**Bacterial Mastitis DNA Extraction from Ruminant Milk Using MagAttract Mastitis Paramagnetic Beads and MagMAX-Flex**

1.0 Purpose

The purpose of this procedure is to define a uniform method of extraction of bacterial DNA from ruminant milk using the MagAttract Mastitis Kit (cat. #947757).

2.0 Responsibility

It is the responsibility of all laboratory technicians to follow this procedure. It is the responsibility of the laboratory management to ensure that this procedure is accurate and up-to-date.

3.0 Scope

This procedure is to all ruminant milk samples requiring mastitis bacterial DNA. This procedure utilizes the MagAttract Mastitis purification kit (cat. #947757).

# **4.0 Reagent preparation**

4.1 All components of this kit are stored at room temperature. When opening a new kit record the box lot number on the Extraction Form (MD-PCR-FORM-007).

4.2 Do not add bleach or acidic solutions directly to the sample preparation waste. Buffer ML and Buffer AW1 contain guanidine hydrochloride and Buffer MVL contains guanidine thiocyanate, which can form highly reactive compounds if combined with bleach.

4.3 Buffer MVL is supplied as a concentrate. Before using for the first time, the appropriate amount of isopropanol (100%) must be added, as indicated on the bottle. Tick the check box on the bottle label to indicate that isopropanol has been added. [how long is MVL stable with isopropanol?]

4.4 Buffers AW1 and Buffer AW2 are supplied as a concentrate. Before using for the first time, the appropriate amount of ethanol (100%) must be added, as indicated on the bottle. Tick the check box on the bottle to indicate that ethanol has been added. Reconstituted Buffer AW1 and AW2 can be stored at room temperature for up to one year.

4.5 MagAttract Suspension G must be shaken and vortexed before use. Shake the bottle and then vortex for 3 minutes. Prepare MVL mixture and Buffer ML mixture according to the MagAttract Mastitis extraction recipe. [located in the Cador Mastitis development folder]. Once these mixtures are prepared keep at room temperature until needed.

4.6 Remove the caps and add **80 µl of Buffer ML mixture** to each needed Collection Microtube with pre-loaded beads. [how long is Buffer ML mixture stable?]

* 1. **Sample preparation**
  2. All samples are prepared in a biological safety cabinet, using sleeves and double gloves.
  3. Thaw and equilibrate milk samples at room temperature (15-25°C).
  4. Mix the sample thoroughly by vortexing, pulse spin.

1. **Plate set up and sample Transfer From Tube to Lysis Plate**
   1. Plate setup:

|  |  |  |  |
| --- | --- | --- | --- |
| **Plate ID** | **Plate Type** | **Reagent** | **Volume per well** |
| Tip Comb | Deep well | Place a tip comb in the plate.  Be sure the comb is flat | |
| **Sample plate** | Deep well | **Lysate** (MVL mixture **500 l +** ML/sample mixture **420** **l**) **920 l total** | |
| **Wash 1** | Deep Well | **Buffer AW1** | **1000 l** |
| **Wash 2** | Deep Well | **Buffer AW2** | **1000 l** |
| **Wash 3** | Deep well | **100% Ethanol** | **1000 l** |
| **Elution** (label with worklist date) | Standard | **Buffer ATE [does this have EDTA]** | **100 l** |

* 1. Prepare the Sample, Wash, and Elution plates with the required reagents as listed above. Cover the plates to reduce evaporation and potential contamination.
  2. Pipet **400** **µl sample** (or PBS for NECs) into the Collection Microtube containing the Buffer ML (80 l, 480 l total**)** mixture in a biological safety cabinet.
  3. After all samples and controls have been added to the tubes, re-cap the tubes with the clear plastic caps supplied with the MagAttract Mastitis kit.
  4. Place a compression mat over the sealed bead tubes to prevent unnecessary movement and leaking when it is placed into the BioSpec. Be sure the shiny side of the compression mat is facing up. Place the box lid over the compression mat.
  5. Place the bead tube rack in the BioSpec with the rack lid facing towards you.
  6. Bead beat the samples for 2.5 min, rest for 5 min, and bead beat for an additional 2.5 min. Be sure to switch off and unplug the BioSpec.
  7. Centrifuge samples at 2,500 rpm for 5 min, using Program 3 on the Sorvall RG3 centrifuge.
  8. Carefully remove the caps from the tubes. Discard the caps into the waste container.
  9. Transfer as much of the **420 µl** lysate to the deep well plate containing MVL mixture, labelled “Sample plate” in the table above. Transfer of small quantities of glass beads will not affect the procedure.
  10. Turn on the MagMAX Flex. Push the up arrow to select “KF\_Flex\_mastitis” program (for MagMAX Flex) and then push start.
  11. Load each of the plates according to the display, pressing start after each plate is loaded. Be sure that the orientation of the plate is correct (A1 is in the A1 location).
  12. Press start and record usage in the log book.
  13. The program runs approximately 28 minutes. The first plate to be removed is the Elution plate, which now contains the eluted DNA. Place a lid over this plate, label it with the worklist date, and seal it with parafilm. Place the plate with the eluted DNA on ice. All other plates can be placed in a ziplock bag and discarded in the biohazard waste, with the exception of the unlabeled plate that held the comb, which can be reused.
  14. Turn off the MagMAX Flex and wipe down with DRNase Free solution.
  15. The elution plate can now be used to perform the PCR assay. The last person to PCR from the elution plate must cover the plate with parafilm and store in fridge overnight and by the endof second day the person who extracted the plate tightly seals the plate and cover with parafilm and store at -20°C. The elution plate is stored for 2 months after it has been created, unless specified otherwise.

1. References

Qiagen MagAttract Mastitis Kit Handbook, October 2016

AAVLD Section 5.4 Test Methods

ISO 17025 Section 5.4 Test Methods

AHDC QM Section 5.4 Test Methods

8.0 Changes from Previous Version

New SOP