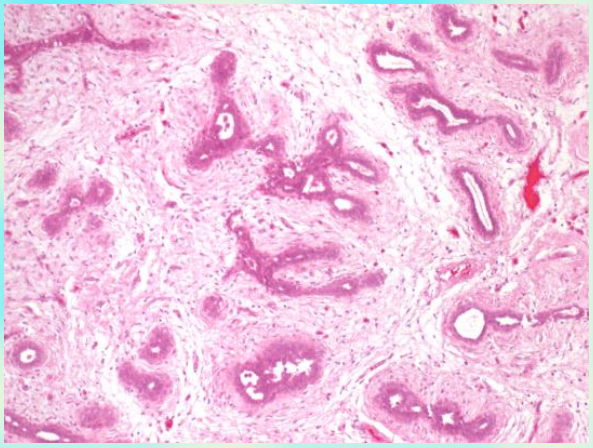
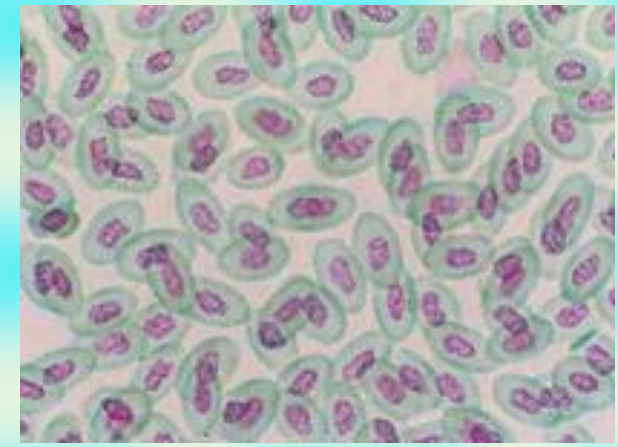
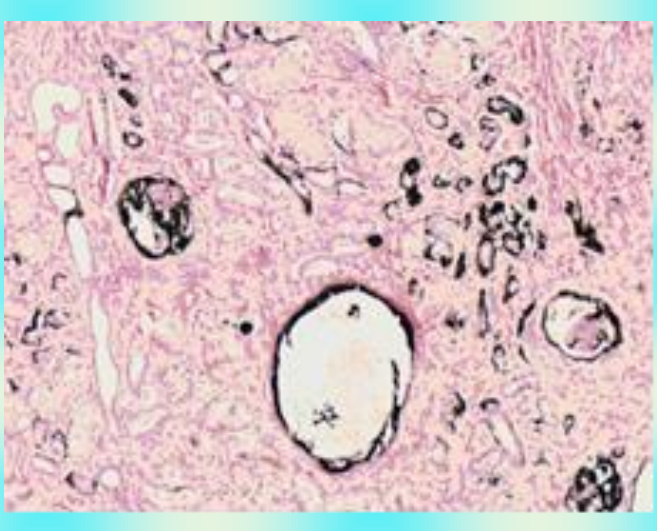
Queen Mary
University of London

BLIZARD INSTITUTE
CORE PATHOLOGY

ATLAS OF TINCTORIAL STAINS







Contents

Introduction

This atlas is a guide to the Tinctorial Stains being carried out at the Blizard Institute Core Pathology Facility. You will find the stains grouped according to tissue components.

Please note if there are stains that you require and are not on the list then please contact the department on, core-pathology@qmul.ac.uk as this list covers the most common stains.

Background to Tinctorial Stains

Biological tissue has little inherent contrast in either the light or electron microscopy. Staining is employed to give both contrasts to the tissue as well as highlighted particular features of interest.

Tinctorial staining (or more commonly known as special staining) has been used to selectively stain cells and cellular components. A variety of compounds used to colour tissue sections include, Safranin, Oil Red O, Congo Red, Fast Green FCF, Silver Salts and numerous natural and artificial dyes that were usually originated from the development of dyes in the textile industry.

