

# Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from bovine colostrum by immunomagnetic separation

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## INTRODUCTION

Newborn calves are most susceptible to infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the cause of paratuberculosis. Colostrum is an important source of this organism due to direct excretion of MAP within the milk gland or indirect contamination with MAP-infected faeces. Feeding of high quality colostrum is essential for Passive immunization and an optimal development of calves because of its high concentration of immunoglobulins and growth factors. For this reason the screening of colostrum reserves for the presence of MAP is important to avoid new infections and to get the paratuberculosis under control.

The cultivation of MAP from Colostrum is time-consuming (12-18 weeks) [1]. A rapid alternative is the specific detection of MAP -DNA on the basis of IS 900-PCR reaction [2]. Colostrum contains high levels of proteins and PCR inhibitors making the extraction of DNA difficult. The immunomagnetic separation (IMS) offers the possibility to isolate cells specific from the surrounded matrix. Therefore the aim of this study was to develop a rapid method based on an IMS-technique for the sensitive detection of MAP in bovine Colostrum

## MATERIAL and METHODS

**Colostrum:** Colostrum samples collected from healthy cows ( Fig. 1 ) were inoculated with a reference strain of MAP (DSM44133). Logarithmic dilution rows of the MAP suspension culture (in Middlebrook 7H9) were used to inoculate the colostrum and to determine the number of CFU/ml by cultivating on Herrolds Egg Yolk Medium.

**IMS/DNA-Extraction:** The IMS was performed with mouse monoclonal antibodies (IgG) against lipoarabinomannan (LAM), a component of the cell wall of mycobacteria, secondary coated onto Dynabeads Pan Mouse IgG. For antibody production the hybridoma cell clone CS35 was cultivated (10 % FCS). Centrifugal filter devices were used to concentrate and purify the antibodies. The IMS (including sample preparation ) and lysis of the cells were carried out as shown in Fig. 2. Following Proteinase K treatment (30 min, 65 °C) the DNA was extracted with modified NucleoSpin Food Kit (Macherey-Nagel).

**IS900-PCR:** The amplification of IS 900 was applied for the specific detection of MAP. The Primers s204, s749 [3] and MP4 [4] were used as follows: single PCR s204 & s749, seminested PCR (external) s204 & MP4 and (internal) s204 & s749. The Amplification conditions were: 1 µl DNA/15 µl reaction mixture; 97 °C, 2 min, (97 °C, 30 s, 60 °C, 30 s, 72°C, 75 s) 40 cycles, 72 °C 5 min. For seminested PCR the first PCR product was diluted (1:100).



Fig. 1. Collection of colostrum within 24 hours after parturition.

