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Updated solution

Prevention and control of paratuberculosis with VetMAX™ *M. paratuberculosis* 3.0 Kit Toolbox Solution

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Introduction

Paratuberculosis, caused by *Mycobacterium avium ssp. paratuberculosis* (MAP), is an incurable chronic enteritis in ruminants. Its diagnosis relies on laboratory-based methods such as ELISA and PCR. In Belgium and France, surveillance and control programs (particularly in cattle) aim to limit disease spread. Although ELISA testing is rapid and cost-effective, its sensitivity remains limited and it is frequently combined with PCR to enable earlier detection of infected animals.

With increasing PCR testing needs, several laboratories have had to scale up their sample throughput and diversify their applications (e.g., environmental PCR). To address this challenge, a series of studies was conducted to develop a method combining ergonomic workflow, flexibility, and efficiency, in collaboration with several specialized laboratories.

Material and method

To optimize our method, partnerships with multiple laboratories enabled a complete review of each key step of MAP diagnosis—from sample preparation to qPCR data analysis.

Four field studies were conducted to evaluate the feasibility of this new toolbox approach:

- **Sample collection**
  In partnership with LDA76, development efforts focused on optimizing sample preparation, including determining the optimal fecal mass, pre-lysis conditions (diluted MagMAX CORE Clarifying Solution), incubation times (short and long), homogenization feasibility, and selection of appropriate consumables for fecal sample collection.
- **High-throughput sample homogenization**
  In collaboration with ARSIA and TERANA Creuse, we developed a 96-well plate grinding approach by evaluating multiple adhesive films and deep-well plate designs. The goal was to identify the optimal combination ensuring absence of cross-contamination while maintaining maximal PCR detection sensitivity.
- **Environmental PCR (ePCR)**
  In partnership with LVD88, environmental samples (bedding, manure from cattle barns) were evaluated using a dedicated preparation protocol involving sampling volumes different from those used for feces. At LVD54, this protocol was also tested on boot covers. The objective was to improve both ergonomics and analytical sensitivity relative to the laboratory's existing routine workflow.

Partnership with ARSIA – High-Throughput Sample Homogenization

ARSIA played a key role in optimizing plate-based homogenization. Their expertise helped refine the high-throughput protocol to ensure efficient nucleic acid extraction while preventing cross-contamination.

- **Test 1: Colorimetric non-contamination test (Figure 1)**
  To identify the optimal "96-well plate + adhesive film" combination for contamination-free grinding, a colorant was placed in every other column while adjacent wells were filled with water to assess potential leakage.
- **Test 2: PCR-based cross-contamination evaluation with strongly positive field samples**
  Multiple replicates of highly positive field samples were tested using MAP qPCR, deposited in alternating columns with MAP-negative samples. This assessment was repeated at the Lissieu laboratory (Thermo Fisher Scientific) using three replicates of four positive samples (plus a positive control), placed randomly across a 96-well plate surrounded by negatives (Figure 5a).
- **Test 3: Confirmation PCR with a diverse field sample panel (Figure 2)**
  Finally, we assessed a panel of samples from various origins and infection levels to confirm robustness.

