Improvements in the diagnosis of contagious bovine pleuropneumonia through the use of monoclonal antibodies

E. BROCCHI *, D. GAMBA *, F. POUMARAT **, J.L. MARTEL ** and F. DE SIMONE *

Summary: Seventeen monoclonal antibodies (MAbs) raised against Mycoplasma mycoides ssp. mycoides small colony type (Mmm SC), the causative agent of contagious bovine pleuropneumonia (CBPP), were partially characterised. Six MAbs recognising a main protein of 70 kDa and showing reciprocal competition were found to be specific for Mmm SC, while the other MAbs showed different patterns of reactivity with Mycoplasma spp. within the mycoides “cluster”.

Sandwich enzyme-linked immunosorbent assay (ELISA) performed with different combinations of MAbs enabled the detection of Mycoplasma of the mycoides cluster in pathology samples and the specific identification of Mmm SC from the initial culture passages, overcoming the necessity for difficult and time-consuming biochemical and growth inhibition assays.

A competitive ELISA based on Mmm SC-specific MAbs was able to measure anti-CBPP antibodies in cattle sera, and detected only the set of antibodies directed at Mmm SC-specific epitopes, thus avoiding the false-positive reactions occasionally observed with the complement fixation test.

KEYWORDS: Contagious bovine pleuropneumonia – Diagnosis – ELISA – Monoclonal antibodies – Mycoplasma mycoides “cluster”.

INTRODUCTION

In the past, contagious bovine pleuropneumonia (CBPP) caused by Mycoplasma mycoides ssp. mycoides small colony type (Mmm SC) was widespread in Europe, but since the beginning of this century European countries have generally been free of the disease. However, sporadic outbreaks of CBPP were reported several years ago in France and Spain, while Portugal has been affected since 1983 (5).

In November 1990, CBPP was also reported in Italy, where several outbreaks have been recorded (4). Control of the disease is primarily based on post-mortem inspection of lungs or pleural lesions at slaughterhouses and serological screening of cattle. Laboratory tests must be used to identify Mycoplasma in the infected organs or specific antibodies in cattle sera. The identification procedures for diagnosis of CBPP are time-
consuming and difficult, due to complex cross-reactions with other *Mycoplasma* within the *mycoides* “cluster” (8). The complement fixation test, normally used to detect antibodies, is well standardised; nevertheless, some false-negative and false-positive results are occasionally reported (7).

Monoclonal antibodies (MAbs) may be useful in increasing the reliability of immunoassays. For this reason, the authors produced MAbs against *Mmm SC* and investigated whether the use of such MAbs could improve the diagnosis of CBPP.

**MATERIALS AND METHODS**

*Mycoplasma strains*

The following international reference strains were used:

- *Mmm SC*: PG1
- *M. mycoides* ssp. *mycoides* “large colony”: YG
- *M. mycoides* ssp. *capri*: PG3
- *M. capricolum*: California Kid (CK)
- *M. sp.*, serogroup 7 of Leach: PG50.

In addition, a collection of field isolates of *Mmm SC* (nine from Europe and seven from Africa), other isolates classified in the *mycoides* cluster and reference strains of other bovine *Mycoplasma* were used (8).

**Antigen preparations**

*Mycoplasma* cultures (1,000 ml) grown in Difco-enriched liquid medium (6), aged 7-10 days, were harvested by centrifugation at 10,000 g for 30 min. The cultures were washed three times in phosphate-buffered saline (PBS) (pH 7.4) and re-suspended in a hundred-fold concentration. The protein concentration was determined using a commercial reagent; the antigens were stored at −70°C. When required, liquid medium cultures were inactivated by binary ethylenimine (1).