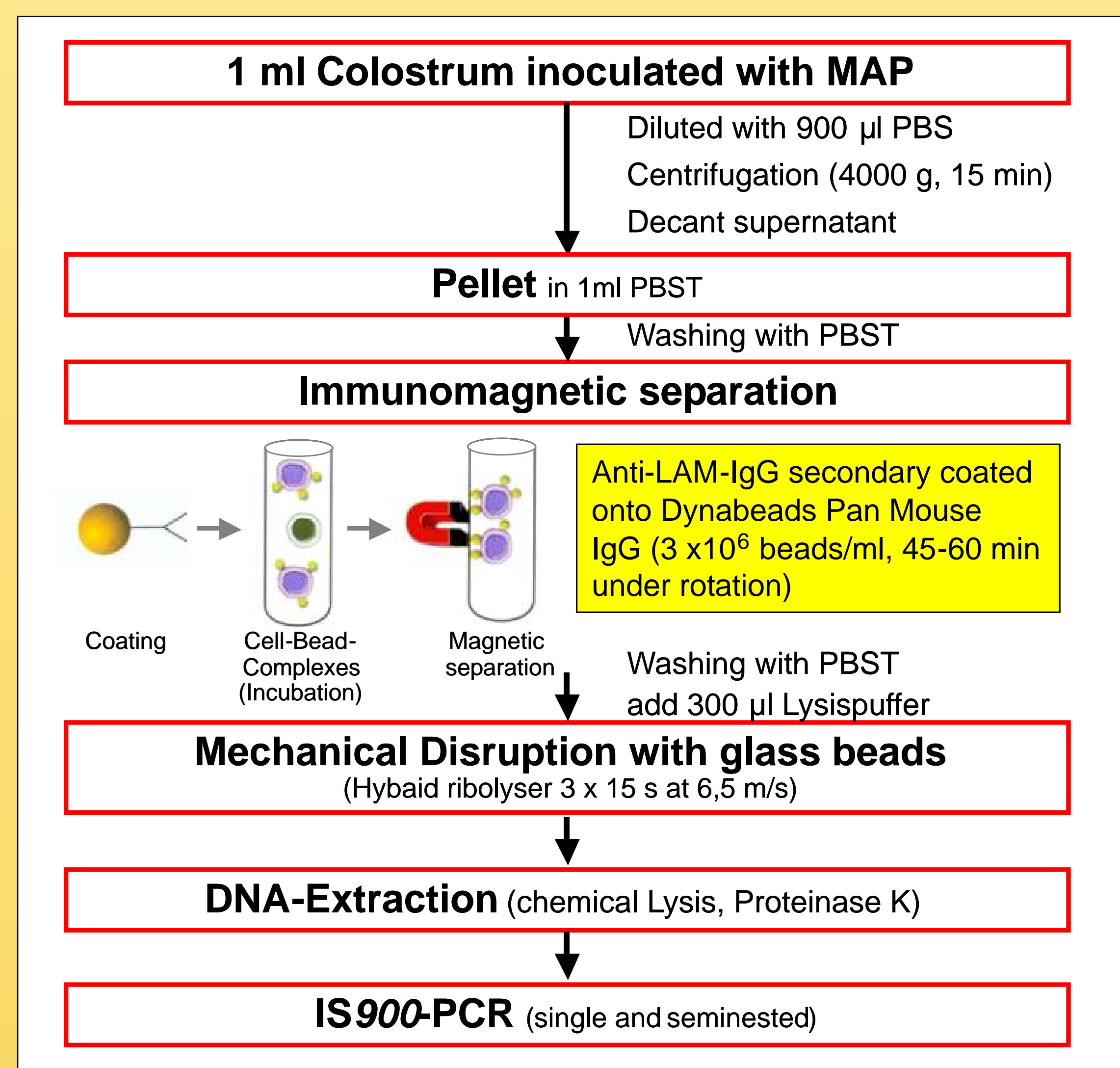


Fig. 2. Sample preparation for detection of MAP in colostrum

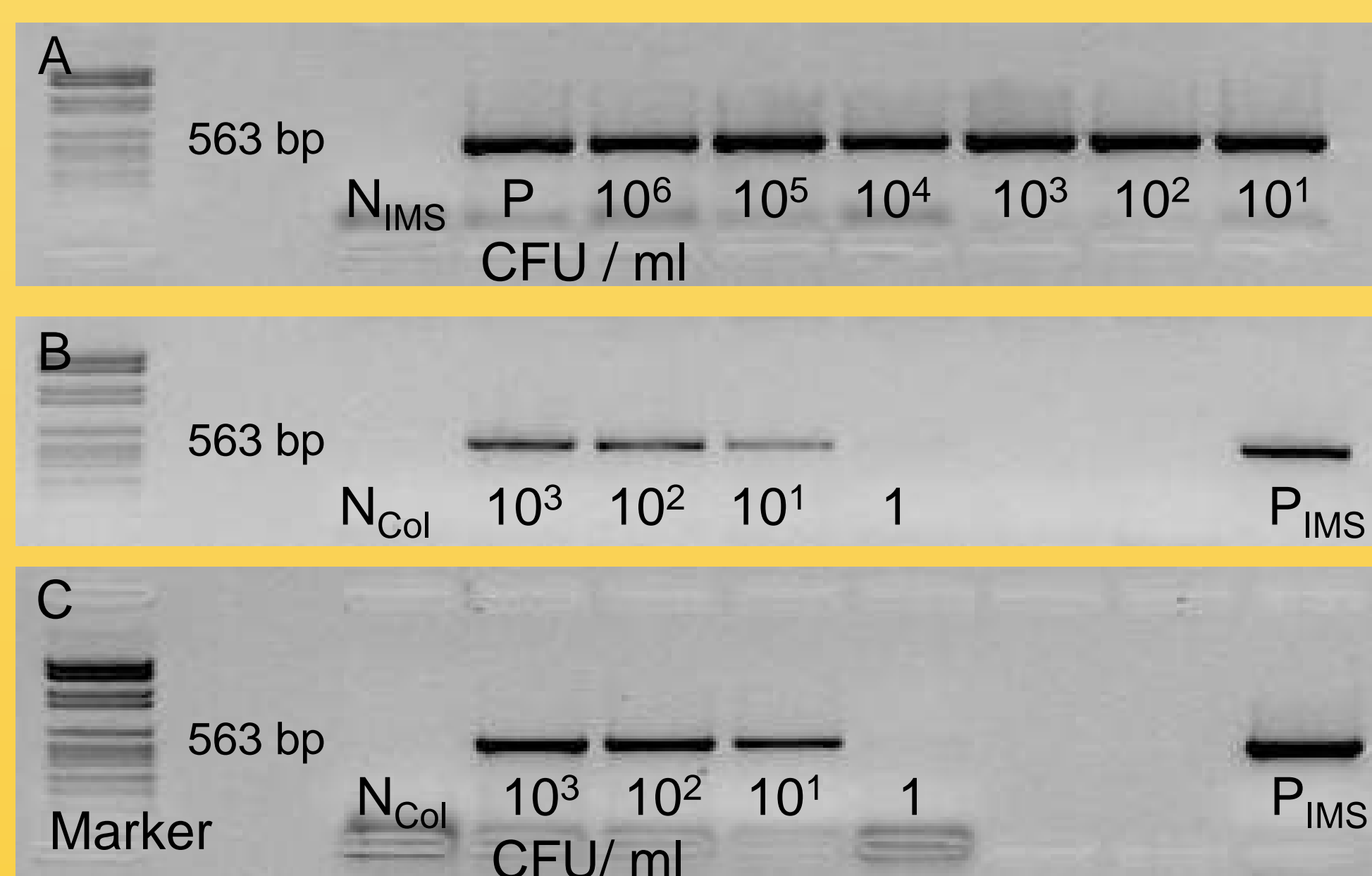


Fig. 3. Recovery of MAP with IMS-PCR from artificially inoculated PBS (A, single PCR) and colostrum (B, single PCR or C, seminested PCR) N<sub>col</sub>= control with MAP-free colostrum; N<sub>IMS</sub>/P<sub>IMS</sub> = negative/positive control for IMS, N / P = negative/positive control for PCR (not shown)