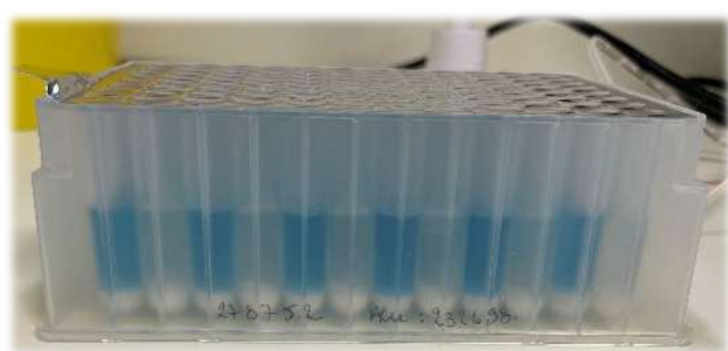

Figure 1. Intercontamination test Colorimetry of a plate after grinding

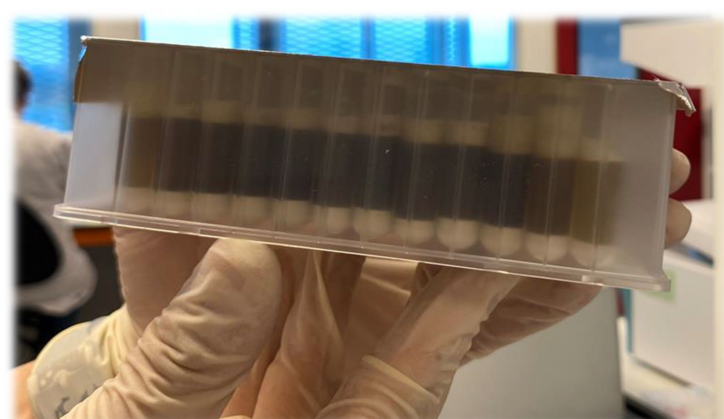

Figure 2. Intercontamination test of field samples Detection of MAP by qPCR

Partnership with LVD88 – Environmental PCR (ePCR)

Environmental sampling involves collecting fecal material from different areas of livestock facilities. These composite samples reflect herd-level infection status and are used to detect MAP presence in the environment of both adult and young animals. Selection of bedding and fecal sampling sites was coordinated by the GDS88 (Figure 3).

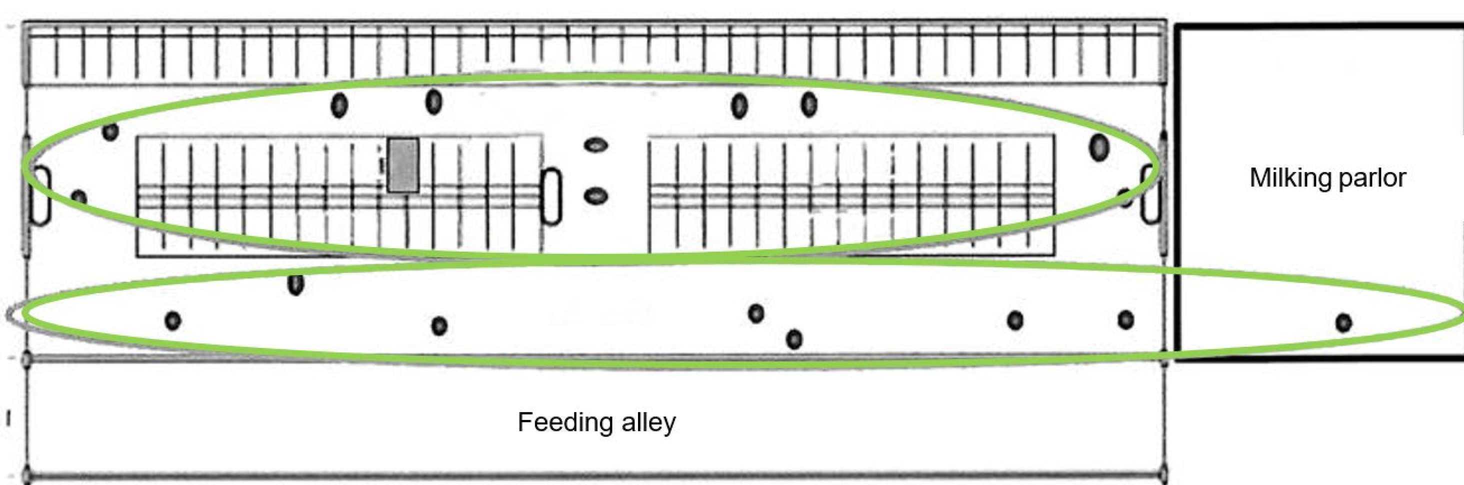

Figure 3. Example of environmental sampling in a dairy cow barn

The optimization of the ePCR method at LVD88 occurred in several phases. An initial ergonomics and sensitivity study on five environmental samples compared the new protocol with the laboratory's routine method to determine optimal sample mass, reagent volumes, and required consumables (Figure 4). Results were then validated using a larger field sample panel. Stability studies were conducted on twelve samples to assess MAP detection over several days following resuspension in the pre-lysis solution.

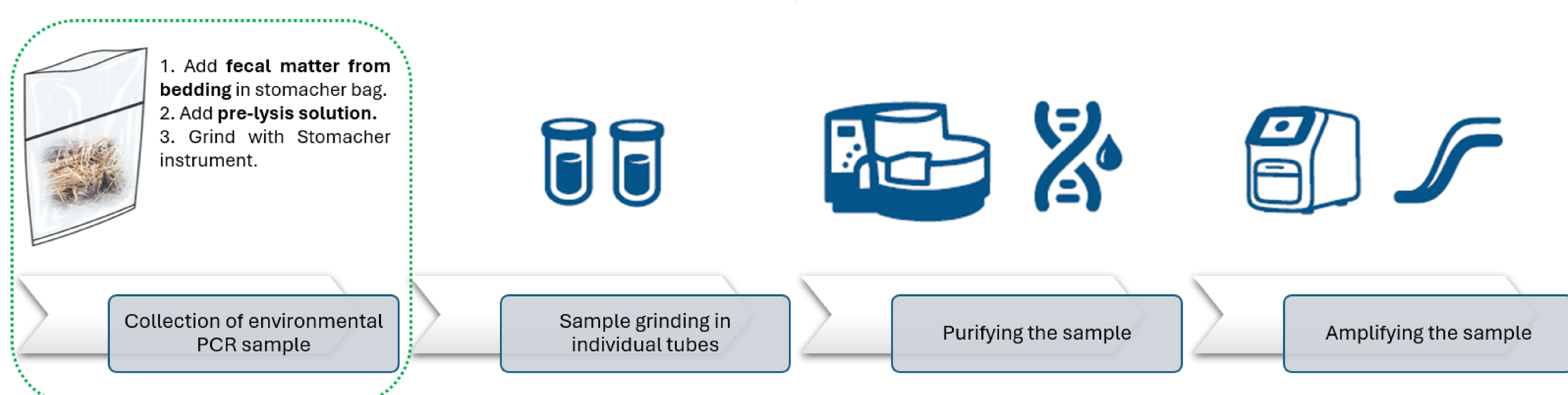

Figure 4. VetMAX M. paratuberculosis 3.0 Kit Toolbox Solution for Environmental Application

Results

High-throughput sample grinding

The three tests performed were essential for identifying a combination of grinding consumables that ensured reliable results:

- **Test 1: Colorimetric non-contamination test**
  After measuring absorbance at 620 nm, no contamination of water-filled wells was observed. This allowed selection of several consumable references (96-well plates and adhesive films) for further evaluation.
- **Test 2: qPCR-based cross-contamination test with strongly positive field samples**
  Following MAP qPCR, only one consumable combination showed complete absence of cross-contamination (Figure 5). No edge effects were observed, and results were reproducible regardless of well position.
- **Test 3: Real-life grinding conditions in 96-well format**
  This test confirmed the robustness and efficiency of the final plate-based grinding configuration.

✓ These tests enabled development of a grinding module adapted to MAP DNA extraction in 96-well format
The selected combination of consumables, a defined quantity of grinding beads, and the sealing system (see Figure 6) ensures efficient mechanical lysis while maintaining well integrity.