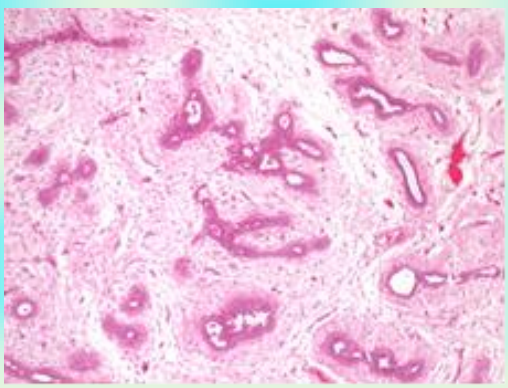
Histochemistry refers to the science of using chemical reactions between laboratory chemicals and components within tissue. Below is a list of the most commonly used stains in a laboratory's repertoire. If you do not see what you require please contact a member of staff as many of the stains can be adapted and there are more available.

Haematoxylin and Eosin Stain (H&E)

The H&E stain is probably the most widely used histological stain. Its popularity is based on its comparative simplicity and ability to demonstrate clearly an enormous number of different tissues.

Gills haematoxylin and Eosin is the standard method I the lab but other stains; like Ehirlichs and Weigert's haematoxylin for nuclei are also available.

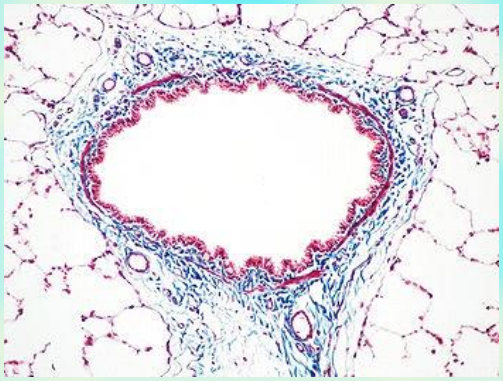
Nuclei stain blue, other elements shades of pink/red.



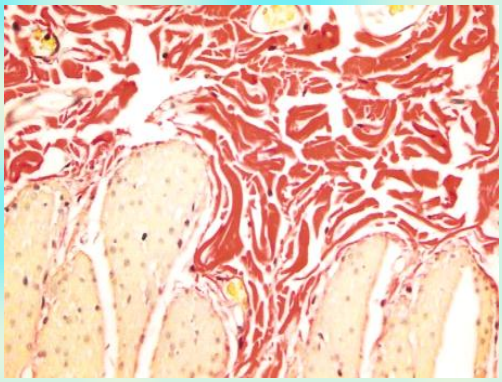
Connective Tissue Stains

Connective tissue's major function is to connect together and support the other tissue of the body. It usually consists of a cellular portion in an enveloping framework of non-cellular substances; there are many techniques available for the demonstration of the different connective tissues.

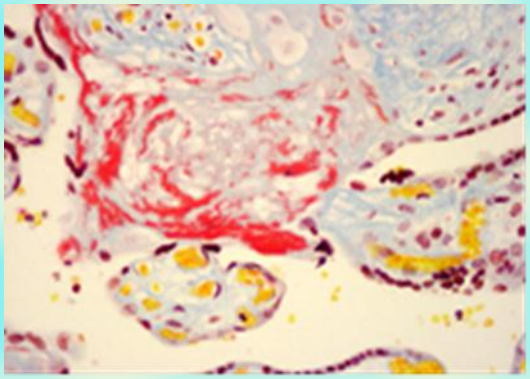
<u>Masson Trichrome</u>
Nuclei: Black
Collagen: Green / Blue
Muscle: Red
Fibrin: Red
Reticulin: Green
Elastic: Red



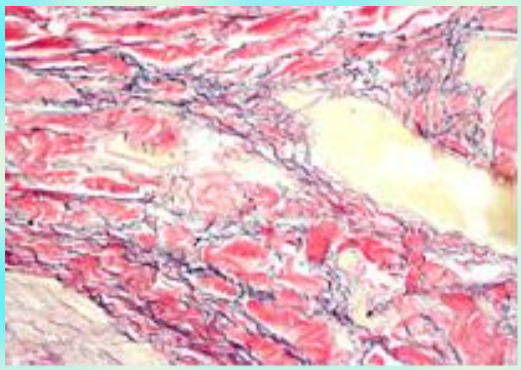
<u>Van Gieson</u>
Nuclei: Black
Collagen: Red
Muscle: Yellow



