**Rumen Papillae Kultivierungsstudie**

**Columbia Blood Medium**

Workflow:

* Prepare all utilities and Agars at least 2h before sampling: Wipe all utensils with microzid-disinfectant; Scissors and Tweezers must be flame treated and wiped with microzid three times alternate; then store each pair in a sterile plastic bag until sampling (for each cow a own pair of scissors and tweezers)
* Fill **10ml sterile PBS into Falcon Tubes** (2 for each cow), put them into Anaerobe-pot. Also prepare one Falcon with PBS for negative control. **Always turn on the Bunsen burner**
* Preparation of cow like for Rumen Papilla Sampling. Shortly: empty the rumen partly to be able to mobilize the rumen wall, fix rumen wall with uterine forceps, clean papilla with sterile PBS.
* Cut papilla with cleaned scissors and tweezers. Piece has the size of a fingertip. Try to get papillae only (no connective tissue). Put cutted papillae right into the first labeled Falcon tube filled with PBS.
* Take one Control sample: stir for 3 sec with scissors and tweezers in Control-Falcon tube
* After sampling is finish: transport the anaerobe-pot to the lab.
* When you start preparing the samples, turn on the Bunsen burner and keep it aside where you work!
* Tubes have to be vortexed for 30 seconds to remove blood, slightly adherent bacteria and digesta
* After vortexing transfer the Papilla with sterile loop into a new Falcon tube, filled with 10ml PBS
* Vortex new Falcon tubes for 3 min, until parts of the papilla get loosened
* After this step the Bacterial-Papilla Suspension has to be plated onto the Columbia Blood Medium:
* put 100µl suspension with a 1000µl pipette onto the middle of the agar and use L-shaped disposable plastic spatula (or selfmade glass spatula) to streak it over the whole plate (always turn plate during streaking to get an **even layer).** First leave the plate with the agar side at the bottom to let it dry; same for Control sample
* Then do a 1:10 dilution of your sample solution: put 9ml of sterile PBS into a 15ml Tube and add 1ml of your sample with the pipette, shortly vortex it, plate another Agar per sample.
* To prepare the PBS Control plate: take 100µl from your PBS out of the bottle and plate it.
* When all samples are plated, put them into anaerob pot with **agar-side on top**! Insert two anaerob bags (GEN box anaer, bioMerieux®) into each Anaerobe-Pot; place the anaerob pot into autoclave bag and close it firmly with two cable binders
* Incubate at 37°C for 48 h.
* The rest of your papilla samples have to be stored: put all tubes into Stomacher bag, add one anaerob-bag, put a second bag around, close with cable binders and store it in the fridge (4°C)
* After 48h check if colonies have been growing, if not you can leave it for another 24 hours.
* After incubation, prepare your camera, take anaerobe-pot out of the incubator
* Check and note findings on the plates, take a picture, describe morphology of colonies and number colonies
* Then the different colonies have to be plated on a single agar. Use disposable loop (white ones, 1µl) to take a part of a single colony = one sample of all the different morphology groups and streak it as single line on a new Blood agar plate. In case of e.g. two little equally looking spots, take both together and plate them. **Be really careful to only take samples from one colony!!!! Otherwise you won’t have a pure culture of one bacterium!!**
* **Work quickly**, so that O2 is affecting your colonies as less as possible! Process plate after plate and put them back to the anaerobe-pot as soon as you are finished with the agar-side on top
* When all pure cultures are on the plates, put two new active-carbon pellets into the anaerobe-pot. And close the autoclaving bag again.
* Incubate the plates again at 37°C for 48h
* Control the grown cultures for purity: Often two colonies grow together, one as a slight smear, the other one as dots on top. If this is the case: try to separately plate those two again! (37°C, 48h, anaerob)
* Reference stock preparation: Use 2ml Kryotubes filled with 800µl Glycerol and 1200µl BHI Medium, use blue disposable loop (10µl), take a good part of the single cultures, stir in the medium to loosen it. freeze them at -80°C

You will need:

**At the cow:**

2-3 people for sampling, Rectal gloves, normal gloves, apron, trolley, buckets for rumen contents, bucket for water, uterine forceps, scissors, tweezers, PBS in wash bottle for cleaning the papillae, urin cup with PBS, tablet, alcohol, Kimtech paper, paper towels

anaerobe-pot, containing:

Labelled 45ml Falcon Tubes with 10ml PBS

Sterile Tweezers and scissors

rack

**In the lab (Kremesberg):**

Columbian Agar Plates

Vortexter

Incubator

Bunsen burner

1000µl Pipette

1000µl Pipette tips

Each two anaerob bags (GENbox anaer, bioMèrieux®)

Gloves

Scissors

L-shaped blue disposable plastic spatula for plating

Glass spatulas

Autoclaving bag

Cable binders

Camera

Edding

Pen, Sth to write on

Disposable plastic loop (white one, 1µl)

Disposable plastic loop (blue, 10µl)

Microzid disinfectant solution

45ml Falcon Tube

Lab book

1x PBS autoclaved (2000ml)

15ml Tubes for diluted sample solution

Lighter

**In the lab (institute):**

Labelled 2ml Kryotubes, filled with:

1200 µl Glycerol

800µl BHI- Medium (Brain Heart Infusion) (Kühlraum)

Pipettes

Pipette tips

Labelled Eppis

Kryoboxes

Space in -80 freezer

**Recipe for BHI medium (1200µl + glycerol 800µl):**

* BHI: Kühlraum (+Y(east)), Oder aus BHI Pulver + ddH2O
* Glycerol: ist 99% Lösung (Chemikalienraum), runter verdünnen mit ddH2O auf 60%
* Im Verhältnis 120ml BHI plus 80ml 60% Glycerol mischen
* Autoklavieren

**Rekultivierung aus Stammsammlung:**

* Probe auftauen, gut vortexen
* 100µl Probe in 10ml Flüssigmedium (BHI oder TSB)
* Über Nacht bei 37°C inkubieren, Kontrolle nächsten Tag: wenn Medium trüb, dann hat sich was vermehrt. Wenn nicht trüb:
* Nochmal 24h inkubieren, anderes Medium, mehr Probe rein

**DNA Extraktion von Platten:**

* Probe mit Öse von Platten nehmen, über Nacht in Pre-Lysis Puffer inkubieren
* Dann weiter mit Kit (Nucleospin?)