HEALTH & DISEASES

This column is taken care of by the "Studygroup for Diseases and the Optimum Keeping and Breeding of Terrarium Animals" of the Belgian Society "Terra". If there is a question concerning health or diseases, feel free to contact the president of the Studygroup: Mr. Hugo Claessen, Arthur Sterckstraat 18, B-2600 Berchem, Belgium. He will try to answer your question in this column to the benefit of all members.

MICROSCOPY, PART II.

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Contents: Staining - Bacteriology - Parasitology - References.

STAINING

In this chapter we shall deal with the most-used stainings and techniques for bacteriology and parasitology. Of the listed stainings you will find many variations, all with advantages and disadvantages. We only list simple stainings which readily give results. With experience you can try modifications, and get an insight into the possibilities. There are also many specific stainings which are not listed. Should the reader be interested in a specific staining, please write to the author.

BACTERIOLOGY

I. Crystal Violet - general staining for bacteria

Solutions

A) Crystal violet 0.5% solution in water
B) 5 g copper sulfate in 25 ml water

Staining procedure

- Stain the slides for 3-4 minutes in A
- Differentiate in solution B
- Allow to dry

Results

Bacteria are dark blue.

II Breed's staining - genaral staining for bacteria

Solution

Methylene blue	0.3 g
Alcohol 95%	30 ml
Phenol	2.5 g
Distilled water	100 ml

Staining procedure

- Stain the slides for 2 minutes
- Differentiate in 90% alcohol
- Allow to dry

Results

- bacteria: dark blue
- background: light blue

III Eosin - Methylene blue - basic fuchsin - general staining

Solution

Eosin 2% in alcohol	2.5 ml
Methylene blue 3% in alcohol	7.5 ml

Basic fuchsin 10% in alcohol	3.0 ml
Alcohol 95%	12 ml
Distilled water	25 ml

Allow the solution to stand for several days, then you must filter it.

Staining procedure

- stain the slides for 1 minute
- rinse with tap water
- allow to dry

Results

Bacteria: *B. typhosus* and paratyphus forms: pink
E. coli: lilac
B. diphterine: pink with lilac granules
B. influenza and *leptothrix*: colourless with lilac granules
Meningicocci: violet to pink

Blood: Erythrocytes: pink to orange
Leucocytes:
- Neutrophyles, cytoplasm: pink
- Lymphocytes, cytoplasm: purple, nucleus: blue
- Basophyles: purple to dark blue, nucleus: dark purple to black
- Eosinophyles granules: pink

Polychromatophile cells: red
Spirochaetes: pink to violet.

IV Ziehl-Neelsen Staining - for acid-fast bacteria

Solutions

A. Basic fuchsin	1 g
Absolute alcohol	10 ml
Phenol	5 g
Distilled water	95 ml
B. Hydrochloric acid (37% HCl)	3 ml
Alcohol 95%	97 ml

C. Methylene blue 1 g
Alcohol 95% 30 ml
Potassiumhydroxyde (KOH) 10 mg
Distilled water 100 ml

Staining procedure

- Stain the slides 3-5 minutes with A while heating to 80-90°C or stain at room temperature for 60 minutes
- Rinse in tap water
- Differentiate with B until light pink
- Rinse in tap water
- Stain with C for 10-30 seconds
- Rinse in tap water
- Allow to dry

Results

Acid-fast bacteria and mycobacteria: red
Other bacteria: blue
Background: colourless to light blue.

V Gram-staining - differentiation between Gram-positive and Gram-negative bacteria

Solutions

A. Gentianviolet 1 g
Absolute alcohol 10 ml
Phenol 2 g
Alcohol 95% 20 ml
Distilled water 100 ml

B. Iodine (I_2) 1 g
Potassiumiodide (KI) 2 g
Distilled water 100 ml

First you carefully mix the iodine and the potassiumiodide, then add water carefully while stirring firmly.

C. Fuchsin stain according to Ziehl-Neelsen (A). Dilute the solution 10X with water.

Staining procedure

- Stain the slide for 2-3 minutes with A
- Wash with B for 1 minute
- Allow to dry
- Differentiate with alcohol or acetone
- Rinse with tap water
- Stain 30 seconds with C
- Rinse with tap water
- Allow to dry

Results

Gram-positive bacteria: blue
Gram-negative bacteria: red with a pink nucleus.