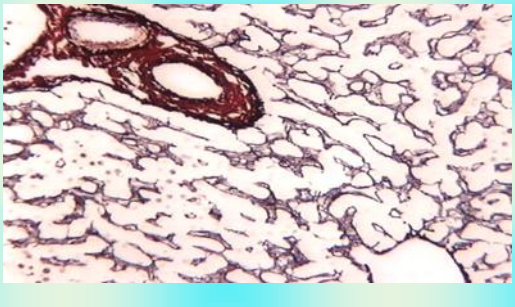
<u>MSB</u>
Fibrin: Red
Collagen: Blue
Muscle: Pale Red
Erythrocytes: Yellow
Nuclei: Blue



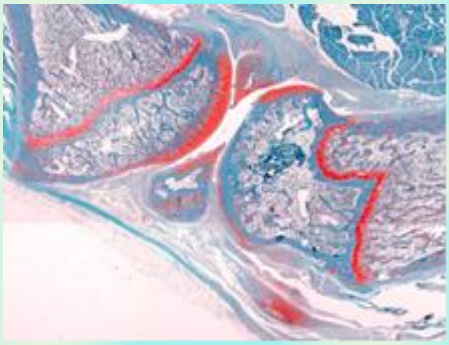
<u>Elastic Van Gieson (EVG)</u>
Elastic: Blue/Black
Nuclei: Blue
Collagen: Red
Muscle: Yellow



Gordon & Sweet's Stain for
Reticulin Fibres
Reticulin: Black
Collagen: Brown (if untoned)



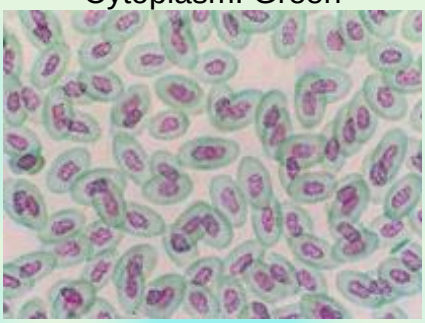
Safranin O
Nuclei: Black
Cytoplasm: Grey/Green
Mucus, cartilage, mast cell granules: Red
Muscle: Green



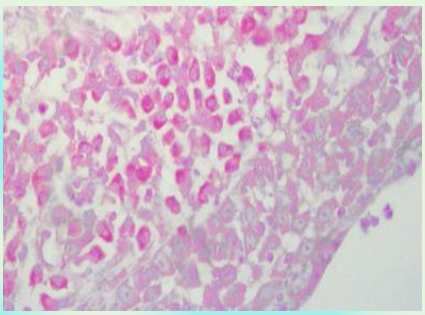
Nucleic Acids

Nucleoproteins are combinations of basic proteins (protamines and histones) and nucleic acids. The two nucleic acids are DNA which is found in the nucleus of the cell and RNA which is located in the cytoplasm of cells, mainly in ribosomes. Both DNA and RNA molecules consist of alternate sugar and phosphate groups with a nitrogenous base being attached to each sugar group. The sugar in DNA is the 5-carbon sugar deoxyribose; in RNA it is ribose.

Feulgen Stain
DNA: Red-Purple
Cytoplasm: Green



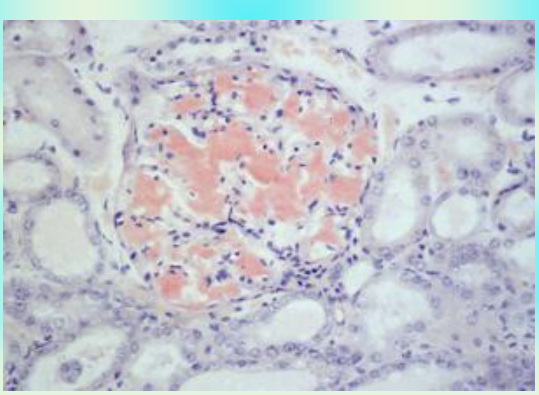
Methyl Green – Pyronin
DNA: Green-Blue
RNA: Red



Amyloid

The classical, Histopathological definition of amyloid is an extracellular proteinaceous deposist exbiting beta sheet strcture. Common to most cross beta type structures they are generally identified by apple-green birefringence when stained with Congo red and seen under polarised light. These deposists often recruit various sugars and other components such as serum amyloid P component, resuting in complex and sometimes ihhomogenous structures.

