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Protocol for bio molecular analysis on mosquitoes samples

- Live mosquitoes must reach the lab in a short time, eventually transported at 4°.
- Mosquitoes can be killed at -20° for 30 minutes and then morphologically identified.
- Pool mosquitoes according to date, location and species, with a maximum number of 200 individuals per pool
- Store pooled mosquitoes in polypropylene cryotubes of 2 ml (sample with 100 exemplars or smaller) or 4.5 ml (more than 100 mosquitoes)
- Every pool must have a unique code or progressive number
- Store at -80°, if not analyzed immediately
- Add three or four 4.3 mm diameter copper plated round balls to each cryotube
- Add different amounts of PBS 1X in each tube according to the number of stored mosquitoes (see table)

Number of mosquitoes	PBS 1x (μ l)
1-30	500
30-100	1000
101-200	2000

- Grind samples for 1 minute in a vortex mixer
- Centrifuge at 4000 g for 8 minutes
- Collect 200 μ l aliquot of mosquitoes homogenates in a 2ml eppendorf tube or plate for extraction
- Proceed with RNA extraction with semi-automatic extractor Bio-Sprint 96 Qiagen or manual extraction with Tizol LS reagent (Invitrogen or equivalent), following the producers instructions.

Dr. Paolo Bonifauri