

Significance of Single and Double Map Agar Gel Immunodiffusion Precipitation Bands

Gilles R. G. Monif^{1,2}

J. Elliot Williams²

¹*Infectious Diseases Incorporated, Bellevue, NE*

²*University of Florida College of Veterinary Medicine, Department of Infectious Diseases and Pathobiology, Gainesville, FL*

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ABSTRACT

A retrospective analysis was performed to determine the significance of single and double MAP AGID immunoprecipitation (IP) bands. Of the 58 sera that exhibited a single MAP IP band, 15.5% lacked corresponding anti-MAP antibodies. Of the 71 sera that tested positive in a MAP AGID test, 13 (18%) exhibited a second IP band. The second MAP IP band occurred only when the OD test reading exhibited a further increase. The test data infers that the first IP band is caused by a dominant cell-wall mycobacterium antigen. The second IP band is specific for MAP isolates. The presence of a positive MAP AGID IP band in an animal with necropsy documented Johne's disease and no anti-MAP antibodies argue for the existence of polyphonic genomic variant of MAP as being a causes of Johne's disease.

INTRODUCTION

Agar gel immunodiffusion test (AGID) was initially developed as a screening test for the detection of Johne's disease in flock ruminants. While AGID positivity has an apparent 100% specificity with the presence of chronic granulomatous enteritis (Johne's disease), it has only 5% sensitivity in detect-

ing infected cows¹⁻³. The development of absorbed enzyme-linked immunoabsorbent assay (ELISA) tests and documentation of their great sensitivity have led to the relative abandonment of AGID in herd management schema of dairy herds¹⁻².

Cows with documented Johne's disease can have a diagnostic MAP ELISA reading and not have a positive AGID test. Conversely, cows with necropsy documented Johne's disease may have a positive immunoprecipitation band and not have a diagnostic MAP ELISA titer. Occasionally, a second immunoprecipitation band is identified in AGID tests.

The purposes of this report are to describe the relationship between the single and the second immunoprecipitation bands as they relate to their corresponding MAP ELISA titers.

MATERIALS and METHODS

Study Populations: The AGID test data, derived from a five year herd management program which was implemented between January 2001 and January 2006, were reviewed to identify the number of cows with a positive AGID test. Immunoprecipitation bands were identified in sera obtained from 71 cows.

Study Material: The corresponding MAP

ELISA titers were identified and tabulated for the cows with a single and double immunoprecipitations bands. Multiple observations on three cows were available in which the presence or absence of the second precipitation band varied.

Nineteen single IP band sera came from cows that had been necropsied at the University of Florida College of Veterinary Medicine. In each case, the histopathology confirmed Johne's disease due to acid-fast bacilli.

AGID: Petri dishes were poured with sterile saline 1% agarose prepared in 0.1 M Tris-HCL buffer at pH 10. Well distances were 8 mm. Well sizes were 4 mm for the six peripheral wells and 3 mm for the central well. The peripheral well received 45 ul of the test serum. The central well was inoculated with 35 ul of a crude protoplasmic antigen (Allied Monitor, Fayette Missouri). Serum from a cow with documented Johne's disease constituted the positive control. Final analytical readings were done at 24 and 48 hours. The appearance of one or more clearly definable precipitation lines before or at 48 hours constituted a positive result. The AGID tests were done with both non-absorbed and *Mycobacterium phlei* absorbed sera.

Pre-absorbed ELISA Test: Test sera were pre-absorbed with *M. phlei*. The ELISA results were calculated from wavelength readings at optical density (OD) 405 nm. All readings less than 1.6 OD were considered negative; readings between 1.5 and 1.9 OD were deemed suspicious/inconclusive; and readings of 2.0 to 2.5 OD were called low positive. A high positive was deemed to be any reading of 2.6 OD or above.

RESULTS

Pre-absorbed MAP ELISA Test: The MAP ELISA test had USDA certification for diagnostic use. Protoplasmic protein derivative from IS 900 MAP isolate (Allied Monitor) constituted the test's antigen core array. Test sera were pre-absorbed with *M. phlei*. The ELISA results were calculated from wavelength readings at optical density (OD) 405 nm. All readings less than 1.5 OD were con-

sidered negative; readings between 1.6 and 1.9 were deemed suspicious/inconclusive; and readings between 2.0 to 2.5 OD were called low positives. A high positive reading was a value 2.6 OD or above.

Overall Relationship of a Single IP Band to MAP ELISA Test Reading: Single IP bands were not tightly correlated to the corresponding MAP ELISA. Of the 54 sera identified as being AGID test positive with a single IP band, 9 (15.5%) lacked the demonstrable presence of anti-MAP antibodies. Another 10 (17.2%) had inconclusive readings. Thirty-eight of the 54 exhibited a strong positive OD reading.

Frequency of a Double IP Band within AGID Positive Tests: Of the 71 sera identified as having a positive MAP AGID test 13 (18%) had a double band.

Relationship of a Double IP Band to its Corresponding MAP ELISA Optical Density Reading: The mean MAP ELISA optical density reading for the 13 sera with a double IP band was 3.88 with a range from 2.3 to 5.8. Only one serum had an OD reading of less than 2.6.

Serial OD Readings on Sera from a Cow Exhibiting a Double IP Band: Serial MAP ELISA OD readings from the same cow were available in three instances.

Cow #4151's initial sera exhibited a single IP band at an OD reading of 2.32. When she developed clinical disease and her OD reading increased to 4.1, a second IP band was present.

Cow #3882's sera exhibited a single IP band at an OD reading of 3.09. When her OD reading increased to 4.96, a second IP band was present. The second band disappeared when the OD reading dropped below 4.0.

Six AGID and MAP ELISA test results were available for Cow #3917. Three sera obtained over a seven day period exhibited a double IP band. Her OD readings were 5.5, 5.0 and 4.1. When her OD readings fell below 4.0, the next three consecutive AGID tests collected over a three day period demonstrated the presence of a single IP band.

DISCUSSION

It had long been presumed that both the MAP AGID and MAP ELISA tests identified similar or corresponding antigens. The absence of an immunoprecipitation band in the face of a high MAP ELISA test reading had been largely ascribed to the amount of MAP antibody required for immunoprecipitation in contrast with that required for a positive ELISA test. Analysis of the double band phenomena challenges this line of reasoning. What is inferred is that the primary/first immunoprecipitation band is due to antigen-antibody complex elicited by an antigenic array common to mycobacteria that are derived from *Mycobacterium avium* (MAA) or *Mycobacterium hominissuis*⁵. It should have been anticipated that in the MAP's evolution from MAA, disease, the process would have produced pathogenic polymorphic genomic variants. IS900 MAP isolates are a cause of Johne's disease, but the sole cause of the clinical/pathological presentation termed Johne's disease. Fifteen point five percent of sera with a single IP band lacked antibodies that identified IS 900 MAP.

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