VI Azur L - staining for fungi

Solutions

Azur L: 0.5% in distilled water

Carnoy's fixer:

Alcohol absolute	60 ml
Chloroform	30 ml
Acetic acid 37%	10 ml

Staining procedure

- Put a piece of mould on a slide
- 10 minutes in Carnoy's fixer
- Dry it by heating
- Stain 2-3 minutes in Azur L
- Rinse with tap water
- Allow to dry

Results

Mould cells: dark blue
Background: light blue

VII Malachite green - Basic fuchsin - bacteria and yeasts.

Solution

Basic fuchsin 10% in alcohol	0.5 ml
Malachite green 0.5% in water	100 ml

Staining procedure

- Stain the slide while heating for 1 minute; change solution regularly
- Rinse with tap water
- Allow to dry

Results

Spores and bacteria: greenish blue
Yeasts: violet to pink

After the slides have been coloured and dried, they can be studied. The best results are obtained with an oil immersion objective (100 X), so that the total enlargement becomes 1000 X or 1500 X. The slides can also be directly enclosed in Eupa-ral, in which they can be preserved. The slides can be kept in wooden or plastic boxes.

PARASITOLOGY

In parasitological studies you can expect best results with living forms. The fact that living parasites move, is the best way to recognize them. That's why you should always study a living preparation first. This is not so with blood, as it has to be stained. You can stain living material using a stain which will not kill the parasites. In the section on faeces research, such a stain is discussed. All the following stains are for dead and fixed material.

I Noland's staining - universal staining for protozoa

Solution

Gentianviolet 2% in distilled water	1 ml
Phenol saturated in distilled water	80 ml
Formalin 40%	20 ml
Glycerin	4 ml

Staining procedure

Mix a drop of preparation suspention and a drop of stain on a very clean slide. Mix with a needle and spread. Allow to dry.

Result

Flagellates: violet.

II Nile blue sulphate - universal staining for protozoa and yeasts

Solution

0.1% nile blue sulphate in water

Staining procedure

- Stain the slide for 5-10 minutes
- Rinse in tap water
- Allow to dry

Result

Organisms become dark blue to light blue.

This staining can be used for living amphibian eggs and larger protozoa.

III Haematoxylin Mallory - amoeba staining

Solution

Haematoxylin 10% in alcohol	1 ml
Phosphotungstic acid 10% in distilled water	20 ml
Distilled water	80 ml

Allow the stain to stand for about 1 month shaking it regularly; or add 0.5 ml hydrogen peroxide.

Staining procedure

- Stain the slide for 30 minutes
- Rinse in tap water
- Allow to dry

Results

Nucleus: dark blue
cytoplasm: light blue.

IV Giemsa staining - universal staining for blood films

Solutions

Giemsa stain - you can buy this solution

Buffered water:

Sodiumdihydrogen phosphate	4 g
Disodium phosphate	6.5 g
Distilled water	1000 ml

Staining procedure

- Stain the fixed slides for 5 minutes with Giemsa that has been diluted 4x with buffered water
- Rinse with buffered water
- Allow to dry

Results

Spirochaetes: red-violet
Leucocytes: purple red nucleus
Erythrocytes: yellow-pink
Chromatin: bright red
Cytoplasm: bright blue

V Haematoxylin - Eosin Staining - universal histological staining

Solutions

A. Haematoxylin 3.5% in alcohol	100 ml
Ammonium aluminium sulphate 6.5% in distilled water	380 ml

Glycerin 80 ml

Allow the solution to stand for at least three months, then you must filter it.

B. Eosin 1% in distilled water.

Staining procedure

- Stain the slides for 10 minutes in A
- Rinse with tap water until it colours blue
- Stain for 2 minutes with B
- Rinse with tap water
- Allow to dry

Results

Nucleus: blue
Cytoplasm: pink to red
Muscle- and collagen filaments: pink
Erythrocytes and keratine: bright red.

REFERENCES

Adam, K.M.G. et al., 1971. Medical and Veterinary Protozoology. Churchill Livingstone, London.

Buttiaux, R. et al., 1962. Manuel de Techniques Bactériologiques. Editions Medicales Flammarian, Paris.

Claessen, H., 1982. Microscopical examination of feces. Litteratura Serpentium, Vol. 2 (4): 184-192.

Gurr, E., 1955. Medical and Biological Staining Techniques. London (328 stainings).

Reichenbach-Klinke, H.H. & E. Elkan, 1974. Color atlas of the Diseases of Fishes, Amphibians and Reptiles. t.t.h. Publications, New York.

Smith, A. & J. Bruton, 1978. A colour atlas of histological staining techniques. Wolfe Medical Publications Ltd, London.

Welsch, U. & V. Storch, 1975. Comparative Animal Cytology and Histology. Sidgwick Jackson, London.