

Sample collection protocols

Blood collection protocol for LC-MS/MS analysis

- A needle will be inserted into the ante-cubital fossa or back of the hand and bloods drawn for hormone analysis.
- Blood needs to be collected in the appropriate collection tube for serum and plasma.
- Blood must be packed well in wet ice after collection until it arrives at the laboratory for processing and must be stored on ice during processing in the laboratory.
- Ideally, sample transport and processing should be performed within 30 minutes.
- Avoid any exposure of samples to ambient temperatures after collection.
- The required serum collection tubes and cryovials will be sent to the study sites at the start of the study.

Saliva collection protocol for LC-MS/MS analysis

Saliva will be collected by passive drool because many of the commercial absorbent swab collection devices have problems with adsorption and give poor recoveries. Passive drool is very simple and cheap and after freeze thawing gives clean samples. This method has the advantage that five steroid hormones can be measured simultaneously (17OHP, androstenedione, testosterone, cortisol and cortisone).

- The proband needs to rinse the mouth before collecting the saliva.
- No food intake 30 mins prior to collection.
- The proband must not brush teeth within one hour before collection.
- The saliva should be allowed to pool in the mouth for 5 min without swallowing.
- The saliva is drooled down plastic drinking straw (cut in half) into a 5 mls screw topped polypropylene tube to collect 1-2 mls of saliva.
- The saliva sample will be stored at -20 C and sent frozen to the study coordinator in Sheffield for shipping in batches to the analysing laboratory.
- Before the steroid hormone analysis the saliva will undergo a freeze/thaw cycle to break down the mucins.