**Monoclonal antibodies**

BALB/c mice were primed subcutaneously with 100 µg of PG1 antigen in complete Freund's adjuvant and boosted intraperitoneally two months later with an identical dose of antigen in PBS. Three days later, 10^8 spleen cells of one mouse were collected and fused with 10^7 NSO myeloma cells using PEG 4000, following the method described by Galfré and Milstein (3). Fused cells, suspended in Dulbecco's modified Eagle medium, supplemented with hypoxanthine/aminopterin/thymidine and 20% fetal calf serum, were distributed over six microplates (200 µl per well). Growing colonies were observed in all wells; hybridoma supernatants were screened using an indirect enzyme-linked immunosorbent assay (ELISA); positive hybridomas were cloned by limiting dilution on a feeder layer of normal mouse spleen cells, in 96-well microplates. For production of ascitic fluid (3), 1 to 2 × 10^6 hybridoma cells were injected intraperitoneally in pristane-sensitised mice. MAbs were purified from ascites by ammonium sulphate precipitation and conjugated to horseradish peroxidase (HRPO) using a modification of the method described by Wilson and Nakane (9).
Enzyme-linked immunosorbent assays

**Indirect ELISA**

*Mycoplasma* antigen preparations (10 µg/ml) were adsorbed on microplates by incubation overnight at 4°C in 0.05 M sodium bicarbonate buffer (pH 9.6). Fifty µl samples of hybridoma supernatants and HRPO-conjugated goat anti-mouse immunoglobulin antibody were sequentially reacted for 1 h at 37°C. The diluting buffer consisted of PBS (pH 7.4) with 0.05% Tween 20 and 1% yeast extract. The substrate solution (orthophenylenediamine 0.5 mg per ml and 0.02% H$_2$O$_2$ in 50 mM phosphate citrate buffer, pH 5) was then added. After 10 min, the colorimetric reaction was stopped by addition of 2 N sulphuric acid; absorbance values were read at 492 nm. Fifty µl per well of reagent was used; three washings with PBS-Tween 20 were performed after each incubation.

**Competitive binding assays**

Fifty µl of hybridoma supernatant or ascitic fluid, at sequential dilutions, was incubated for 1 h at 37°C in PG1-coated microplates together with 25 µl of a concentration of labelled MAbs which had given an absorbance value of 1-1.5 in a previous titration. The colorimetric reaction was achieved as described above.

**Sandwich ELISA**

Microplates were coated overnight at 4°C with the trapping MAb (10 µg/ml). Sequential dilutions of a liquid medium culture of PG1 were then incubated for 1 h at 37°C, followed by MAb-HRPO conjugate (1 h at 37°C). Each conjugate was tested with each MAb as coating antibody. Diluting buffers, reagent volumes, washing and colorimetric reactions were as for the indirect ELISA.

**Competitive ELISA for antibody assessment**

Microplates were sensitised with 50 µl per well of capture MAb, as for the sandwich ELISA. After three washings, the selected dilution of PG1-inactivated liquid culture (50 µl per well) was incubated for 1 h at 37°C. Following three washings, three-fold dilutions of test serum (50 µl per well) were reacted for 1 h at 37°C with the trapped antigen, followed directly by the addition of 25 µl per well of the competitor labelled MAb. After incubation and washing as before, the colorimetric reaction was performed. The serum titre was calculated as the dilution which competed to a 50% level for the antigen binding. Predetermined concentrations of antigen and conjugated MAb giving an absorbance value of 1.5 were used.

**Western blotting**

The PG1 antigen preparation was separated on 10% reducing sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (10). Filters were blocked overnight with 2% bovine serum albumin in PBS and incubated for 1 h with hybridoma supernatants. After several washings and incubation with HRPO-conjugated goat anti-mouse immunoglobulin antibody, 4-chloro-1-naphthol and H$_2$O$_2$ were added as substrate.

**Membrane filtration dot immunobinding**

Membrane filtration (MF) dot immunobinding tests were performed following the method described by Poumarat and colleagues (8).
**Bovine samples**

Sera were examined from various Italian breeds of cattle. Some of the samples were taken from CBPP-free herds, while others were taken from herds known to be infected with CBPP.

Lung and lymph node samples were collected at slaughterhouses during the outbreaks of CBPP which have occurred in Italy since 1990. These were taken from animals which were either serologically positive for CBPP or showed clinical symptoms and CBPP lesions.

**Complement fixation test**

The complement fixation (CF) test was performed in microplates following the standard technique used in member countries of the European Community (2).

**Bacteriological isolation and identification**

Isolation and identification of *Mmm* SC field strains was performed following the methods described by Perreau (6).

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**RESULTS**

**Characterisation of monoclonal antibodies against PG1**

Seventeen MAbs reacting with *Mmm* SC were produced following one fusion experiment. Preliminary characterisation of the MAbs by Western blotting analyses, competitive binding assays and cross-reactivity tests was performed in order to select those MAbs most suitable for diagnostic use.

The results of Western blotting are shown in Figure 1. A group of seven MAbs showed an identical pattern of reactivity, recognising a main protein of approximately 70 kDa. Two MAbs (2D12 and 1E3) reacted reproducibly with several bands. One MAb (3A8) detected a 40 kDa protein, while two MAbs (6C4 and 7B8) showed a weak reaction with bands apparently overlapping with the 70 kDa band. The five remaining MAbs (not shown in Figure 1) were non-reactive, possibly due to low concentration or to the conformation of the target epitopes.

Competitive binding assays were performed among the seven MAbs which reacted with the 70 kDa protein, in order to study the relationships of the respective epitopes. The seven MAbs competed with each other to a high degree, while no other MAb was able to inhibit them (data not shown).

In order to further characterise these MAbs, they were tested against five antigenically-related *Mycoplasma* of the *mycoides* cluster, using an indirect ELISA (Table I). Six of the seven MAbs which reacted with the 70 kDa protein were found to be specific for PG1. Six other MAbs reacted with all the *mycoides* cluster strains. The seventh MAb which recognised the 70 kDa protein (3B2) showed greater reactivity with the homologous strain than with other *Mycoplasma*. The four remaining MAbs each showed a different pattern of reactivity with the heterologous strains. Three of the six PG1-specific MAbs were examined using MF dot immunobinding in order to check the
Western blot analyses

Mycoplasma mycoides ssp. mycoides cells (PG1) were separated under reducing conditions by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, then transferred to nitrocellulose and incubated with the monoclonal antibody shown. Arrows show positions of molecular weight markers.

specificity and antigenic stability of the target epitopes against a collection of the following known Mycoplasma:

- 16 field isolates of Mmm SC, originating from European and African countries
- 57 field-derived Mycoplasma strains, classified in the mycoides cluster
- 11 reference strains of other important species of bovine Mycoplasma or Acholeplasma (8).

The MAbs reacted with all the Mmm SC strains, but did not react with any other Mycoplasma.

Application of monoclonal antibodies to antigen detection

Since the aim of this work was the development of more convenient diagnostic tests, the possibility of using MAbs in a sandwich ELISA to detect specific antigens was evaluated. In a preliminary experiment, combinations of MAbs used either as coating or conjugated antibodies were tested for their ability to detect the reference strain PG1 in a liquid medium culture (Table II). Three of the pairs which successfully detected PG1 were selected as the most suitable for diagnostic applications. The first pair (6B6/3F3) combined a strong signal with absolute specificity for Mmm SC, while the other two pairs (6C4/7B8 and 3A8/3A8), although cross-reactive with the antigenically-related Mycoplasma of the mycoides cluster, showed the highest sensitivity.
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**Table I**

*Reactivity of monoclonal antibodies against five antigenically-related Mycoplasma of the mycoides cluster, studied by indirect enzyme-linked immunosorbent assay*

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Monoclonal antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2B3, 6B6, 3F3, 7H12, 4A5 and 5F2</strong></td>
<td>2B3, 6B6, 3F3, 7H12, 4A5 and 5F2</td>
</tr>
<tr>
<td><em><em>3B2</em>, 6C4, 1E3, 7B8, 2D12, 7D8 and 3A8</em>*</td>
<td>3B2*, 6C4, 1E3, 7B8, 2D12, 7D8 and 3A8</td>
</tr>
</tbody>
</table>

