New product

Development of an Efficient and User-Friendly Workflow for the Extraction of Mycobacteria from Various Sample Types

Julie Charrot[1], Angela Burrell[2], Emeline Ripoche[1], Élodie Pautet[1], Aurore Blanc[1], Leigh Barton[3], Sandrine Moine[1]
[1]Thermo Fisher Scientific, Lissieu, France
[2]Thermo Fisher Scientific, Austin, USA
[3]Thermo Fisher Scientific, GB - Warrington (KG), Cheshire

Introduction

Johne's disease is caused by the bacterium Mycobacterium avium ssp. Paratuberculosis (MAP). It is an incurable chronic enteritis that primarily impacts ruminants, often tested for using fecal and environmental samples. Many countries have implemented control plans to manage the disease and limit its transmission resulting in an increased demand for high throughput testing workflows. Historically, fecal testing workflows have required laborious pre-processing steps in individual tubes, making high-throughput testing difficult. We have developed a method to improve the testing workflow to enhance the usability, flexibility, and efficiency of the sample collection workflow and dramatically simplifying the sample prep and testing process.

Materials and Methods

To optimize our method, we established partnerships with various laboratories, allowing us to refine each key step of the MAP testing process, from sample preparation to result analysis by qPCR.

The organization of four field studies enabled the evaluation of the feasibility of the new method:

- **Sample collection:** In partnership with LDA76, the development focused on sample preparation to determine the optimal fecal weight, pre-lysis conditions (diluted MagMAX™ CORE Clarifying Solution), short and long incubation times, feasibility of pooling, and appropriate consumables for fecal samples collection.
- **High-throughput disruption:** In collaboration with the ARSIA laboratory and LDA23, we developed a disruption workflow in 96-well plates by selecting various adhesive films and deep-well 96-well plates. The goal was to determine the optimal combination ensuring no cross-contamination between wells and maximizing the sensitivity of sample detection by PCR.
- **Environmental PCR (PCRe):** In collaboration with LVD88, we tested environmental samples (ex. fecal matter from bedding) using a specific preparation protocol with different sampling volumes than those used for feces. A protocol was also tested by LVD54 on boot covers. The objective was to improve the ergonomics and sensitivity of the laboratory's routine protocol.

Collaboration with ARSIA - High-throughput sample disruption
The ARSIA laboratory played a crucial role in optimizing the disruption step in 96-well plates. Their experience allowed us to refine the high-throughput disruption protocol, helping to ensure efficient nucleic acid extraction and the absence of cross-contamination.

✓ **Test 1:** Colorimetric cross-contamination test (Figure 1) To determine the optimal combination of 96-well plate and adhesive film, enabling sample disruption without contamination, we initially conducted a colorimetric test. A dye was added to every alternate column of the plate to check for leaks into adjacent wells that were filled with water.

✓ **Test 2:** PCR cross-contamination test with strong positive field samples. Several replicates of these samples were tested by qPCR MAP, placed in every other column, alternating with columns containing MAP-negative samples. This test was repeated at the Thermo Fisher Scientific laboratory with 3 replicates of 4 positive samples, and a positive control randomly placed in a 96-well plate surrounded by negatives (Figure 5a).

✓ **Test 3:** PCR Confirmation test with a panel of various field samples (Figure 2) Finally, we evaluated a panel of various samples, including samples from different origins and infection levels.

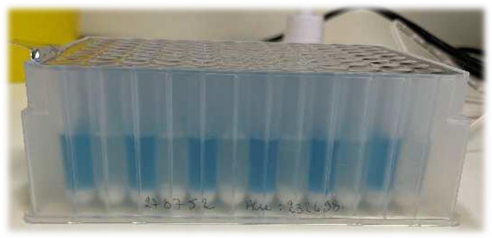

Figure 1. Cross-contamination test: colorimetry of a plate after disruption

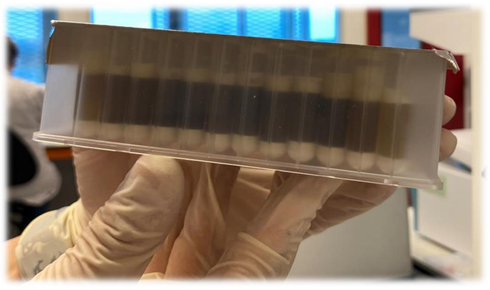

Figure 2. Cross-contamination test: field samples detection of MAP by qPCR

Collaboration with LVD88 – Environmental PCR (PCRe)
Environmental sampling was conducted by collecting fecal samples at various points within the livestock facilities. These samples, consisting of a pool of feces from different animals, are good indicators of the health status of the herd. They are used to identify the presence of MAP in the animals' environment, regardless of animal age. The selection of bedding and fecal sampling sites was managed by the project leader, GDS88. (Figure 3).