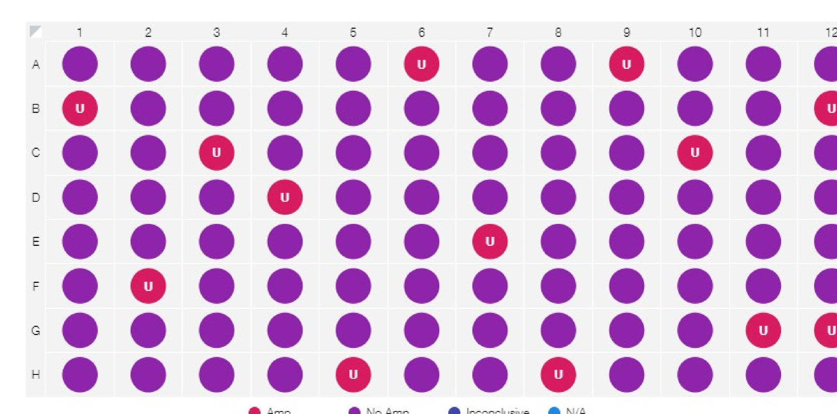

Figure 5a. Amplifications of high MAP positives (red) and no amplification of negatives (purple)

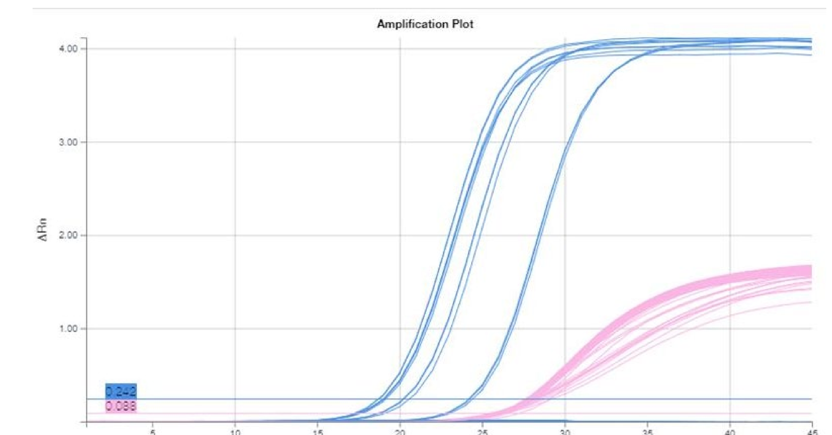

Figure 5b. MAP (blue) and IPC (pink) target amplifications

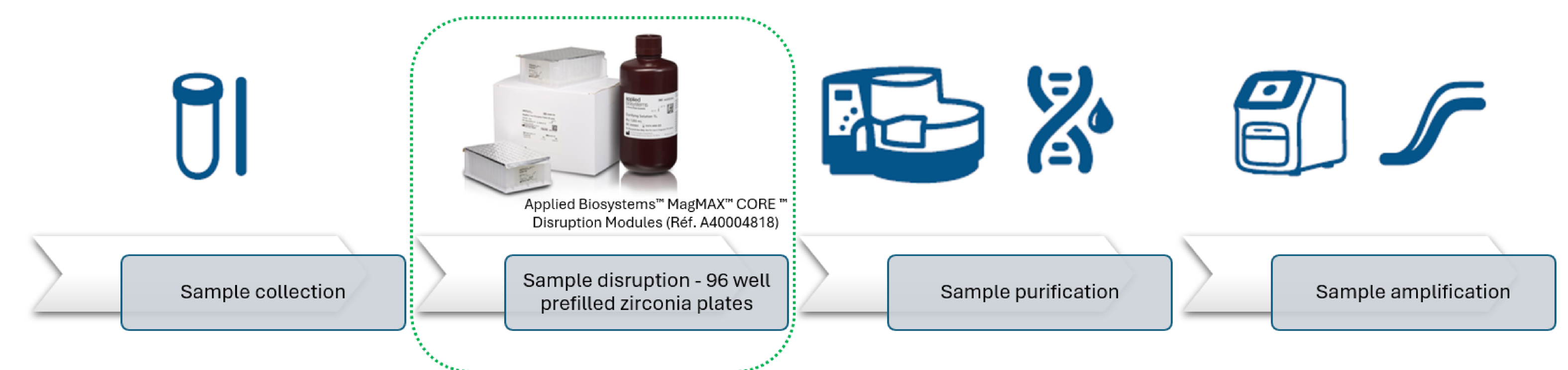

Figure 6. VetMAX Method M. paratuberculosis 3.0 Kit Toolbox Solution for High-Throughput Feces Application

