

Antigen *Burkholderia mallei* (*Pseudomonas mallei*) for BRC

Composition: Clear, yellowish extract of the *Burkholderia mallei* (*Pseudomonas mallei*) culture, preserved by phenol.

Antigen is calibrated by the standard positive and negative serum and its capacity is modified so that it may grant the visible reaction with the positive serum (in the working dilution) containing the minimum specific antibody quantities, dilution 1:5. The antigen of working dilution may not show false positive reactions with the negative serum.

Indication: For serologic diagnostics of glanders, using binding reaction of complement (BRC).

Testing: The concentrated Meyer solution is used for dilutions during the test; the Meyer solution is diluted before use by the sterile re-distilled water in the 1+4 ratio.

Titration of hemolytic amboceptor: We prepare the basic dilution 1:1000 and then the auxiliary series of dilutions up to 1:10.000 or even higher, based on the basic dilution. Starting by the highest dilution, we pipette 0.2 ml of the amboceptor of the relevant dilution into the titration set test tubes. Then 0.2 ml of 2% suspension of ram's blood cells, 0.4 ml of the Meyer solution and 0.2 ml of the complement (diluted 1:20) are added into all test tubes. At the same time the series is extended by the three test tubes of standard controls: amboceptor, complement and blood cells. Everything is shaken thoroughly and inserted immediately into the water bath 37 °C. The result is read after 30 minutes of incubation.

Auxiliary amboceptor dilution

Test tube No.:	1	2	3	4	5	6	7	8	9	10
Resultant amboceptor dilution	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
Amboceptor 1:1000 in ml	1	0,5	0,5	0,5	0,5	0,25	0,25	0,25	0,25	0,25
Meyer solution ml	-	0,5	1,0	1,5	2,0	1,25	1,5	1,75	2,0	2,25

Amboceptor dilution	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000	K1	K2	K3
Doses of individ. dilutions in ml	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
Meyer solution in ml	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,6	0,6	0,8
Guinea pig's complement 1:20 in ml	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	-	0,2	-
2% suspension of ram's blood cells in ml	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2

20 min. incubation in the water bath at the temperature of 37 °C.

K1 – amboceptor control; K2 – complement control; K3 – blood cell control

Evaluation of the reaction:

+++ - complete suppression of hemolysis /+4/
 ++, +, - suppression of hemolysis with descending intensity /+3, +2, +1/
 - 100% hemolysis - full blood cell dissolving

The minimum hemolytic dose of the amboceptor (MHD) is contained in 0.2 ml of the highest amboceptor dilution, which in case of complement excess dissolves 0.2 ml of the used ram's blood cell suspension completely (the last fully cleared test tube). For blood cell sensitization the amboceptor is diluted so that it may contain 4 MHD in 0.2 ml. The hemolytic system is prepared by mixing 2% of blood cell suspension with the identical amboceptor volume in the working dose of 4 MHD.

Complement titration: Diluted close before use by the cooled diluting solution and stored in the fridge. Based on the basic dilution 1:25. Complement titration is carried out in conformity with the Table below.

Test tube No.	1	2	3	4	5	6	7	8	9	10	K1	K2	K3
Complement 1 + 24	0,20	0,18	0,16	0,14	0,12	0,10	0,08	0,06	0,04	0,02	0,20	-	-
Meyer solution	0,40	0,42	0,44	0,46	0,48	0,50	0,52	0,54	0,56	0,58	0,60	0,60	0,80
Hem. amboceptor 4 MHD	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20	-	0,20	-
2% ram's blood cells	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20
Incubation in the water bath for 30 minutes at 37 °C													
% complement	4%	3,6	3,2	2,8	2,4	2,0	1,6	1,2	0,8	0,4	++++	++++	++++

The result is read after 30 min. incubation in the water bath at 37 °C. The complement titre is the least complement quantity, able to dissolve all hemolytic system blood cells under the set conditions. The complement at the dose of 1.5 MHD is used as the working dilution (e.g. complement titre is 2.4% and the working dilution is 3.6%).

Controls are as follows:

K1 - complement control; K2 - amboceptor control; K3 - blood cell control

The examined horse serum must be inactivated prior to start titration in the water bath at 60 °C for 30 min. Serum of the equidae other than the horse ones must be inactivated in the water bath at 63 °C for 35 min. The examined serum is diluted 1 + 4 (titre 1:5), 1 + 9 (titre 1:10), etc. To the prepared serum dilutions the identical antigen quantity is added (in its working dilution), then the identical part of the titrated complement (1.5 MHD) is added - the whole volume is let incubate in the water bath at 37 °C for 30 min. Then the twice as high volume of the hemolytic system is added (the identical part of 2% ram's blood cells and hemolytic amboceptor - 4 MHD). Incubation in the water bath for 30 minutes at 37 °C follows. The test for examined serum anticomplementarity is carried out by the same method. In this series the antigen is replaced by the Meyer diluting solution. Check of the guinea pig's complement, hemolytic system and used antigen with the control positive serum in its working dilution is the integral part of titration.

Tested serum	Dilution	1:5	1:10	1:20	1:40	-	-	Serum of working d.
Dose	0,2	0,2	0,2	0,2	-	-	0,2	-
Antigen in working dilution	0,2	0,2	0,2	0,2	-	-	0,2	0,2
Complement 1.5 MHD	0,2	0,2	0,2	0,2	-	-	0,2	0,2
Incubation at 37 °C for 30 minutes								
Hemolytic system	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4
Mayer solution				0,4	0,6			0,2
Incubation at 37 °C for 30 minutes								

Test assessment: Evaluation of reaction:

+++ - full prevention of hemolysis
 ++, +, - prevention of hemolysis with descending intensity
 - complete blood cell dissolving

Pack: 1×10 ml, 5×10 ml

Storage: In a dry and dark room at the temperature from 2 to 8 °C.

Container disposal: Disposal of the containers and any medicinal product residues must be carried out in conformity with the valid regulations.

Shelf life: 2 years

Marketing authorization holder: Bioveta, a. s., Komenského 212, Ivanovice na Hané, Czech Republic

Date of last revision of the text: February 2006



Rozměr š × v (mm):

2 x A6

Barevnost:

černá

Připravil:

Michlíček

Datum:

21. 3. 2012

Schválil(a):

Dne: