Protocol for Collection of Coprolites for DNA Analysis

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The recovery of ancient DNA from coprolites requires us to protect the samples from modern DNA contamination. Each of us is a DNA volcano, spewing out vapors and sloughing off cells containing our DNA. Anything we touch or get close to for any period of time is probably contaminated with our DNA. The following protocols must be followed to restrict the amount of this contamination to acceptable levels. Ideally we would all be wearing white suits, face masks, booties, gloves and hoods as we excavate. However, that is not possible under normal field conditions in the Great Basin or other hot and dry locations like the Columbia Plateau due to the heat that these non-permeable clothes generate. Closely following the procedures below is the next best thing.

Collect 4 hairs with roots on from each crew member. If people are concerned about privacy issues you may assign personal identification numbers (PIN) rather than give the person’s actual name to the DNA laboratory. Only the Project Director need know the person’s name and corresponding PIN. Freeze the hair and send with coprolite samples to DNA lab. This is a tracking measure used only if modern contamination is later detected.

Excavators:

1) Each person will excavate in their assigned unit exclusively, do not change units with your partner or anyone else unless the supervisor approves it. Any variation from this pattern should be clearly noted on your paperwork and in your notebook along with the Unit, quad, and level that the change occurred.

2) When excavating, try to uncover coprolites or other specimens which could be tested for ancient DNA (chewed fibers [quids], menstrual pads, etc.) in situ. Do not touch it with your trowel if at all possible. Your trowel is contaminated with your DNA whether you are wearing work gloves or not, because you have handled both the gloves and the trowel. When you identify a coprolite large enough to be human in your excavation stop digging and get away from it immediately, placing your trowel pointing at the coprolite—but not touching it—so that the Coprolite Collector can identify it easily when they come to get it. If you do not recognize something you have found, back off and call in a supervisor. While they are determining what it is write a note that they entered your quad to look at something. If it is a coprolite or something to be collected for DNA analysis you will be the Collector’s assistant while they work in your unit.
Coprolite Collector:

1) The Coprolite Collector should carefully put on a pair of latex lab gloves first, trying to avoid touching the fingers and palm as much as possible, then pull on the Tyvek hazmat coveralls. Next, put on your face mask, then cover your head with the hood. The assistant, usually the excavator, rips open the paper container holding the sterile surgeon’s gloves and, being careful not to touch the gloves, holds them out to the Collector who removes them—L for left, R for right—from the sealed packet. Touching only the inside of the sleeves, pull them on carefully over the latex lab gloves. The assistant should then peel back the disposable sterile forceps package exposing the conjoined end for the Collector to grasp and remove from the package. Next, the assistant should carefully peel-back the clear plastic wrapper of a sterile lab cup exposing the top to the Collector who can grasp it with sterile gloved fingers. Avoid touching the cup or breathing on it. It is best for the assistant to wear a face mask to contain their breath.

4) The Collector walks to the specimen careful to avoid raising dust as much as possible, bends and, opening the cup just before, picks up the specimen with the disposable forceps and places it in the cup, closing it immediately thereafter. The Assistant tapes the press-on top shut with masking tape or duct tape and writes all the provenience data on the cup with a Sharpie. Dispose of the forceps and gloves and use new ones for each coprolite collection. Do not put more than one coprolite in a single specimen cup even if there are multiple fragments that look like they could be the same bowel movement. Each gets its own container because you do not know for a fact that they are the same.

5) Next, collect about 1/2 cup of sediment from under the coprolite employing a trowel previously soaked in bleach for 2 minutes. Again, at the end of each collection phase the forceps are contaminated with DNA and should be disposed of. Gloves are thrown away at the end of the collection procedure.

6) Record the precise location and elevation of the *in situ* specimen on your paperwork in the collected specimen column and on the grid area on the paperwork. This must be done for all specimens and each must have a unique number. If you think you may have contaminated the specimen someway please note what and how the possible contamination occurred.

Screeners:

1) If you see a coprolite in the screen stop immediately and carefully set the screen down. You need not suit up since the coprolite may well have already been contaminated. At this stage we simply want to avoid any further contamination if possible.

2) Put on a mask, prepare a specimen cup and then, following the procedures noted above, pull on a glove or gloves, pick up the specimen with the disposable forceps and place it in a sample cup. Carefully close and tape the cup as quickly as possible.
3) Note on your sample cups the site, unit, quad, level, date, your name, and fact that the coprolite was recovered from the screen. Dispose of the gloves and forceps, they are contaminated.

Materials needed for Sterile Coprolite Collections

1) Disposable Paper Tyvek coveralls (large or x large best)
2) 125 or 250 ml sterile plastic (see through) specimen cups
3) Disposable forceps
4) Sterile face masks
5) Sterile surgeons gloves (8 or 8.5 most common sizes)
6) Purple nitrile lab gloves (medium or large best)