Factors affecting the detection of Mycobacterium avium subsp. paratuberculosis (MAP) by PCR in raw milk and their interactions were investigated. Three day old bulk tank raw milk (50 ml) samples were seeded with MAP at a level of an estimated 30 CFU/ml. Heat-treatment of raw milk before centrifugation significantly affected the partitioning of MAP in the cream, whey and pellet fractions. Based on the IS900 PCR results, MAP preferentially partitioned into the cream fraction in unheated raw milk, and into the pellet fraction in the heat-treated milk. Treatment with 0.75% hexadecylpyridinium chloride (HPC) helped collect MAP in cream fraction. Heat treatment, use of pooled cream and pellet fractions and treatment with HPC improved the detection by PCR significantly, while washing of pellets prior to DNA extraction did not. The limit of detection using our optimized procedure was an estimated 15ï 50 CFU in 50 ml, or Ò1 CFU/ml.