ORCEIN TECHNIQUE FOR HEPATITIS B (AUSTRALIA) ANTIGEN AND COPPER ASSOCIATED PROTEIN

INTRODUCTION

This method will selectively demonstrate the ‘ground glass’ hepatocytes associated with hepatitis B (Australia) antigen. Acidified orcein will also demonstrate copper associated protein, elastic fibres and lipofuscin, which may cause confusion. Lipofuscin may be demonstrated by other methods, such as PAS, long ZN or Schmorl’s technique for reducing substances, though the coarse granularity and distribution of lipofuscin should not be confused with hepatitis B antigen staining.

SECTION PREPARATION

Formalin fixed paraffin sections cut at 3 to 4 microns. A known positive control section must be stained in parallel.

REAGENTS REQUIRED

1. acidified potassium permanganate
   - 0.5% potassium permanganate: 47.5 ml
   - 3.0% sulphuric acid: 2.5 ml
   Acidified potassium permanganate deteriorates with prolonged storage. Label the solution with the preparation date and discard any solution not used within four weeks.

2. orcein stain
   - orcein (synthetic, BDH): 0.5gm
   - concentrated hydrochloric acid: 1 ml
   - 70% alcohol: 49 ml
   Dissolve the acid in the alcohol and then add the orcein. The orcein stain must be prepared fresh before each use. For ease of preparation, maintain a stock of 2% acid alcohol, together with vials of 0.5gm of orcein, so that a 50cc amount may be prepared as required.

3. 1% oxalic acid

SAFETY NOTES

Most of the reagents used in this method are hazardous. Refer to the relevant COSHH data for the handling of acids, oxidising agents, flammable substances and poisons. In particular, use caution when preparing acid solutions – always add the acid component to the water, never the water to the acid.
TECHNICAL NOTES

1. In steps 8 and 9, sections are stained progressively in the orcein stain, controlling microscopically, until the required intensity is achieved. Excess background staining may be removed by controlled differentiation in the dehydrating alcohol. Ground glass hepatocytes should stain intense reddish brown against a pale background.

2. It is important that only BDH synthetic orcein is used. Other synthetic or natural orceins are not as effective.

METHOD

1. Sections to distilled water.

2. Oxidise in acidified permanganate for 5 minutes.

3. Wash in distilled water

4. Decolourise in oxalic acid for 5 minutes.

5. Rinse in distilled water and then wash in tap water for 5 minutes.

6. Rinse in 70% alcohol

7. Stain in orcein solution at 37°C for up to 1.5 hours (Check after 30 mins)

8. Differentiate and dehydrate in absolute alcohol (refer notes)

9. Clear in xylene and mount in synthetic resin.
RESULTS

Australia antigen, copper associated protein, lipofuscin and elastic tissue..............deep reddish brown

The distribution of Australia antigen can occur as:

- Fine granules diffusely distributed in the cytoplasm of sublobular clumps of hepatocytes
- Round or oval cytoplasmic clumps in scattered single hepatocytes

REFERENCE