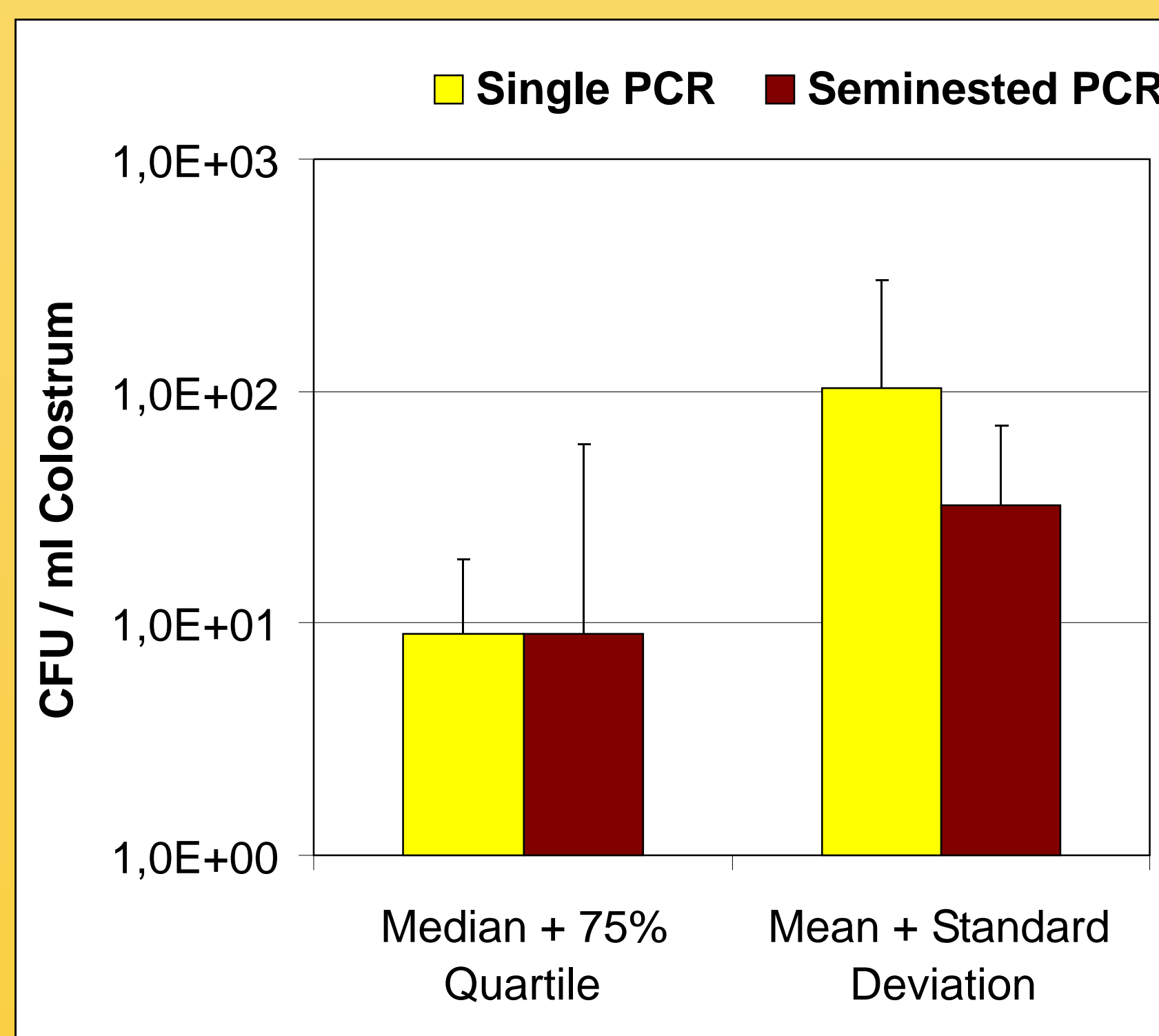


Fig. 4. Averaged detection sensitivity of single and seminested IMS-PCR (n = 5).

