Swab collection for vaginal microbial DNA extraction

Basic principle

Vaginal microbiome promotes host protection from pathogenic agents potentially involved in urinary tract infections and disorders. For instance, microenvironmental disequilibrium, such as increased facultative and anaerobic organisms and reduced *lactobacilli* abundance, promotes bacterial vaginosis, which in turn promotes low birth weight and higher predisposition towards acquiring bacterial infections (Saraf et al., 2021). In other mammals, such as humans, pregnancy is associated with a higher abundance of *Lactobacillus* and low bacterial diversity (Alhabardi et al., 2021). Vaginal microbiomes can be characterized by using sequences generated from the 16S ribosomal genes.

Required equipment and consumables

a) Sterile rayon or nylon-flocked swabs (e.g., Copan FLOQSwabs®)
b) Sterile transport tubes (e.g., 2 mL screw-cap tubes with DNA/RNA-free buffer or dry tubes)
c) DNA/RNA stabilization buffer (e.g., DNA/RNA Shield™, RNAlater®)
d) Personal protective equipment (PPE): gloves, lab coat
e) Restraining equipment (e.g., sheep/goat handling chute or stand)
f) Sterile saline or PBS (optional, for moistening swab if needed)
g) Cooler with ice packs (if there is no immediate access to -20°C or -80°C)
h) Labels and permanent markers

Procedure

1) Pre-Collection Preparations

a) Restrain the animal in a comfortable, safe position using appropriate handling techniques.
b) Wear clean gloves for each animal to prevent cross-contamination.
c) Label sample collection tubes in advance with animal ID, date, and sample type.

2) Swab Collection Procedure

a) Clean the vulva using sterile gauze and warm sterile saline to remove debris and contaminants. This step is crucial to reduce contamination from external microbiota.
b) Insert a sterile swab gently into the vaginal canal (~5–8 cm deep).
    i) Rotate the swab 360 degrees for 15–30 seconds, ensuring contact with vaginal walls.
    ii) Remove the swab carefully and place it immediately into the pre-labeled transport tube:
c) If using dry tubes, snap the swab tip into the tube without buffer.
d) If using a buffer, immerse the swab in the stabilization buffer and snap off the stick.
e) Secure the cap and temporarily store on ice or at 4°C. As soon as possible, transfer to -80°C for long-term storage.

3) Post-Collection

a) Change gloves and use a new swab for each animal.
b) Record metadata: animal ID, age, breed, estrous stage (if known), health status, and environmental conditions.
c) Clean the collection area and dispose of biohazard waste properly.

Precautions

a) Avoid pre-moistening swabs unless vaginal secretions are very low. If needed, use sterile PBS.
b) Avoid cotton swabs – they can trap DNA and inhibit downstream PCR.
c) Sampling should be performed before antibiotic treatment or hormonal manipulation.

Applications

DNA can be collected for 16S rRNA gene sequencing, enabling microbial quantification and characterization.

Bibliography

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