

GAHMU sample collection protocol

There are 4 tubes to be filled per great ape feces:

1. One 50 ml tubes containing RNA-later (“purple cap”)
2. One 15 ml tube containing glycerin (marked “G”)
3. One 15 ml tube containing formalin (marked “F”)
4. One 50 ml tube containing silica beads for drying the feces (marked “S”)

Other material provided:

- Wooden spatula or spoon
- Gloves
- Parafilm for closing tubes
- Pipettes
- Data sheet

The 4 different tubes, one spatula, gloves and parafilm provided in one plastic bag per individual. After filling the tubes should be placed back into the same plastic bag and the bag well closed. The tissue should stay in the plastic bag, it will absorb all possible leakage.

Which feces is of interest?

For this first screening it is sufficient to collect samples from great showing no symptoms with “normal” feces.

BUT: If you encounter an individual with diarrhea or other signs of diseases, these samples would be of special interest. For diarrhea it may be difficult to put the feces into the tubes, however, just take what you can and if the feces are very liquid, just put the whole leaf(s) “carrying” the feces into the tube. If there is not enough stool for all 4 tubes, the “priority list” would be:

RNA-later > Glycerine > Formalin > Silica

How to collect the feces?

From chimps with “normal feces” please try to take the upper part of the feces, which means the part which did not touch the ground.

If you encounter great apes with **liquid feces**, just collect what you get, even if it had contact to the ground or leaves. In this case we are not looking for the “normal flora” but for “pathogens”, so we can be sure not to mix up pathogens from the environment with those found in feces.

Data taken down:

The data taken down per sample are listed on the data sheet which is enclosed. Please try to fill in all the information requested, it will be important to know under which conditions the samples were taken and stored.

Procedure of filling tubes:

1. Take out the 4 different tubes and put on the gloves.
2. Using the plastic spoon or spatula, fill the tubes containing the liquids up to the marked level, which is up to the 10 ml level for the smaller tubes and up to the 40 ml level for the bigger 50 ml tubes.
The silica tubes should be filled with feces of the size of a Cola nut (or hazel nut, or palm fruit), anyway there is not too much space left over the silica so you can't put too much in.
3. Close all tubes well and put the parafilm around the cover. It is easy to use the parafilm: Just hold one end with the thumb and pull the parafilm with the other hand until it reaches its full length (it is very flexible) and wrap it around the cover/tube gap at the same time. If you press it well against the tube it will not come off any more and cling to the tube just by itself.
4. Shake the tubes vigorously to maximize contact between the sample and the storage solution.
5. Mark the tubes with sample number (1, 2, 3, ...), the name of the individual (if known) and the date of sampling.
6. Put all 4 tubes of one individual back into the plastic bag (containing some tissue to suck up liquid in the case of leakage) and close the bag well.
7. If possible, store the tubes in a fridge (about 4°C). If this is not possible, keep as cool as possible in the dark.
8. Shipment: Include the "Import Permit" and the data sheet and ship as soon as possible after collection at room temperature, to:

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Some background information:

Why these 4 different tubes?

1. Tube containing RNA-later (purple cap) = Extraction of DNA/RNA
2. Tube containing glycerin (marked "G") = Culturing of bacteria
3. Tube containing formalin (marked "F") = Classical parasitology AND electron microscopy for viruses (and possibly other pathogens)
4. Tube containing silica beads for drying the feces (marked "S"): Extraction of DNA/RNA and antibodies.