

Rapid Plate Agglutination Procedure for Serotyping *Pasteurella haemolytica*

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Received for publication 2 September 1977

A specific, rapid plate agglutination procedure for serotyping *Pasteurella haemolytica* was performed. The procedure did not require special antigen preparation and yielded essentially the same results as the indirect hemagglutination procedure.

Pasteurella haemolytica is associated with several diseases of domestic animals, particularly pneumonia in cattle and pneumonia and septicemia in sheep. In addition to being isolated from diseased animal tissues, *P. haemolytica* is frequently isolated from the nasal passages of healthy cattle and sheep. Biberstein et al. (2) divided the species into 10 serotypes with an indirect hemagglutination (IHA) procedure; later, this was expanded to 12 serotypes (1, 4). Smith (10, 11) divided the species into biotypes A and T on the basis of both colonial morphology and fermentation of arabinose and trehalose. Later, all 12 serotypes were grouped under the biotypes A and T (1, 4). For identifying isolates or for epidemiological purposes, *P. haemolytica* is usually serotyped by the IHA procedure, and then its biotype designation is assigned according to its serotype (4, 7-9, 12, 14, 15).

The IHA procedure is a specific and sensitive method of serotyping *P. haemolytica*. However, one must prepare antigen from each culture to be serotyped and adsorb the antigen to erythrocytes (RBC).

This report presents a rapid plate agglutination (RPA) procedure for serotyping *P. haemolytica*, which yields essentially the same results as the IHA procedure.

MATERIALS AND METHODS

Cultures of known serotype. Lyophilized strains of 12 *P. haemolytica* serotypes were received from E. L. Biberstein, University of California, Davis, California, including the type 12 strain (S209) isolated by D. A. Thompson, Moredun Institute, Gilmerton, Edinburgh, Scotland. The strains were suspended in tryptose broth (Difco Laboratories, Detroit, Mich.) and grown on blood agar base (Difco) supplemented with 5% defibrinated bovine blood.

Typing antisera. Antiserum to each of the 12 serotype strains was prepared in rabbits as described by Biberstein et al. (2) except that Formalin-killed cells were used. Antisera were stored at -20°C.

IHA procedure. The IHA procedure described by Biberstein et al. (2) was adapted to a microtiter system in which 0.05 ml of diluted serum and 0.05 ml of sensitized bovine RBC were used. Tests were read after a 2-h incubation period at 37°C followed by overnight incubation at room temperature. The last serum dilution at which agglutination occurred was considered the end point, and titers were expressed as the reciprocal of the serum dilution.

RPA procedure. A drop of antiserum (approximately 10 μ l) was placed on a clean glass surface, and then a small amount of *P. haemolytica* colony from blood agar was picked up on an inoculating needle and mixed with the serum. A strong positive reaction in the form of clumping and clearing occurred as the mixture was stirred with the needle. Negative reactions remained turbid. Strongly positive sera were diluted in 0.85% NaCl containing 1:10,000 thimerosal. Reactions were run at room temperature.

RPA titration of typing antisera. Sera were serially diluted twofold in 0.85% NaCl containing 1:10,000 thimerosal, and 10 μ l of each dilution was tested. Titers were expressed as the reciprocal of the last serum dilution at which positive agglutination occurred.

Field isolates of *P. haemolytica*. Bovine and ovine field isolates of *P. haemolytica* were serotyped by both procedures. Of these, 38 were bovine isolates from Iowa, Nebraska, Minnesota, Colorado, Illinois, and Maryland; 23 were ovine isolates from Maryland, Montana, California, and Scotland. All of the above isolates had been lyophilized. In addition, 46 were selected fresh bovine isolates collected by nasal swabs from cattle originating in Tennessee.

RESULTS

Specificity of the RPA procedure. The 12 serotype strains were reacted with homologous and heterologous rabbit antisera by the RPA procedure (Table 1). Antisera, both undiluted and diluted to a concentration that reacted in 5 to 10 s, were used. The serotype strains reacted specifically with their homologous antisera. One major cross-reaction occurred as serotype 7 reacted with antiserum 12. Minor cross-reactions

occurred with serotype 4 and undiluted antiserum 10 and with serotype 3 and diluted antiserum 10. All cross-reactions were quantitated by titration of the antisera against the cross-reacting serotypes (Table 2). All such cross-reactions were one way, and, as can be seen by comparing titers in Tables 2 and 3, homologous titers were higher than heterologous ones. The one exception was antiserum 12, which gave the same titer with both serotypes 7 and 12. A one-way cross-reaction also occurred between serotype 7 and antiserum 12 by the IHA procedure (Table 2). However, antiserum 12 against serotype 12 gave a much higher titer than with serotype 7 (1:640 versus 1:20).

