

# Sample Handling Substantially Affects Johne's ELISA

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## Introduction

The Johne's enzyme-linked immunosorbent assay (ELISA) is designed for rapid detection of antibody to *Mycobacterium avium* subspecies *paratuberculosis* (MAP), the causative agent of Johne's disease in ruminants. However, there can be significant variation in ELISA values between wells, test kits and laboratories. The objective of the present study was to determine if sample handling could significantly affect ELISA values to MAP.

## Materials and Methods

Blood samples were collected from nine cows that previously had high positive ELISA values (>2.5) to MAP. The samples were subjected to different transportation temperatures (on ice, 26°C), storage temperatures (4°C, -20°C), storage times (one day, one week) and preparation methods (serum separated, hemolyzed and serum separated, whole blood). Blood samples were also collected from 12 cows that previously had inconclusive to low positive (0.4 to 1.5) ELISA values to MAP. The

samples were subjected to three treatments – short-term (overnight) or medium-term (1 week) storage in the refrigerator (4°C) or freezer (-20°C).

## Results

For the highly ELISA positive cows, significant difference in ELISA values to MAP was present among the different handling methods by univariate analysis ( $p=0.000$ ). Among the second group, significant difference in ELISA values to MAP was also seen for different sample handling treatments by univariate analysis ( $p=0.037$ ).

## Significance

The results indicate time to process blood samples, as well as blood sample storage duration and temperature, can lead to significant discrepancies in ELISA values to MAP. Such discrepancies will inevitably result in improper classification of MAP-infected cattle, impeding both biosecurity measures on uninfected farms and MAP control programs.