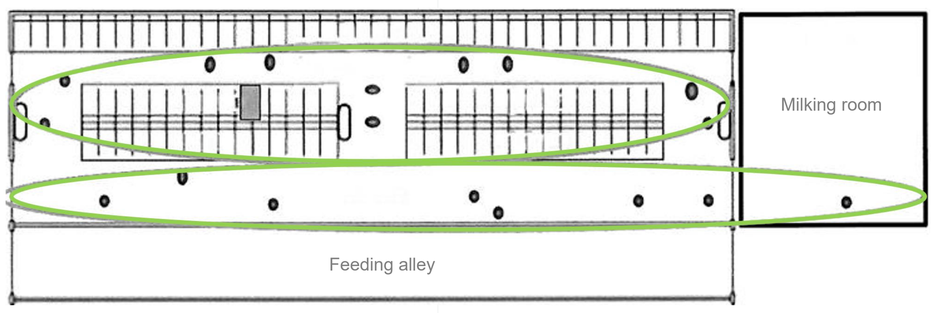

Figure 3. Example of environmental sampling in a dairy cow barn

The optimization study of PCRe was conducted at LVD88 in several steps. Initially an ergonomics and sensitivity test was performed on 5 environmental samples, compared to the laboratory's routine method, to determine the optimal initial weight, reagent volumes, and necessary consumables (Figure 4). The results were then confirmed on a larger panel of field samples. Then, stability tests were conducted on twelve samples to evaluate their status several days after being resuspended in our MagMAX™ CORE pre-lysis solution.

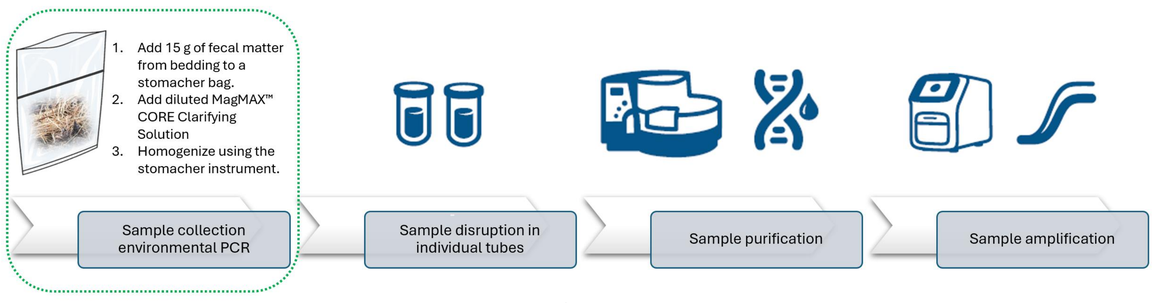

Figure 4. MAP new method: protocol for environmental application

Results

High-throughput sample disruption
The three tests conducted were essential to identify a combination of disruption consumables that facilitate reliable results:

- **Test 1**: Colorimetric cross-contamination test: after reading the absorbance (λ 620 nm), no contamination was observed by the dye in the wells filled with water. This allowed us to select several optimal consumables (96-well plates and adhesive films) for further study.
- **Test 2**: PCR cross-contamination test with strong positive field samples: after the MAP qPCR test, only one combination of consumables showed a complete absence of cross-contamination between wells (Figures 5). Additionally, no edge effect was observed after disruption, with results being repeatable regardless of their positions.
- **Test 3**: PCR Confirmation test with a panel of various field samples: this test of real-world conditions allowed us to confirm the robustness and efficiency of disruption in plates in the final configuration.

✓ All these tests allowed us to create a module adapted for MAP DNA extraction in a 96-well format: the choice of consumables combined with a defined quantity of disruption beads and the sealing system (Reference Figure 6) enables effective mechanical lysis while supporting consistent results.

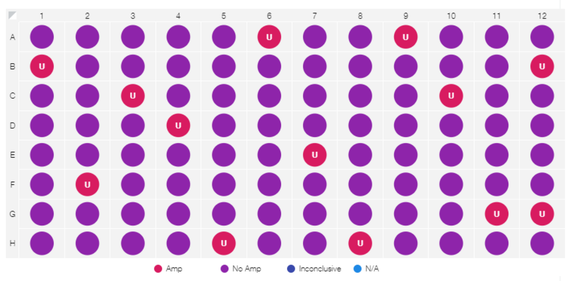

Figure 5a. Amplifications of strong MAP positives samples (red) and absence of amplification in negatives (purple)

