

## Rapid Detection of Bovine Mycoplasma Antigens by Counterimmunoelectrophoresis

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Twelve reference strains of mycoplasma and acholeplasma previously reported to have been recovered from cattle were tested against hyperimmune rabbit serum by counterimmunoelectrophoresis. This technique detected antigen by the formation of precipitin lines with antibody within 1 h and promises to be a useful technique for detecting and identifying mycoplasma isolates in either pure or mixed cultures.

Mycoplasma isolates are presently classified on the basis of cultural, biochemical, and antigenic characters (8). The antigenic relationships of bovine mycoplasmatales have been examined by means of metabolic inhibition (3), immunodiffusion, growth precipitation, and growth inhibition (2). However, these techniques require at least 12 h to 8 days for the detection of mycoplasma antigens or antibodies. Therefore, a faster, reliable, serological method was sought.

Counterimmunoelectrophoresis is widely used in many laboratories to detect hepatitis-associated antigen (4) and other viral and bacterial antigens and antibodies. We applied this technique for the rapid detection of bovine mycoplasmatales antigens with hyperimmune rabbit serum as the source of antiserum, and favorable results were obtained.

The strains used in the production of antisera are listed in Table 1. Strains B12PA and B142P were supplied by J. L. Al-Aubaidi (1), and the other strains were supplied by the National Collection of Type Cultures (London, England). The antigens and hyperimmune antisera were prepared according to the methods of Langford and Leach (7). The antigens used in the counterimmunoelectrophoresis were suspended to a final protein concentration of 10 mg/ml.

The counterimmunoelectrophoresis tests were carried out on glass plates (75 by 50 mm) covered with 10 ml of 1% Ionagar with 0.02% sodium azide in tris(hydroxymethyl)aminomethane-barbital-sodium barbital buffer (pH 8.8, ionic strength 0.025). Three sets of parallel rows of wells, 3 mm in diameter, spaced 7 mm apart were cut in the agar. The antigen was placed in the wells on the cathode side, and antiserum

was placed in the anode wells. Electrophoresis was carried out at room temperature at 250 V.

Figure 1 illustrates a pattern of counterimmunoelectrophoresis. A single, weak precipitin line generally became visible within 15 min of starting electrophoresis; however, stronger or multiple lines, or both, appeared at the termination of electrophoresis, usually 1 h. Table 1 shows the reactions of antigens of 12 reference strains with homologous and heterologous antisera. All of the antigens tested reacted with their homologous antisera: Antigens prepared from *Mycoplasma bovis*, *Acholeplasma laidlawii*, *M. bovirhinis*, and *M. bovum* cultures reacted only with their homologous antiserum. However, *A. modicum* and *Mycoplasma* sp. Group 7 (Leach) antigens cross-reacted with anti-*M. bovis* serum, but the reverse reactions did not occur. Similarly, such one-way cross-reactions were observed with *M. agalactiae* var. *bovis* antigen against anti-*M. bovirhinis* serum; with *A. modicum* antigen with anti-Leach Group 7 antiserum; and with Leach Group 7 with anti-*M. alkalescens* antiserum. These one-way cross-reactions may be explained as follows. (i) *M. bovis* may contain common antigens with *A. modicum* and Leach Group 7 in sufficient amounts to elicit an antibody production in rabbits. However, the antigens are not sufficient in quantity to react with the heterologous antisera, or (ii) *A. modicum* or Leach Group 7 have antigen(s) which react with anti-*M. bovis* antiserum, but the antigen(s) do not elicit enough antibody response to react with *M. bovis* antigen. Thus, the concentration of cross-reacting antigen and the amount of cross-reacting antibody might determine the

TABLE 1. Cross-reaction of bovine mycoplasma antigens with hyperimmune rabbit sera by counterimmunoelectrophoresis

Species or subgroup	Strain or reference no.	Antiserum <sup>a</sup>											
		10122	10116	10118	10131	10134	10133	10135	10125	10129	10141	B12PA	B142P
<i>M. bovis</i>	10122	+ <sup>b</sup>	-	-	-	-	-	-	-	-	-	-	-
<i>A. laidlawii</i>	10116	- <sup>c</sup>	+	-	-	-	-	-	-	-	-	-	-
<i>M. bovirhinis</i>	10118	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> var. <i>bovis</i>	10131	-	-	+	+	-	-	-	-	-	-	-	-
<i>A. modicum</i>	10134	+	-	-	-	+	+	-	-	-	-	-	-
<i>Mycoplasma</i> sp. Group 7 (Leach)	10133	+	-	-	-	-	+	+	-	-	-	-	-
<i>M. alkalescens</i>	10135	-	-	-	-	-	-	+	-	- <sup>d</sup> , + <sup>d</sup>	-	+	+
<i>M. dispar</i>	10125	-	-	-	+	- <sup>e</sup>	-	-	+	-	-	-	-
<i>M. arginini</i>	10129	-	-	-	-	-	-	+	-	+, +	-	+	+
<i>M. bovoculi</i>	10141	-	-	-	-	-	-	-	-	-	+	-	-
<i>M. gateae</i>	B12PA	-	-	-	-	-	-	+	-	+, +	-	+	+
<i>M. gallinarum</i>	B142P	-	-	-	-	-	-	+	-	- <sup>d</sup> , + <sup>d</sup>	-	+	+

<sup>a</sup> Antisera were prepared in rabbits using organisms grown in Difco PPLO broth supplemented with boiled yeast extract (10% vol/vol of a 25% aqueous extract) and 20% rabbit serum, except for *M. dispar*, which was grown in the media described by Gourlay and Leach (5) in which the fetal calf serum was replaced by rabbit serum. Serological test antigens were grown in the same media supplemented with horse serum and fetal calf serum, respectively.