Environmental PCR (PCRe)

✓ **The optimized ePCR protocol is simpler and more sensitive than the previous version**
Removing centrifugation and supernatant transfer steps significantly streamlined the workflow. Among 20 bedding samples tested (negative by both methods; not shown), two additional positives were detected with the optimized MAP PCR protocol compared with the routine method (Figure 7, samples 11 and 12). Overall analytical sensitivity improved, as indicated by lower mean Ct values with the optimized method (Figure 7 "MOY").
✓ **Stability testing demonstrated strong sample integrity**
Samples preserved in the pre-lysis solution maintained detectable MAP levels for at least five days.

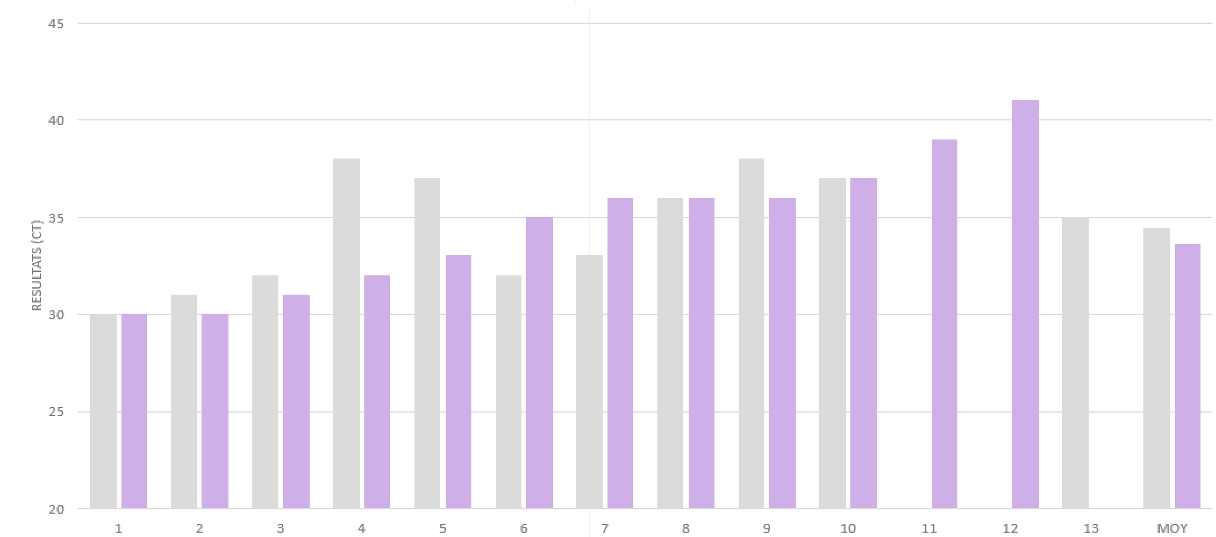

Figure 7. Comparison of the former method (grey) vs the optimised method (purple)

Conclusions

The VetMAX™ M. paratuberculosis 3.0 Kit – Toolbox Solution, developed with the support of multiple testing laboratories and adopted by laboratories such as ARSIA and LVD88, enables highly sensitive and specific MAP detection by PCR. Suitable for both low- and high-throughput workflows (up to 2×96 samples), it simplifies sample collection, preparation, and purification, and enables rapid amplification, reducing total processing time by half.

Validated across diverse sample types (including feces and environmental samples) and species (ruminants, camelids), and evaluated in pooled samples (up to 10 bovine fecal samples), this solution provides a streamlined and flexible response to large testing volumes, supporting herd-level health management and improved control of paratuberculosis.

VetMAX™ M. paratuberculosis 3.0 Toolbox Format 500 reactions (ref. A40004117)
Also available in 100 reactions ref. A40004116

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