Congo Red
Amyloid: Red
Nuclei: Blue
Eosinophils, Elastin and Keratin: Red

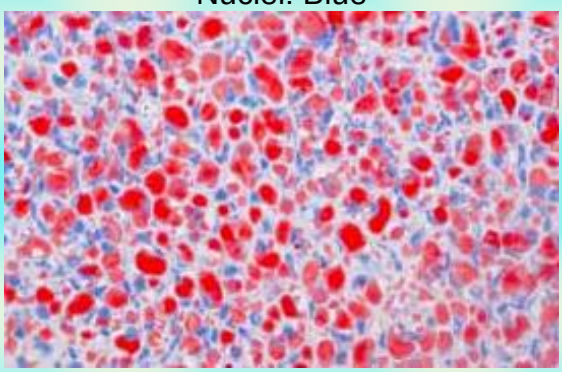


Lipids

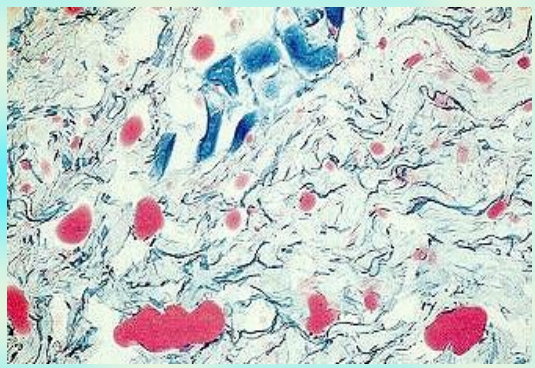
Lipids have been defined, Baker 1946, as naturally occurring fat-like substances that are soluble in organic solvents but not in water, but any no means all lipids resemble fats. Free cholesterol for example, is crystalline and some of the phospholipids are even water soluble. The definition now which is more preferred is, Lovern 1955, lipids are actual potential derivatives of free fatty acids and their metabolites. It is now possible with Tinctorial staining to identify individual each groups of lipids.

Please note lipid stains must be carried out on unfixed frozen sections as processing the samples will dissolve out majority of lipids.

Oil Red O
Unsaturated hydrophobic lipids: Red
Phospholipids: Pink
Nuclei: Blue



Nile Blue
Neutral Lipids: Red-Pink
Acidic Lipids: Blue



Carbohydrates (Glycogen and Mucins)

The two main entities to be considered in tissue carbohydrate demonstration are glycogen and mucins, the latter of which incorporates mucopolysaccharides, mucosunstances and glycoconjugates.

Glycogen is a simple polysaccharide which consists of branched or straight chain D-glucose units and can be found in two main forms alpha and beta. A third form has also been described and is a non-particulate glycogen found between beta particles in the alpha rosettes.

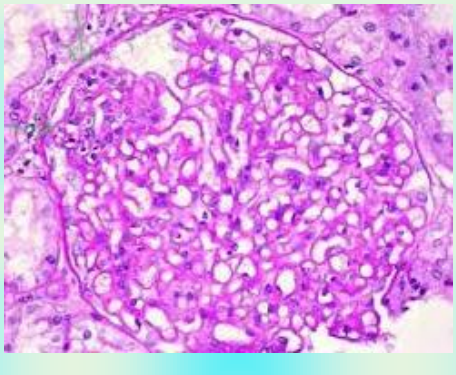
Mucins cover a variety of substances as mentioned but to try and simply put it; these are hexosamine-containing polysaccharides covalently bound to varying amounts of protein. Free hexose groups are often available, together with certain acidic moieties, the presence of which influences the histochemical reactivity. The different mucins may be present as a single type or more commonly as a mixture.

