
ORCEIN STAIN - INCLUSION BODIES - HEPATITIS B ANTIGEN

PURPOSE: Viruses themselves are very small and visible only with the electron microscope. Viruses can be described as parasites since they can only reproduce in host cells. These virus particles inside host cells are called "viral inclusion bodies" and are visible with light microscopy. The HBsAg (hepatitis B surface antigen) occurs most frequently as cytoplasmic inclusions in hepatocytes. Kupffer cell cytoplasm may also show the antigen.

If many hepatocytes are affected, the antigen appears as fine granules either diffusely spread through-out the cytoplasm or concentrated in the cytoplasm peripheral to the sinusoid space. This is called the 'ground glass' appearance. In single cell involvement, the HBsAg appears as oval, round, or irregularly shaped aggregates in the cytoplasm, especially in the peri-nuclear region.

This procedure also stains copper associated proteins.

PRINCIPLE: Orcein stains the inclusion bodies, gives a "ground glass" appearance.

CONTROL: A known hepatitis positive liver (carrier). A known copper control.

FIXATIVE: Any well fixed tissue.

TECHNIQUE: Cut paraffin sections 4-5m.

EQUIPMENT: Coplin jars, rinse all glassware in D.I. water.

REAGENTS:

Potassium Permanganate

Potassium permanganate 0.15 gm
Distilled water 100.0 ml
3% sulfuric acid 5.0 ml

5% Oxalic Acid

Oxalic acid 5.0 gm
Distilled water 100.0 ml

Mix well. Label, stable for 1 year.

Mix right before use, discard.

CAUTION: Irritant, avoid contact and inhalation.

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MICROORGANISIMS

ORCEIN

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0.5% Periodic Acid

| | |
|-----------------|----------|
| Periodic acid | 0.5 gm |
| Distilled water | 100.0 ml |

Differentiating Solution:

| | |
|-------------------|----------|
| 70% alcohol | 100.0 ml |
| Hydrochloric acid | 1.0 ml |

Mix well. Label, stable for 1 year.

Make fresh, discard after use.

CAUTION: Avoid contact and inhalation.

CAUTION: Flammable, irritant, corrosive.

Orcein Solution:

| | |
|-------------------|----------|
| Orcein, natural | 1.0 gm |
| 70% alcohol | 100.0 ml |
| Hydrochloric acid | 1.0 ml |

The pH should be 1 to 2. Allow this solution to age at least 48 hours, filter before use. Stable for 6 mos.

CAUTION: Flammable, irritant.

SAFETY: Wear nitrile gloves, goggles and lab coat, avoid contact and inhalation.

Potassium permanganate: Skin and eye irritant, ingestion will lead to severe gastrointestinal distress. Strong oxidant.

Sulfuric acid: Strong irritant to skin, eyes and respiratory system. Inhalation can produce target organ effects on skin, respiratory, reproductive and fetal systems. Corrosive.

Oxalic acid: can cause severe burns of the eyes, skin or mucous membranes. Toxic by inhalation and ingestion. Target organ effects on kidneys and cardiovascular system, repeated exposure can cause dermatitis. Corrosive.

Hydrochloric acid: Strong irritant to eyes, skin, and respiratory system. Target organ effects via inhalation on skin, respiratory, reproductive and fetal systems.

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Potassium permanganate solution, 10 minutes.
3. Wash in water.
4. 5% oxalic acid, until colorless.

5. Wash in tap water, rinse in distilled.
6. 0.5% Periodic acid for 5 minutes.
7. Wash in tap water, rinse in distilled.
8. *Orcein solution, microwave, low power, 30-45 sec, allow to stand for 30 minutes. Check under the microscope, replace in stain if not dark enough.
9. Rinse in 70% alcohol.
10. Differentiating solution if needed, check microscopically.
11. Rinse in 70% alcohol.
12. Dehydrate, clear, and coverslip.
* Conventional method: Orcein solution, 4-16 hours, room temperature.

RESULTS:

| | |
|-----------------------------|-----------------------|
| HBsAg, elastic fibers: | dark brownish-purple |
| Copper associated protiens: | dark purple |
| Background: | light brownish-purple |

REFERENCES:

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, pp 247-248, Battelle Press Ohio

Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

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Luna, L, Histo-Logic, 1978, Vol. VIII. No. 4. Recommended Procedure for Demonstrating Hepatic B Antigen in Parafin Sections, LAB-TEK Div. Miles Lab. Inc.

Prepared: _____ By: _____

Approved: _____ By: _____

PROCEDURE CARD

ORCEIN STAIN - INCLUSION BODIES - HEPATITIS B

CONTROL: A known hepatitis positive tissue. A known copper control. Orcein will stain elastic fibers dark brown, which can be used as a built in control.

TECHNIQUE: Cut paraffin sections 4-5m.

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
 2. Potassium permanganate solution, 10 minutes.
 3. Wash in water.
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- * Conventional method: Orcein solution, 4-16 hours, room temperature.

RESULTS:

HBsAg, elastic fibers: dark brownish-purple
Copper associated proteins: dark purple
Background: light brownish-purple

REAGENTS:

Potassium Permanganate

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|------------------------|----------|
| Potassium permanganate | 0.15 gm |
| Distilled water | 100.0 ml |
| 3% sulfuric acid | 5.0 ml |

Mix right before use, discard.

CAUTION: Irritant, avoid contact and inhalation.

Orcein Solution:

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|-------------------|----------|
| Orcein, natural | 1.0 gm |
| 70% alcohol | 100.0 ml |
| Hydrochloric acid | 1.0 ml |

The pH should be 1 to 2. Allow this solution to age at least 48 hours before use. Stable for 6 months.

CAUTION: Flammable, irritant.

5% Oxalic Acid

| | |
|-----------------|----------|
| Oxalic acid | 5.0 gm |
| Distilled water | 100.0 ml |

Mix well. Label, stable for 1 year.

CAUTION: Irritant, avoid contact and inhalation.

0.5% Periodic Acid:

See PAS

CAUTION: Avoid contact and inhalation.

Differentiating Solution:

| | |
|-------------------|----------|
| 70% alcohol | 100.0 ml |
| Hydrochloric acid | 1.0 ml |

Make fresh, discard after use.

CAUTION: Flammable, irritant, corrosive.

ORCEIN SOLUTION:

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CAUTION: Flammable, irritant.

DATE: _____

TECH: _____

EXPIRATION: _____

5% OXALIC ACID

| | |
|-----------------|----------|
| Oxalic acid | 20.0 gm |
| Distilled water | 400.0 ml |

Solution is stable for 1 year.

CAUTION: Corrosive, avoid contact.

TECH: _____

DATE: _____

EXPIRATION: _____