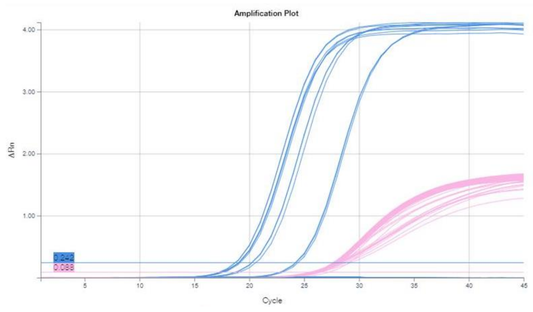

Figure 5b. MAP target amplifications (blue) and IPC (pink)

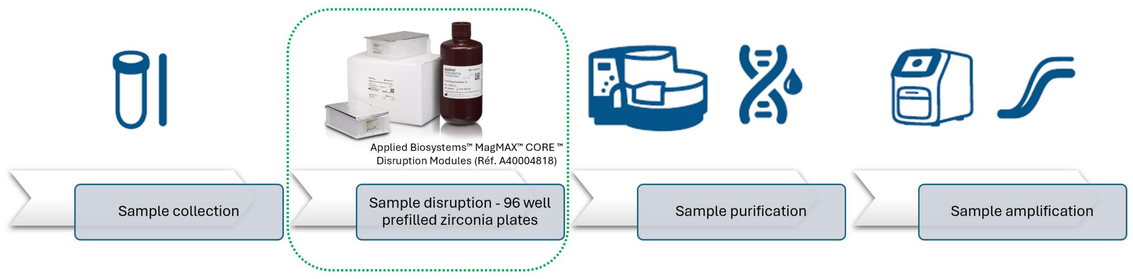

Figure 6. MAP new method: module reference for high-throughput disruption

Environmental PCR (PCRe)
✓ This study allowed us to optimize the PCRe protocol, making it simpler (eliminating the centrifugation steps of the sample and transfer of the supernatant) and more sensitive than the previous version. Out of the 20 bedding samples tested, 2 additional samples were detected positive for MAP PCR compared to the old method (Figure 7, samples 11 and 12). Additionally, the analytical sensitivity is generally improved, with an average reduced Cq for the optimized method (Figure 7, "Average").
✓ Stability tests showed very promising results, with samples maintaining their integrity for at least 5 days after being resuspended in the MagMAX™ CORE pre-lysis solution.

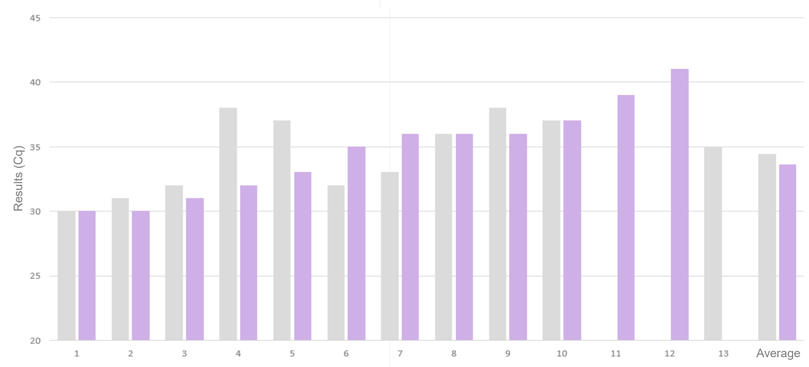

Figure 7. Comparison of the old method (gray) vs optimized method (purple)

Conclusions

The new workflow for the extraction of the MAP bacterium nucleic acid is fully compatible with a 96-well plate format, allowing nucleic acid extraction of up to 188 fecal or environmental samples at once, plus controls, with no loss of yield. The number of steps in the nucleic acid purification protocol has been significantly reduced as well. The workflow has been validated on various sample types including feces and environmental samples from multiple ruminant and camelid species. It is compatible with pooling of up to 10 bovine fecal samples in a single extraction. This solution provides a flexible and adaptable approach to handling large volumes of samples, supporting herd health management with the aim of better controlling Johne's disease.

Acknowledgements

We would like to thank all the experts and veterinary laboratories for their active support and commitment to this project, as well as for providing field samples.

Fabien Grégoire, Pascale Cuvelier - ARSIA Laboratory, Belgium. Bruno Paquant, Elodie Gueribout, Elodie Barbier, Julie Creusot - Veterinary Departmental Laboratory of Vosges (LVD88). David Le Berre, Emily Pecetto - Departmental Analysis Laboratory of Seine-Maritime (LDA76). Eric Guillemot, David Rouchon - Departmental Analysis Laboratory of Creuse (LDA23). Sabine Pelzer, Valérie Schuster, Nathalie Jachacz - Departmental Veterinary and Food Laboratory of Meurthe-et-Moselle (LVAD54).

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.