<u>Periodic Acid Schiff (PAS)</u>

Glycogen and other periodate-reactive carbohydrates : Mangenta

Nuclei: Blue

Glycogen can be digested out with pre-treating slide with diastase



<u>Alcian Blue for Acid Mucins</u>

Acid Mucopolysaccharides: Blue

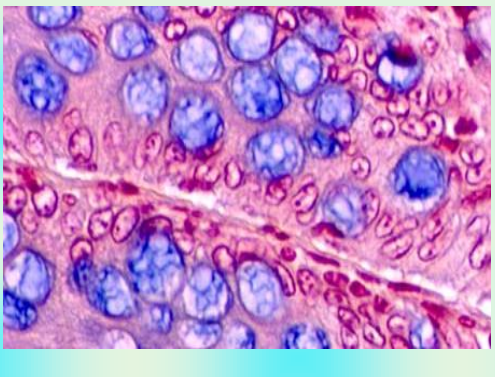
Nuclei: Red

The pH of the solution can be changed according to the type of mucins you wish to demonstrate

pH0.2: Strongly sulphated mucins.

pH1.0: Weakly and strongly sulphated mucins.

pH2.5: Most acid mucins except some strongly sulphated mucins.



Pigments and Minerals

It is tradition to consider pigments under the following headings

Artefact pigments

Deposits of pigments which are artefactually produced as a result of the action of some chemical substance, such as the fixative formalin.

- Formalin pigment – this is present as a brown or brownish black deposist in some tissues. The deposit is likely to occur in blood-rich tissues such as spleen, areas of haemorrhage, or tissue which are heavily congested with blood. The pigment is birefringent in polarise light. It can be removed by treating the slide with alcoholic picric acid for a few hours followed by washing in running water.
- Mercury pigment – some fixatives such as Zenker's and Helly's fluid, Heidenhain's Susa and formal-sublimate contain mercuric chloride. Tissues fixed with these can develop a uniform granular black deposits. This can be easily removed by Lugol's iodine and subsequently in sodium thiosulphate.
- Dichromate deposits – tissues fixed in potassium dichromate containing fixatives like Zenker's must be washed in running tap water for some hours, because if the tissue is transferred directly to alcohol an insoluble yellow-brown oxide is precipitated within it. It is possible to remove some of it but not all in a 1% HCL in 70% IMS.

Endogenous Pigments

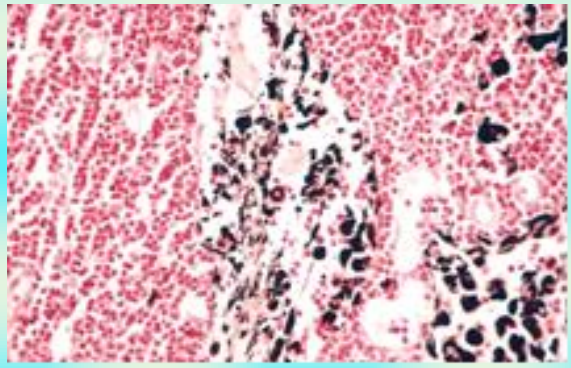
Pigments which are produced within the tissue and which serve a physiological function, or are by-products of normal metabolic processes.

- Bile – it is important to identify bile pigmented in the examination of liver, where it is important to distinguish from lipofuschin as both appear yellow/brown on H&E.

Fouchet
Bile: Green
Other Elements: As counterstain

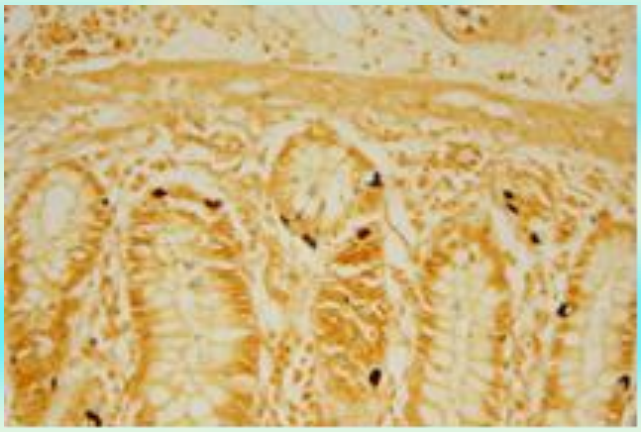
- Melanin – is a group of brown-black pigmented whose exact chemical structure is mot know. The positive identification of melanin and melanin producing cells can be done by reducing methods like Masson Fontana, enzyme methods and fluorescent methods. The reducing methods demonstrate both formed melanin and also melanin precursors.

