HEPATITIS B STAIN KIT PROCEDURE

Item# KTHEP (Revised 04/19/11)

KIT COMPONENTS INCLUDED:

- 30 ml. 5% POTASSIUM PERMANGANATE
- 30 ml. 3% SULFURIC ACID
- 100 ml. 2% OXALIC ACID
- 100 ml. DIFFERENTIATING SOLUTION

PRINCIPLE: This kit demonstrates the Hepatitis B surface antigen (HBsAg).

SPECIMEN: Any well fixed paraffin embedded tissue cut at 6 microns.

QUALITY CONTROL: American MasterTech Recommended Control Slide:
Hepatitis B Surface & Core, CSH0225P

SOLUTIONS:

OXIDIZING REAGENT:

- Distilled water ........................................... 50 ml
- 5% POTASSIUM PERMANGANATE ............. 5 ml
- 3% SULFURIC ACID ..................................... 3 ml

Combine reagents in the listed order and mix thoroughly.

PROCEDURE:

1. Deparaffinize slide with Xylene or Xylene Substitute and hydrate through alcohols to Tap water.
2. Place slide in freshly prepared Oxidizing Reagent for 10 minutes.
3. Rinse slide in running Tap water for a few seconds.
4. Place slide in 2% OXALIC ACID for 10 minutes; section should be colorless after this step.
5. Rinse slide in running Tap water for 1 minute.
6. Place slide in ORCEIN STAIN for 2 hours.
7. Rinse slide in 70% Reagent Alcohol.
8. Place slide in DIFFERENTIATING SOLUTION for a few seconds.
9. Rinse slide in 70% Reagent Alcohol, then check section using microscope for correct differentiation.
   Repeat Step 8 if necessary.
10. Dehydrate slide through 3 changes of Absolute Alcohol.
11. Clear slide through 3 changes of Xylene or Xylene Substitute.
12. Coverslip using a permanent mounting media.

RESULTS:

Hepatitis B surface antigen (HBsAg) appear as fine, irregularly-shaped, dark brown aggregates in the cytoplasm of hepatocytes and occasionally in Kupffer cells.