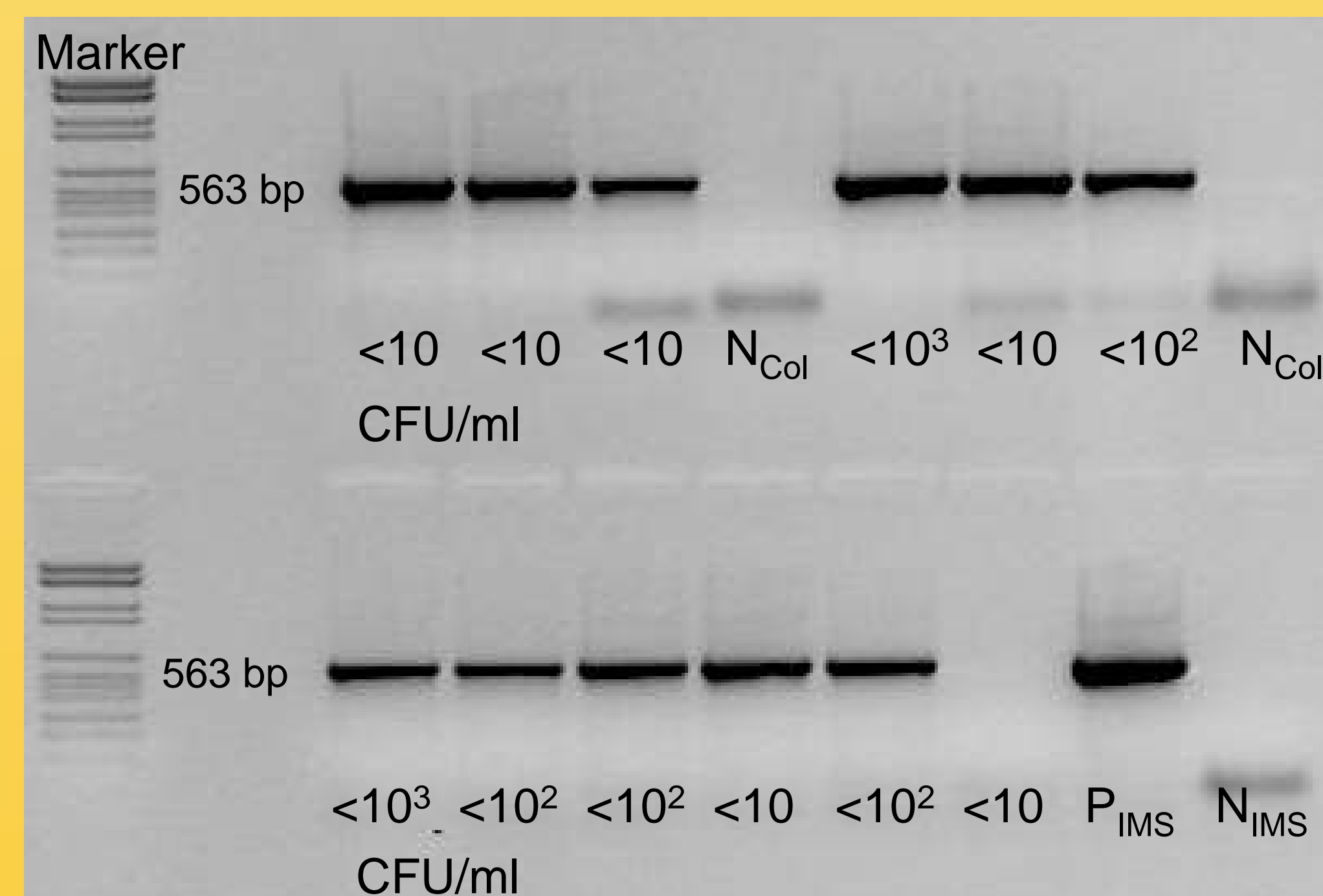


Fig. 5. Results of a blinded analysis with MAP inoculated colostrum samples (single PCR reaction). N<sub>col</sub>= control with MAP-free colostrum; N<sub>IMS</sub>/P<sub>IMS</sub> = negative/positive control for IMS, N / P = negative/positive control for PCR (not shown)

## RESULTS and CONCLUSION

In pre-experiments MAP-DNA was safely detected in inoculated PBS containing ~10 CFU/ml to check the efficiency of the IMS method ( Fig. 3 ). Experiments with colostrum samples showed that IMS in conjunction with IS 900-PCR recovered MAP from artificially inoculated colostrum containing between 10 and 100 CFU/ml ( Fig. 3 and 4 ). The detection sensitivities of the single and seminested PCR were similar, however the seminested PCR led to more pronounced signal bands at low MAP concentrations. The averaged detection limits of both PCR reactions are shown in Fig. 4, calculated on the basis of different independent MAP inoculation experiments with colostrum. In three blinded analysis with 33 artificially inoculated colostrum samples to check the reliability of the IMS-PCR MAP was detected in samples containing >10 CFU/ml safely and in 7 of 11 (seminested 9/11) samples containing < 10 CFU/ml ( Fig. 5 ).

This developed method offers a sensitive rapid detection system to screen reserves of colostrum for the presence of MAP as part of a herd-level paratuberculosis control programme.

### References

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