Masson Fontana
Melanin, Argentaffin, Chromaffin, Some Lipofuscins: Black
Nuclei: Red



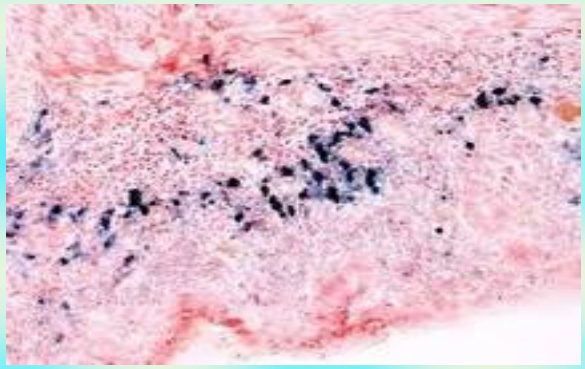
- Chromaffin, argentaffin and argyrophil granules – it is traditional to incorporate these into the heading of endogenous pigments, although the granules are completely lacking in pigment. The names were given to these granules as a result of their staining reactions with chrome and silver salts, resulting in brown and black coloration respectively. Chromaffin cells contain granules which have an affinity for chrome salts. Argentaffin cells have the ability to directly reduce silver solutions with the production of insoluble black metallic silver without the assistance of an external reducing agent. Argyrophil cells need the assistance of an external reducing agent to reduce the silver solution.

Grimelius
Agryrophil Granules: Black
Pancreatic α2 Cells: Black
Background: Yellow



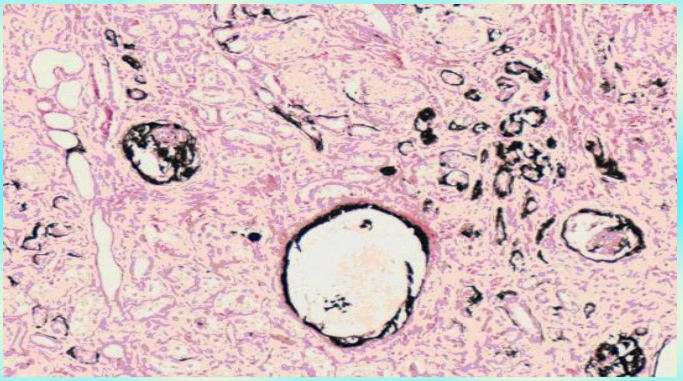
- Haemosiderin and Iron – iron is an important component of the human body, being vital constituent if the oxygen carrying haemoglobin, but also occurring in myoglobin and certain enzymes. Bit all tissue iron is histologically demonstrable; iron which is very tightly complexed protein, as in haemoglobin or myoglobin, cannot be demonstrated unless the iron is released by pre-treatment by as strong agent like hydrogen peroxide. Particulate metallic iron, or inert iron oxide, sometimes introduced into tissues by industrial exposure, cannot be demonstrated in that form but various mechanisms within tissues release some of the iron in a demonstrable form, such as deposits surrounded by hemosiderin. The iron can be easily stained when it exists in the ferric form, as in hemosiderin.

