Oct 18, 2020

BACTERIAL ENDOTOXINS

DOI
dx.doi.org/10.17504/protocols.io.bnjcmciw

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DOI: https://dx.doi.org/10.17504/protocols.io.bnjcmciw

Protocol Citation: Jiaxin Li 2020. BACTERIAL ENDOTOXINS. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bnjcmciw

Protocol status: Other
We attempted this protocol, but could not get it to work in our workspace

Created: October 18, 2020

Last Modified: October 18, 2020

Protocol Integer ID: 43332

Troubleshooting

Confirmation of the labeled lysate sensitivity

1 Confirm in 4 replicates the labeled sensitivity λ, expressed in IU/mL, of the lysate solution prior to use in the test. Confirmation of the lysate sensitivity is carried out when a new lot of lysate is used or when there is any change in the test conditions which may affect the outcome of the test.

2 Prepare standard solutions of at least 4 concentrations equivalent to 2λ, λ, 0.5λ and 0.25λ by diluting the standard endotoxin stock solution with water for BET.

3 Mix a volume of the lysate solution with an equal volume of 1 of the standard solutions (such as 0.1 mL aliquots) in each tube. When single test vials or ampoules containing lyophilized lysate are employed, add solutions of standards directly to the vial or ampoule. Incubate the reaction mixture for a constant period according to the recommendations of the lysate manufacturer (usually at 37±1°C for 60±2 min), avoiding vibration.

4 Test the integrity of the gel: for tubes, take each tube in turn directly from the incubator and invert it through approximately 180° in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if an intact gel is not formed.

5 The test is considered valid when the lowest concentration of the standard solutions shows a negative result in all replicate tests. The end-point is the lowest concentration in the series of decreasing concentrations of standard endotoxin that clots the lystae. Determine the geometric mean end-point concentration by calculating the mean of the logarithms of the end-point concentrations of the 4 dilution series, take the antilogarithm of this value, as indicated by the following expression:

Geometric mean end-point concentration $= \log\text{-}1(\Sigma e/f)$
Σe = sum of the log end-point concentrations of the dilution series used,
f = number of replicates.

6 The geometric mean end-point concentration is the measured sensitivity of the lysate solution (IU/mL). If this is not less than 0.5λ and not more than 2λ, the labeled sensitivity is confirmed and is used in the test performed with this lysate.

Test for interfering factors

7 Prepare solutions A, B, C and D as shown in Table 1, and use the test solutions at a dilution less than the MVD, not containing any detectable endotoxins, operating as described under (i) Confirmation of the labeled lysate sensitivity.

	Solution	Endotoxin concentration/soluti	Diluent	Dilution	Endotoxin concentratio	Number of

		on to which endotoxin is added		factor	n	replicates
	A	None/Test solution	-	-	-	4
	B	2λ/Test solution	Test solution	1	2λ	4
				2	1λ	4
				4	0.5λ	4
				8	0.25λ	4
	C	2λ/Water for BET	Water for BET	1	2λ	2
				2	1λ	2
				4	0.5λ	2
				8	0.25λ	2
	D	None/Water for BET	-	-	-	2

Solution A = solution of the preparation being examined that is free of detectable endotoxins.
Solution B = test for interference.
Solution C = control of the labeled lysate sensitivity.
Solution D = negative control (water for BET).

8 The test is considered valid when all replicates of solutions A and D show no reaction and the result of solution C confirms the labeled lysate sensitivity.
If the sensitivity of the lysate determined with solution B is not less than 0.5λ and not greater than 2λ, the test solution does not contain interfering factors under the experimental conditions used. Otherwise, the test solution interferes with the test.

LIMIT TEST

9 Prepare solutions A, B, C and D as shown in Table 2.

	Solution	Endotoxin concentration/Solution to which endotoxin is added	Number of replicates
	A	None/Diluted test solution	2
	B	2λ/Diluted test solution	2
	C	2λ/Water for BET	2

	D	None/Water for BET	2

Interpretation

10 The test is considered valid when both replicates of solution B and C are positive and those of solution D are negative.

11 When a negative result is found for both replicates of solution A, the preparation being examined complies with the test.

12 When a positive result is found for both replicates of solution A, the preparation being examined does not comply with the test.