<sup>b</sup> (+), Positive reaction.

<sup>c</sup> (-), Negative reaction.

<sup>d</sup> Anti-*M. arginini* serum (supplied by H. Erno) cross-reacted with other arginine-positive, glucose-negative strains (*M. alkalescens*, *M. gateae*, and *M. gallinarum*), whereas our initial serum reacted only with homologous strain *M. arginini*. However, serum collected after further immunization reacted with *M. arginini* and *M. gateae* (see text).

<sup>e</sup> (+), Against whole cell antisera; (-), against cell membrane antisera (see text).

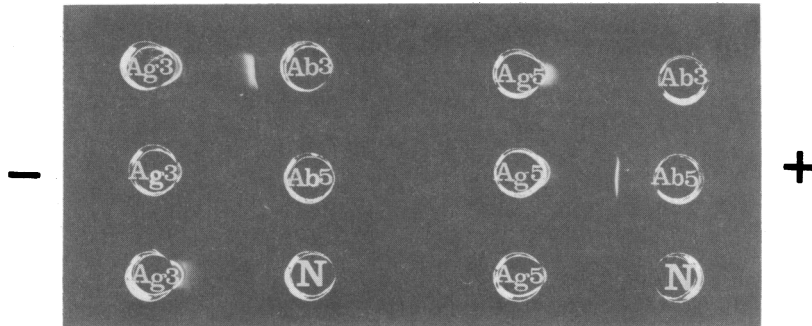


FIG. 1. A pattern of counterimmunoelectrophoresis: *A. laidlawii* (Ag3) and *M. agalactiae* var. *bovis* (Ag5) antigens were reacted with anti-*A. laidlawii* (Ab3), anti-*M. agalactiae* var. *bovis* (Ab5), and with normal rabbit serum (N); (+) anode; (-) cathode.

one-way or reciprocal cross-reaction. It is interesting to note that *M. dispar* antigen reacted with anti-*M. agalactiae* var. *bovis* serum when the antiserum was prepared by immunizing the rabbit with whole cell antigen, whereas when the antiserum was prepared by immunizing the rabbit with a cell membrane antigen preparation from *M. agalactiae* var. *bovis*, *M. dispar* antigen did not react with it. This result probably indicates the specificity of the membrane preparation.

Several workers have studied cross-reactions between mycoplasma species using double immunodiffusion (2, 6). Kenny (6) observed recip-

rocal cross-reaction among *M. gallinarum*, *M. arginini*, and *M. gateae*. These organisms are the arginine-positive, glucose-negative species. On the other hand, Erno and Jurmanova (2) observed reciprocal cross-reaction among *M. arginini*, *M. gateae*, and the other arginine-positive, glucose-negative species *M. alkalescens*. However, these three organisms did not cross-react with anti-*M. gallinarum*, and *M. gallinarum* did not react with anti-*M. gateae*, which observations are contrary to the findings of this study and of Kenny (6). In the present study, reciprocal cross-reactions were observed among *M. alkalescens*, *M. arginini*, *M. gateae*,

and *M. gallinarum* (Table 1). It was observed that when three different rabbit antisera against *M. arginini* were reacted with these four arginine-positive, glucose-negative species, our first serum reacted only with the homologous strain. After further immunization of the rabbit, the serum reacted more intensely with the homologous strain and also cross-reacted with *M. gateae*. However, anti-*M. arginini* serum (supplied by H. Erno) reacted with all of these four strains. This shows that the amount of antibody is a factor in determining one-way cross-reaction or reciprocal cross-reaction.

In the present experiment, it was generally observed that the homologous reactions were stronger and usually consisted of more lines than were observed in the heterologous reactions.

In the counterimmunoelectrophoresis, antigen which shows a negative charge and migrates anodally will react with antiserum. Antigen which does not migrate or migrates cathodally will not show precipitin lines, contrary to double immunodiffusion.

It was demonstrated that counterimmunoelectrophoresis is a more sensitive test than growth inhibition and growth precipitation tests. Anti-*M. agalactiae* var. *bovis* rabbit antiserum was titrated using these three tests. By the counterimmunoelectrophoresis test, antibody was detected up to serum dilution of 1:40, whereas by growth inhibition and by growth precipitation tests, the antibody was detected only up to serum dilution of 1:5.

This counterimmunoelectrophoresis is simple and rapid; therefore, it could be utilized as a preliminary method for detecting and identifying bovine mycoplasmas recovered from biological specimens either in pure or in mixed cultures. It may be used in conjunction with or as a replacement for other cultural, biochemical, or

serological tests used. In a preliminary experiment, mycoplasma isolates were cultured in 25 ml of broth media, and the cultures were concentrated by centrifugation 2 to 3 days later. The pellets were suspended in saline and reacted against a battery of hyperimmune, rabbit anti-mycoplasma typing sera in counterimmunoelectrophoresis tests. The results of this experiment indicate that such a test is feasible. The counterimmunoelectrophoresis test also promises to be a useful confirmatory test for classifying mycoplasma isolates.

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