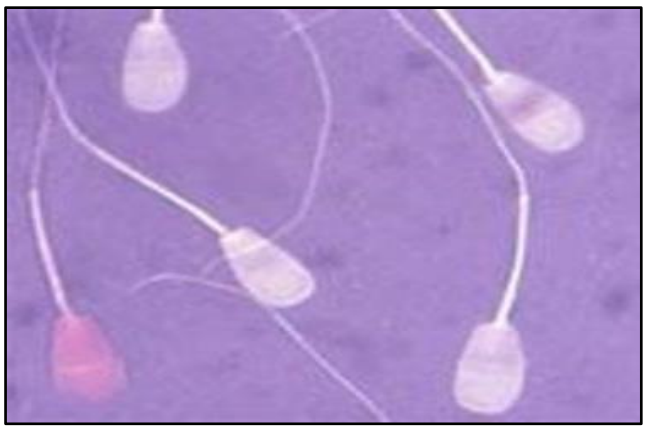

Figure 3. Dead, non-viable sperm staining pink and viable sperm staining pale against a dark background when stained with eosin-nigrosin

In electron microscopy (EM), negative staining is a simple and rapid method for examining the morphology of organelles, macromolecules and viruses. The same principle is used for EM

although the preparations are in a solid state. The method consists of embedding electron-transparent objects such as rotaviruses in a structureless electron-dense matrix such as potassium phosphotungstate (Figure 4).

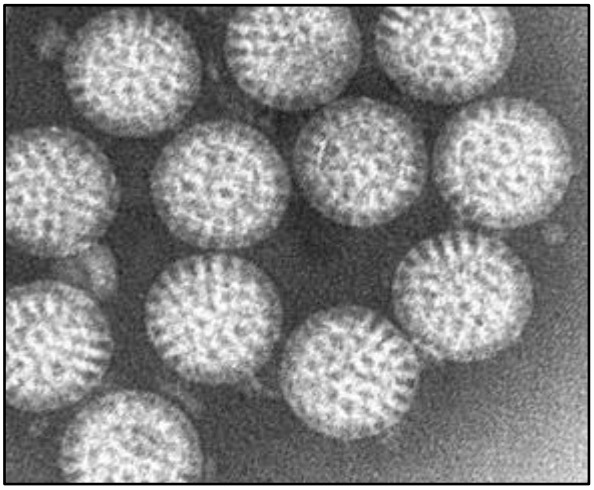

Figure 4. Negative staining of rotavirus at EM level

Further reading

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