*M. mycoides ssp. mycoides*  
"small colony": PG1  
-  +  +  +  +  +  +

*M. mycoides ssp. mycoides*  
"large colony": YG  
-  -  +  +  +  ±  -

*M. mycoides sp. capri*: PG3  
-  -  +  +  -  -  ±

*M. capricolum*: California Kid (CK)  
-  +  -  -  ±  -

*M. sp. serogroup 7 of Leach*: PG5  
-  +  +  ±  ±  +

* reactivity with PG1 higher than with other antigens

**Table II**

*Sandwich enzyme-linked immunosorbent assay performed with pairs of monoclonal antibodies (MAbs)*

*MAb combinations were tested for their capacity to detect Mycoplasma mycoides ssp. mycoides SC (PG1) in a liquid culture*

<table>
<thead>
<tr>
<th>Coating MAb(s)</th>
<th>3F3*, 5F2 and 6B6</th>
<th>3B2</th>
<th>7D8</th>
<th>6C4</th>
<th>7B8</th>
<th>3A8</th>
<th>2D12</th>
</tr>
</thead>
<tbody>
<tr>
<td>6B6, 5F2, 3F3 and 2B3</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3B2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7D8</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>6C4</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>7B8</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3A8</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>1E3 and 2D12</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>5E12</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

* MAbs in bold-faced type were selected for diagnosis of contagious bovine pleuropneumonia

Absorbance values at 492 nm:  
+++ > 2
+++ ≥ 2
++ 1-2
+ 0.5-1
- < 0.5

The following MAbs were not tested: 4A5, 7H12, 1D4, 7D3 and 5A8
The three pairs of MAb s were then used in sandwich ELISAs to test a number of positive and negative samples, and the results were compared with those of traditional isolation and identification procedures. The following results were obtained (Table III):

- Samples not containing Mycoplasma of the mycoides cluster reacted negatively ($A_{492} < 0.1$) with all ELISAs, both as tissue extractions and as cultures.

- Samples from which the agent of CBPP was isolated reacted positively with all three pairs after only a few days of culture in liquid medium; all samples from the 54 outbreaks which have occurred in Italy in recent years exhibited this pattern. However, when examined directly as tissue extractions, these samples always reacted negatively with 6B6/3F3 (the PG1-specific pair of MAb s). All samples were positive with 6C4/7B8, except those containing so few antigens as to require a second culture passage for traditional detection by isolation of the agent. However, not all the samples identified as positive by 6C4/7B8 ELISA reacted with 3A8/3A8.

- A positive result in the sandwich ELISA performed on the pathological samples was occasionally combined with a negative result for both Mycoplasma growth and ELISA reactivity from the corresponding culture.

**Application of monoclonal antibodies to antibody assessment**

A competitive ELISA (C-ELISA) for the detection of specific antibodies was developed by modifying the sandwich ELISA performed with the Mmm SC-specific pair 6B6/3F3, as described above.

Assuming that the epitope target of 3F3 is immunogenic, a positive serum is expected to inhibit the binding of this MAb to the antigen. The reliability of this test was investigated by examining a large number of sera collected from both CBPP-free and CBPP-infected herds. Results were compared with those from the CF test (Fig. 2). The assay of 466 CF-negative sera from 20 healthy herds enabled the authors to establish an ELISA cut-off corresponding to 1:80. Of 1,551 sera from 10 infected herds, 392 (25.5%) indicated a positive result.

