User-Developed Protocol:

Isolation of DNA from formalin-preserved stool samples using the QIAamp® DNA Stool Mini Kit

This protocol has been adapted by customers and is for use with the QIAGEN® QIAamp® DNA Stool Mini Kit. **It has not been thoroughly tested and optimized by QIAGEN.**

The protocol describes the preservation and concentration of stool samples (in preparation for microscopic examination for intestinal parasites), using two systems from Meridian Biosciences, followed by isolation of DNA from the stool samples for pathogen detection using the QIAamp DNA Stool Mini Kit.

Please be sure to read the *QIAamp DNA Stool Mini Kit Handbook* and the detailed "Protocol using Stool Tubes for Isolation of DNA from Stool for Pathogen Detection" carefully before use.

Procedure

Preservation and concentration of stool samples

1. **Using the Para-Pak® ULTRA System (Meridian Biosciences), select and sample an appropriate area of fresh stool with the collection spoon provided in the cap of the vial containing 10% buffered neutral formalin preservative.**
2. **Add sufficient stool to the vial to bring the liquid level up to the "fill to here" level. This will result in approximately 5 ml of sample.**
3. **Mix the sample thoroughly.**

   The sample is now ready for processing using the Para-Pak Macro-Con® Stool Concentration System (Meridian Biosciences).
4. **Remove the protective cap from the vial.**
5. **Add 10 drops of surfactant if the sample appears to be mucoidal. Swirl the vial gently to mix.**
6. **Insert a 50 ml conical tube into the top of the Para-Pak ULTRA vial. Invert and filter the sample by squeezing the vial until at least 3–5 ml of the sample has passed through the filtration device which is contained in the conical tube.**

   If desired, the entire sample may be filtered into the conical tube.
7. **Holding the vial and conical tube at a 30° angle, remove the vial from the tube.**
8. **Add 6–8 ml of 10% formalin to the tube, mix thoroughly, and allow to stand for 5 min.**
9. **Add 3 ml of ethyl acetate to the tube, then stopper and shake the tube vigorously for at least 30 s. Carefully remove the stopper.**
10. **Centrifuge at 2000 rpm for 10 min.**

11. **Four layers will appear in the tube:**

    Top layer: ethyl acetate

    Second layer: plug of debris

    Third layer: formalin

    Bottom layer: sediment

12. **Remove the debris from the sides of the tube with a cotton swab, and carefully decant the three top layers from the tube. While keeping the tube inverted, a cotton swab may be used to remove any remaining debris from the sides of the tube.**

13. **Add a few drops of 10% formalin solution to resuspend the remaining sediment. If the resulting slide is too dense (printed text should be readable through it), more formalin may be added. The concentrated stool samples can now be examined microscopically for the presence of intestinal parasites. The remaining sample can be used for the isolation of DNA and subsequent analysis by PCR.**

Isolation of DNA for pathogen detection

1. **Centrifuge the 50 ml conical tube at 3000 rpm for 10 min.**
2. **Carefully remove the tube from the centrifuge, avoiding agitation of the contents, and place it upright in a test tube rack.**
3. **Pipette 0.2 ml of the stool sediment into a labeled 2 ml microcentrifuge tube (cut off the ends of the pipet tips first).**
4. **Add 1.6 ml PBS to the tube and vortex for 15 s.**
5. **Centrifuge the tube at full speed for 5 min to pellet the stool sample.**
6. **Discard the supernatant and repeat steps 4, 5, and 6.**
7. **Add 1.6 ml Buffer ASL to the stool pellet. Vortex continuously for 1 min or until the sample is homogenized.**
8. **Continue with step 4 of the "Protocol using Stool Tubes for Isolation of DNA from Stool for Pathogen Detection" in the *QIAamp DNA Stool Mini Kit Handbook*.**