Sensitivity of the RPA procedure. Antisera titrated higher with the IHA procedure than with the RPA procedure (Table 3). Ratios of IHA to RPA titers ranged from 1.25 to 160, with most being from 10 to 80 (Table 3). Strong, positive RPA reactions with undiluted serum usually occurred within 5 to 10 s during mixing. As sera were diluted, reactions took more time

to begin and to reach completion (Table 3). Lesser degrees of agglutination (smaller clumps, less clearing) were observed only in very low-titrated or diluted serums.

Serotyping field isolates by RPA and IHA. Of the 103 isolates, both lyophilized and freshly isolated, 95 were of the same serotype as determined by both methods and 5 were untypable (Table 4). Three isolates were typable by RPA but not by IHA. There was no apparent difference in the reactions of long-term-stored cultures by either serotyping method as compared with the reactions of freshly isolated cultures. The bovine RBC sensitized with the type 12 isolate were hemolyzed in the IHA reaction, making interpretation difficult. Hemolysis occurred even though the antiserum had been heat inactivated and adsorbed with bovine RBC. None of the isolates was serotype 6 or 10.

DISCUSSION

The RPA procedure is a rapid, specific method for serotyping *P. haemolytica* colonies

TABLE 1. RPA reactions of rabbit antisera against heterologous and homologous *P. haemolytica* serotypes

Serotype no.	Reaction of type antiserum											
	1	2	3	4	5	6	7	8	9	10	11	12
	8 ^a	-2 ^b	8	32	2	4	64	32	1	16	4	16
1	+	-	-	-	-	-	-	-	-	-	-	-
2	-	+	-	-	-	-	-	-	-	-	-	-
3	-	-	+	-	-	-	-	-	-	-	-	-
4	-	-	-	+	-	-	-	-	-	-	-	-
5	-	-	-	-	+	-	-	-	-	-	-	-
6	-	-	-	-	-	+	-	-	-	-	-	-
7	-	-	-	-	-	-	+	-	-	-	-	+
8	-	-	-	-	-	-	-	+	-	-	-	-
9	-	-	-	-	-	-	-	-	+	-	-	-
10	-	-	-	-	-	-	-	-	-	+	-	-
11	-	-	-	-	-	-	-	-	-	-	+	-
12	-	-	-	-	-	-	-	-	-	-	-	+

^a Reciprocal of serum concentration used for the RPA procedure.

^b Antiserum 2 was concentrated twofold.

^c Reaction was slight but never became positive.

TABLE 2. RPA and IHA titers of rabbit antisera against cross-reacting heterologous *P. haemolytica* serotypes

Serotype no.	Type antiserum no.	RPA reaction at serum dilution of:								IHA titer
		1	2	4	8	16	32	64	128	
7	12	+	+	+	+	+	+	-	-	20
12	7	-	-	-	-	-	-	-	-	0
3	10	-	sl(30) ^a	sl(25)	sl(25)	sl(45)	sl(45)	sl(45)	-	0
10	3	-	-	-	-	-	-	-	-	0
4	10	+	+	+	+	sl(30)	sl(30)	sl(30)	sl(30)	0
10	4	-	-	-	-	-	-	-	-	0

^a Time in seconds in which a slight reaction began but did not become positive with more stirring. sl, Slight reaction.

directly from a blood agar plate. The procedure works well with long-term-stored isolates as well as with those freshly cultured from clinical material. The procedure yields essentially the same information as the IHA procedure (3) and requires no special antigen preparation.

None of the cross-reactions led to confusion, nor did they detract from the usefulness of the procedure. The major cross-reaction, occurring between serotype 7 and antiserum 12, was strictly one way. Only serotype 7 reacted with antiserum 7, whereas both serotypes 7 and 12 reacted with antiserum 12. This particular cross-reaction was not a peculiarity of the RPA procedure because it was also detected by the IHA

procedure when higher concentrations of antiserum 12 were tested. Minor cross-reactions between serotypes 3 and 4 with antiserum 10 were very weak and also strictly one way.