**Table III**

**Sandwich enzyme-linked immunosorbent assay performed on bovine samples using three monoclonal antibody (MAb) pairs for the diagnosis of contagious bovine pleuropneumonia (CBPP)**

<table>
<thead>
<tr>
<th>Strain of Mycoplasma in sample</th>
<th>MAb 6B6/3F3</th>
<th>MAb 6C4/7B8</th>
<th>MAb 3A8/3A8</th>
<th>Occurrence</th>
<th>Reactivity with tissue samples</th>
<th>Reactivity with isolates in liquid culture medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. mycoides ssp. mycoides SC</td>
<td>-(ic)</td>
<td>+</td>
<td>+</td>
<td>54 CBPP outbreaks</td>
<td>- (ic)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(ic)</td>
<td>(ic)</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>M. mycoides cluster</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Seldom observed</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>(non-viable?)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Negative samples*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Regularly observed</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

ic: occurrence of immunocomplexes

*possibly containing strains other than the mycoides cluster
FIG. 2

Results of competition enzyme-linked immunosorbent assay (ELISA) and the complement fixation (CF) test on cattle sera

Sera collected from herds infected with contagious bovine pleuropneumonia (CBPP) and CBPP-free herds are divided according to negativity or positivity by CF. The frequency of ELISA titres observed is shown.
reacted positively by both CF and C-ELISA, while 1,062 (68%) were negative with both tests; 84 (5.5%) were identified as positive by C-ELISA and negative by CF and, conversely, 15 (1%) sera positive by CF were negative using C-ELISA.

The specificity of C-ELISA was studied by examining a sample of 375 sera from seven herds in which, although CBPP was not demonstrated, a total of 40 sera with CF titres ranging from 1:10 \( (n = 24) \) to 1:1,280 \( (n = 1) \) were recorded. All sera gave negative results with C-ELISA.

**DISCUSSION**

The characterisation of MAbs enabled the identification of seven MAbs directed against a main protein of 70 kDa. Reciprocal competition was observed, suggesting that these MAbs recognise overlapping epitopes. Moreover, since six of the MAbs reacted specifically with \( M_{mm} \) SC and three recognised all of the field strains tested (even those originating from very different geographical areas), these MAbs must identify an antigenic domain which may be considered extremely stable and an exclusive marker for this *Mycoplasma* species.

A MAb-based sandwich ELISA was employed for identification of \( M_{mm} \) SC in pathological tissues. Varying results were obtained depending on the particular monoclonal pair and biological material (tissue extractions or first culture passages) used.

MAb pairs 3A8/3A8 and 6C4/7B8 were generally able to detect \( M_{mm} \) SC when reacted directly with extractions of pathological tissues and with the culture media derived from them. However, these pairs react with all members of the mycoides cluster, whereas a specific reactivity with \( M_{mm} \) SC was achieved using 6B6/3F3. With the latter, it was possible to detect \( M_{mm} \) SC only after culture of infected tissue extractions, probably due to inadequate sensitivity or the occurrence of immunocomplexes (ic). The hypothesis that ic were responsible is strengthened by the demonstration of antibodies in positive sera able to inhibit the reaction of 6B6/3F3 with the antigen. On the other hand, MAb pair 6C4/7B8 must identify non-immunogenic epitopes, since the occurrence of ic did not interfere with the detection of antigen by this pair. The MAb pair 3A8/3A8 may or may not react with positive samples, depending on whether immunocomplexed antigen is present alone or in combination with antibody-free *Mycoplasma*. On rare occasions, a positive sandwich ELISA was achieved with tissue extractions without subsequent isolation of the specific agent. Since these samples were collected from animals showing CBPP-specific lesions and/or seroreactivity, failures could be due to the presence of non-viable *Mycoplasma* of the mycoides cluster or, possibly, overgrowth in the culture of *Mycoplasma* other than \( M_{mm} \) SC but often associated with this strain.

The comparison of C-ELISA and CF for the detection of specific antibodies in sera from infected herds showed that the two tests correlated in 93.5% of cases; C-ELISA detected 5.5% more seropositive animals than CF, while only 1% of CF-positive sera were not detected by C-ELISA.