<u>Perls' Prussian Blue</u>
Ferric Iron: Blue
Nuclei: Red



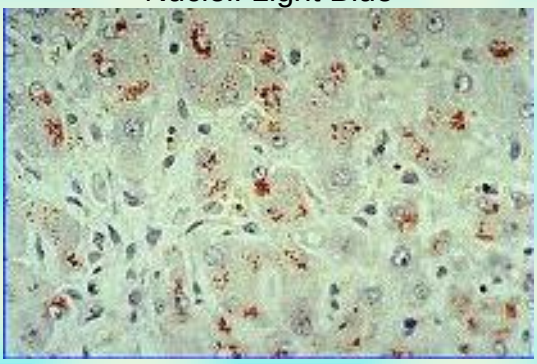
- Calcium – calcium is present in the body in large quantities, a proportion of which is circulating in the blood in free ionic form. This proportion is not demonstrable histochemically. The most important site of bound (demonstrable) calcium is in the bone where it exists combined with phosphates, carbonates and other anions. Calcium appears blue-black on H&E stained sections, like many metallic cations, it produces a blue black lake with haematoxylin. The von Kossa method is a silver reduction technique and in fact demonstrates phosphates and carbonates but since the demonstrable forms of these anions are almost always found combined with calcium it can be regarded as a stain for calcium.

<u>Von Kossa</u>
Calcium Deposits: Black
Other Elements: As counterstain



- Copper – is a normal constituent of many tissues and is an important structural component of some of the oxidase enzymes. It is usually present in small amounts and cannot be demonstrated histochemically. In some diseases copper slowly accumulates in certain organs until sufficient amounts are present to permit histochemical demonstration.

Rhodanine
Copper: Red
Nuclei: Light Blue

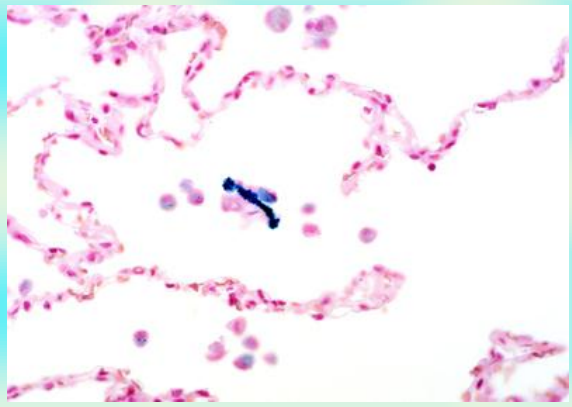


Exogenous pigments

These are pigments which gain access to the body accidentally and serve no physiological function. They usually enter by inhalation into lings or implantation into the skin during industrial exposure; most exogenous pigments are minerals.

- Asbestos – is the name given to a special form of silica which exists in the form of long, thin, crystalline fibres.

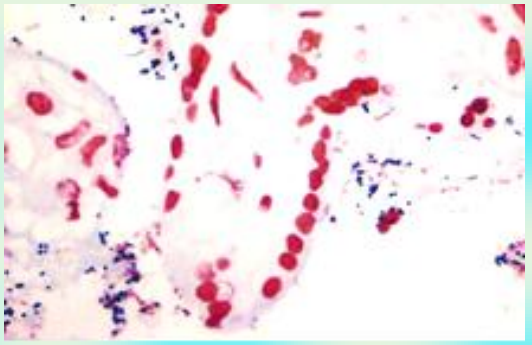
Perls' Prussian Blue
Ferric Iron: Asbestos bodies
Nuclei: Red



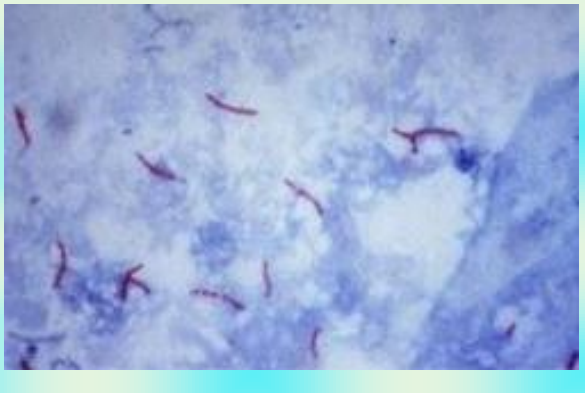
Micro Organisms and Fungi

The detection and identification of microorganisms on histological sections can be of diagnostic importance, although it is rarely as satisfactory or sensitive as microbiological culture of the tissue. The detection of particular infecting organism in a tissue section is a difficult problem due to the fact that many infecting organism are too small to be seen by light microscopy, those that are large enough cannot be seen on a H&E so special stains have to be used and sometimes the numbers of the organisms are so small that even with the special stains they are sometimes not noted.