The three field isolates typable by RPA but not with IHA were those to which the typing antisera were the weakest. However, since five isolates were untypable by both methods, the RPA procedures probably will not be useful in placing many untypable isolates into existing serotype groups.

A distinct advantage of the RPA procedure is that RBC are not required. Certain field isolates, such as the serotype 12 isolate, may cause hemolysis even when heat inactivated, RBC-adsorbed antiserum is used. Such hemolyzed IHA reactions are difficult to interpret. Hemolysis did not occur when the same antiserum was reacted with S209, the type 12 serotype strain.

Reactions obtained with the RPA procedure depend upon the diffusible surface material (S-substance) described by Biberstein et al. (2, 3); this is also the basis for typing by IHA. Correlation between the two procedures (97%) is strong evidence that both detect the same antigens. Widely distributed and less specific somatic antigens may be involved in the one-way cross-reactions between type 7 cells and type 12 antiserum and in the minor reactions involving cells of types 3 and 4 and type 10 antiserum. Biberstein et al. (2) recognized a low-order reaction with types 4 and 10, demonstrable with the IHA method, but they were uncertain as to the mechanism involved.

Some information is available on the serotype distribution of *P. haemolytica* in the United States, Canada, and Great Britain. All 12 serotypes plus untypable isolates have been isolated from sheep (2, 7-9, 12). The predominant sero-

TABLE 3. RPA and IHA titers of rabbit antisera against homologous *P. haemolytica* serotype strains

Sero-type strain	Sero-type no.	RPA titer	RPA re-action time ^a	IHA titer ^b	IHA titer/RPA titer
I29	1	16	15, 20	320	20
J28	2	2	60, 90	20	10
863	3	32	30, 45	80	2.5
S	4	128	30, 45	160	1.25
G13	5	4	20, 30	40	10
A30	6	8	15, 25	640	80
H1	7	256	15, 30	2,560	10
H21	8	128	15, 25	2,560	20
B1	9	2	10, 20	320	160
JF2	10	64	20, 30	160	2.5
KC282	11	16	30, 40	320	20
S209	12	32	15, 25	640	20

^a The first number denotes the time in seconds in which the reaction began; the second denotes time of completion. These times refer to the dilution of serum listed under the column RPA titer.

^b Titer determined after overnight incubation at room temperature.

TABLE 4. Serotypes of *P. haemolytica* field isolates by RPA and IHA procedures

Host	Tissue	No. of <i>P. haemolytica</i> of serotype:													
		1	2	3	4	5	6	7	8	9	10	11	12	UT ^a	
Lyophilized cultures															
Bovine	Nasal passages	30	1	0	0	0	0	0	0	0	0	0	0	0	0
	Lung	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	Trachea	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Ovine	Nasal passages	1	2	0	1	0	0	1	4	0	0	1	1	0	0
	Lung	0	1	1	1	0	0	1	0	0	0	0	0	0	0
	Udder	0	1 ^b	0	0	2	0	2	0	0	0	0	0	0	0
	Blood	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Fresh cultures															
Bovine	Nasal passages	21	19 ^b	0	0	0	0	0	0	1 ^b	0	0	0	5	
No. positive by both procedures		57	22	1	2	2	0	5	4	0	0	1	1	0	
No. positive by RPA only		0	2	0	0	0	0	0	0	1	0	0	0	0	
Total		57	24	1	2	2	0	5	4	1	0	1	1	5	

^a UT, Untypable.

^b One isolate in each group was positive with the RPA but not the IHA procedure.

type isolated from cattle is type 1, along with some type 2 and untypable isolates (2, 4-6, 13-15). However, serotypes 11 (13-15), 7 (14, 15), and 3 and 6 (15) have been isolated, as well as serotype 9 (this paper). In a survey of cattle, sheep, and goat isolates in Kenya (A. U. Mwangota, M.S. thesis, University of Nairobi, Nairobi, Kenya, 1975), all serotypes were isolated from sheep and goats, whereas mostly type 1 and untypable isolates, some type 2, and one type 4 were isolated from cattle.

On the basis of the above information, all bovine isolates should be reacted against type 1 antiserum, and then the negative isolates should be reacted against type 2 antiserum. Those remaining negative will probably be untypable, but some may fall into one of the other established serotypes. If the RPA procedure were widely used in diagnostic laboratories, more extensive information on serotype distribution of *P. haemolytica* could be easily gained.

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