With regard to specificity, the authors found that positive results occasionally recorded by CF in sera from herds where the disease has never been demonstrated, were not confirmed by C-ELISA thus indicating that C-ELISA may be the more specific test.
The inactivated antigens used to perform C-ELISA may be either unpurified liquid cultures or purified cells. However, when unpurified cultures were used, variations were observed in serum titres with different batches of antigen preparation. Work is in progress to study the origin of these variations.

CONCLUSIONS

The authors demonstrated that the use of MAbs can considerably improve diagnostic techniques for CBPP.

A set of very simple sandwich ELISAs, performed with selected MAbs, were standardised. With these tests, it is possible to detect Mycoplasma of the mycoides cluster directly in biological samples and to unequivocally identify Mmm SC after culture for only a few days, thus avoiding tedious and time-consuming biochemical and growth inhibition assays.

Furthermore, satisfactory results were achieved using a MAb-based C-ELISA for the detection of specific antibodies. This test detects a set of antibodies directed against Mmm SC-specific epitopes, avoiding the false-positive reactions occasionally observed with CF, and also shows a high level of sensitivity.

The authors thank Drs S. Giovannini and P. Alatri for CF test serology, and Dr M. Boldini for biochemical and culture characterisation of field isolates.

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Résumé : Dix-sept anticorps monoclonaux dirigés contre Mycoplasma mycoides ssp. mycoides «small colony» (Mmm SC), l'agent responsable de la péripneumonie contagieuse bovine, ont fait l'objet d'une caractérisation partielle. Six anticorps monoclonaux, qui reconnaissent une protéine principale de 70 kDa, et en compétition réciproque, se sont avérés spécifiques de Mmm SC, tandis que les autres anticorps ont présenté différents profils de réactivité vis-à-vis de Mycoplasma spp. au sein du groupe «cluster» mycoides.

Un test enzyme-linked immunosorbent assay (ELISA) «sandwich», réalisé avec différentes combinaisons d'anticorps monoclonaux, permet la détection des mycoplasmes du groupe mycoides dans des prélèvements pathologiques et l'identification spécifique de Mmm SC dès les premiers passages en culture cellulaire, si bien qu'on peut se dispenser désormais des tests biochimiques et d'inhibition de croissance, à la fois difficiles et longs à mettre en œuvre.

Grâce à un test de compétition ELISA, utilisant des anticorps monoclonaux spécifiques de Mmm SC, on peut titrer les anticorps de la péripneumonie contagieuse bovine dans les sérum de bovins et ne détecter que le groupe d'anticorps dirigés contre les épitopes antigéniques spécifiques de Mmm SC. Cette technique permet ainsi d'éviter les erreurs par excès parfois observées lors des épreuves de fixation du complément.

* * *


Resumen: Diecisiete anticuerpos monoclonales dirigidos contra Mycoplasma mycoides ssp. mycoides en pequeña colonia (Mmm SC, small colony), que es el agente causal de la pleuroneumonía contagiosa bovina, fueron objeto de una caracterización molecular parcial.

Seis anticuerpos monoclonales, que reconocieron una proteína principal de 70 kDa y presentaron una competición recíproca, se mostraron específicos de Mmm SC, mientras que otros anticuerpos manifestaron distintos tipos de reactividad respecto de Mycoplasma spp. dentro del «racimo» mycoides.

Una prueba sandwich inmunoenzimática (ELISA), realizada con distintas combinaciones de anticuerpos monoclonales, permite la detección de los micoplasmas del racimo mycoides en muestras patológicas y la identificación específica de Mmm SC desde las primeras aplicaciones en cultivos, de manera que se puede prescindir actualmente de las pruebas bioquímicas y de inhibición de crecimiento, difíciles y lentas.

Por otra parte, una prueba de competición ELISA basada en anticuerpos monoclonales de Mmm SC puede medir los anticuerpos de la pleuroneumonía contagiosa bovina en los sueros de bovinos y detectar sólo el conjunto de los anticuerpos dirigidos contra los epitopios de Mmm SC. Esta técnica permite así corregir errores por falsas reacciones positivas observados con las pruebas de fijación del complemento.


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REFERENCES


