ELEMENTARY BACTERIOLOGICAL BENCHWORK.

By W. J. HATCHER.

A methodical system of working is essential, both for the safety of the worker and accuracy of results obtained. Firstly, the bench or table should be cleared of everything except the actual requisites required for carrying out the various manipulations, which with practice should become largely automatic, leaving the whole attention free to concentrate on the work in hand. The essential requisites for carrying out the routine bacteriological work of a small hospital laboratory are as follows: a Bunsen gas burner, a platinum wire, which is mounted in a suitable holder. This instrument is used for picking out colonies of growing bacteria, inoculating culture medium with infected material, and for transplanting from one culture to another. Two wires are certainly preferable to one, one being much thicker than the other and the end bent in the form of a small loop. In addition a quantity of three inch by one inch microscope slides will be required and a few Petri dishes, a stock of staining solutions, a supply of culture medium, a good microscope, and of course that essential item, the bacteriological incubator, which maintains a constant temperature of 37 degrees Centigrade. The examination of bacteriological material can usually be divided into two parts. Firstly, the direct examination made immediately on arrival at the laboratory. This is done by means of smearing with the platinum loop a small drop of the material on to a microscope slide, staining by a suitable method and examining under the microscope. From this investigation is gleaned such valuable information as the relative number of different bacteria present, if it is a mixed infection the presence or absence of pus cells, leucocytes, epithelial, or red blood cells.

For the second portion of the examination, a little of the material is spread over a culture plate and grown in the incubator overnight. The following morning this plate will, unless the material is quite sterile, be found to be spotted with a number of colonies which appear as white or yellow dots, sometimes large but more often quite small. Smears are now made from each type of colony and the organism identified, the results of this investigation being then compared with the report obtained on direct examination.

Making the Smear.

Specimens of sputum or pus are very frequently sent to the laboratory in ridiculously tiny bottles, having an even smaller neck. The skill exhibited by the sender in persuading a sticky sputum to enter such a narrow aperture is apt to be a source of wonderment.

It is, however, quite impossible to select suitable portions for examination under such conditions, and the contents should be tipped into a sterile Petri dish. Petri dishes are, as you most probably know, quite common articles in the laboratory. They are made of glass and consist of two flat dishes, one slightly larger than the other, which sits on the top, doing duty as a lid. They are usually sterilised in a copper container or simply wrapped in newspaper. Having tipped the material into the Petri dish, with the thick platinum wire transfer a small portion to the centre of a microscope slide and emulsify with a tiny drop of clean water, slowly working the platinum wire about until a thin paste is formed which will dry in the form of a smear. Don't forget the necessity of thoroughly sterilising the platinum wire by heating to a dull red in the Bunsen in between each manipulation. A word of warning: never suddenly plunge a loop full of infected material straight into the heart of the flame. It may splutter, scattering infection over the worker and the bench. When the smear is quite dry it is fixed to the slide by means of gentle heat, such as passing through the top of the Bunsen flame three times.

Staining.

Practically all material sent for bacteriological examination is always first stained by Gram's method, for all bacteria are classified Gram positive or negative according to whether or not they are stained by the primary dye used in this method of staining.

After the smear has, as directed, been carefully fixed to the slide it may be laid flat on two glass rods fixed across a convenient sink and flooded with the staining mixture, or alternatively it may be put in a pot of the staining solution. The first method is usually the most convenient, the second perhaps the cleaner.

Gram's Method of Staining.

Stain in ½ per cent. Methyl Violet (watery) sol. 1 minute.

This is the primary stain and the bacteria, which take this, or rather, which retain it, are Gram positive, and they are stained a violet colour. The others, the Gram negative, are stained by the counterstain and appear a faint red colour.

Tip the stain off and flood with Lugol's Iodine Solution, 1 minute. Tip off and decolourise with methylated spirit until no more colour comes away.

Wash in water, counterstain in ½ per cent. Neutral Red (watery), 1 minute. Wash in distilled water, and dry by blotting.

Formula of Lugol's Iodine Solution.

Iodine	... ...	1 gram.
Potassium Iodide	...	2 grams.
Distilled Water	...	100 c.c.

Gram's Iodine Solution is often used, but Lugol's is more rapid, Gram's solution being only a third of the strength of Lugol's.

Ziehl-Neelsen's Stain for Tubercle Bacilli.

This is a specific stain—that is to say, it only stains one type of bacteria, in this case the acid and alcohol-fast bacteria, which to all practical purposes means the tubercle and leprosy bacilli.

Make and fix smear by the usual method.

Stain in Carbol Fuchsin, heat but do not boil, 5 minutes.

This may be done by staining in a pot and leaving in the incubator for 15 minutes. This is longer, but cleaner than the direct heating.

Wash the stain off in tap water.

Decolourise in Acid-Alcohol.

Counterstain in ½ per cent. Methylene Blue (watery), ½ minute. Dry and blot.

The tubercle bacilli are stained a bright red colour. Other bacteria and any cells present will be stained blue

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