13 Chelex Extraction of DNA

This procedure will allow for the extraction of DNA from samples containing semen. The Chelex extraction is an efficient and quick method to obtain the DNA from the samples. The addition of DTT will allow for the digestion of samples that contain semen. Samples may or may not contain semen.

Safety

Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Standards, Control, and Calibration

One reagent blank must be processed for each extraction batch as a negative control.

Please refer to SOP #5 (Evidence Evaluation and Handling) for guidelines on general evidence handling, and the consumption of evidence in particular.

Equipment, Materials, and Reagents

- scissors, scalpel, tweezers
- 5% Chelex
- Proteinase K (10 mg/ml)
- 0.39 M DTT
- tubes – 1.5 ml, microcentrifuge
- microcentrifuge with rotor for 2 ml tubes at room temperature
- water bath or dry bath ~56.0 °C
- water bath or dry bath ~100.0 °C
- vortex
- stir plate

Procedure

Portioning

A portion of all swabs provided will be taken for Chelex extraction.

1. Set the evidence onto paper.
2. Portion ~1/8 of all swabs for a particular piece of evidence.
3. Place all portions into a microcentrifuge tube.
4. Label microcentrifuge tube with incident number, item number, and initials.

Chelex Extraction

1. Make sure Chelex beads are evenly distributed in solution.
    a. If necessary, re-cap and mix stock by inversion periodically during processing.
    b. Alternatively, chelex solution may be made in a sterile bottle and a reagent stir bar used to keep the beads distributed.
2. Add approximately 200 uL of 5% Chelex solution.

   a. Ensure the substrate is completely saturated. Additional Chelex solution may be needed to completely submerge the swab.
   b. If additional Chelex is needed, the same volume must be added to reagent blank
3. Add 10 uL of Proteinase K and 40 uL of 0.39M DTT
4. Vortex sample for ~10 seconds.
5. Incubate at ~56.0 °C for 30-45 minute.
6. Vortex
7. Incubate at ~100 °C for 8 minutes.
8. Briefly vortex, then centrifuge the tube to pull beads to the bottom of the tube.
   a. Chelex beads and substrates should be pulled to the bottom of the tube. If a supernatant layer is not visible then centrifuge again. A higher centrifugation setting may be necessary.
9. Carefully transfer ~50 uL of supernatant into a new 1.5ml microcentrifuge tube. Be sure to not transfer any Chelex beads.
   a. If any of the beads are transferred, pipette the supernatant back into the Chelex solution. Repeat steps 7 – 9.
10. Store samples refrigerated or frozen until ready to perform Plexor HY analysis.