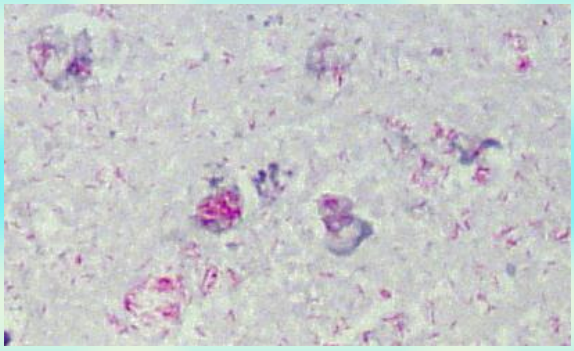
Gram Stain
Gram Positive Organisms: Blue
Gram Negative Organisms: Red
some fungi, keratohyalin and keratin may also stain blue



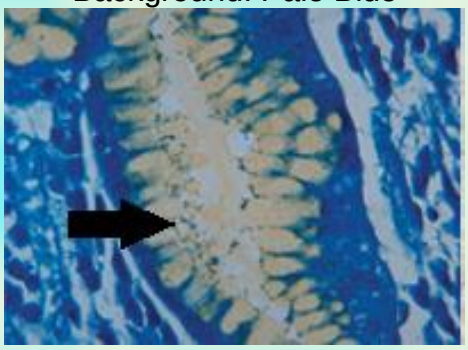
Ziehl-Nelson
Tubercle Bacilli: Red
Background: Pale Blue



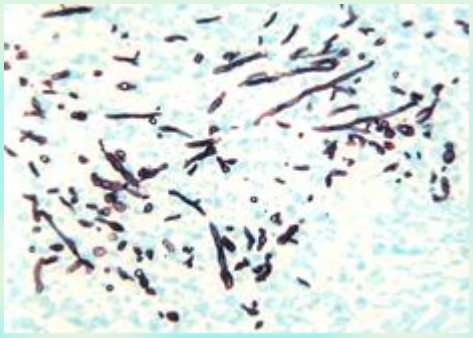
Wade-Fite
Leprosy and Other Mycobacteria: Red
Background: Light Blue



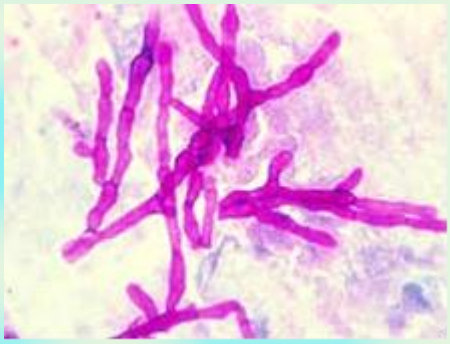
Alcian Yellow Toludine Blue
Mucin: Yellow
Helicobacter Pylori: Blue
Background: Pale Blue



Grocott
Fungi: Black
Pneumocystis: Black
Other Elements: As counterstain
Cellulose, chitin, amoeboe, some mucins, melanin, glycogen and starch may also be balck



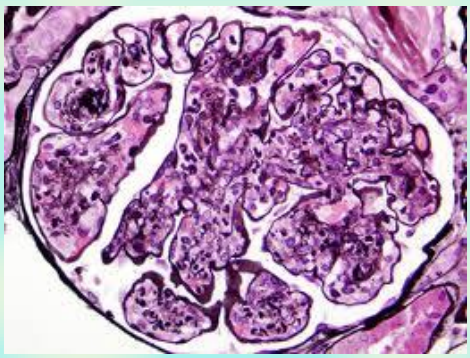
PAS
Fungi: Mangenta
Nuclei: Light blue



Other

Apart from the categories mentioned above, there are many other special stains that are used in both the diagnostic and research world. Please contact us if you cannot see what you are looking for in this atlas as we may be able to suggest an appropriate stain.

Hexamine Silver
Basement Membrane: Black
Other Elements: As counterstain



Luxol Fast Blue Cresyl Fast Violet
Myelin: Deep Bright Blue
Red Blood Cells: Turquoise
Lipofuscin: Bright Blue
Nissl Granules: Purple
Background: Colourless