## Supplementary Materials: Endotoxins from a Pharmacopoeial Point of View

Elvira Franco, Verónica Garcia-Recio, Pilar Jiménez, Manuel Garrosa, Tomás Girbés, Manuel Cordoba-Diaz and Damián Cordoba-Diaz

Table S1. Comparative analysis of the BET conditions between IP, USP, JP, EP and also BP and RFE

Conditions		IP	USP	JP	EP	BP	RFE
Apparatus				Hot-air	oven		
				(30 min at	t 250 °C)		
Reagents		Amoebocyte lysate	Amoebocyte lysate		Amoebocyte lysate	Amoebocyte lysate	Amoebocyte lysate
		Water for bacterial	Water for bacterial		Water for bacterial	Water for bacterial	Water for bacterial
		endotoxins test (BET)	endotoxins test (BET)		endotoxins test (BET)	endotoxins test (BET)	endotoxins test (BET)
		Lysate solution	Lysate solution		Lysate solution	Lysate solution	Lysate solution
	Standard	From the WHO International	From a USP Endotoxin	From Japanese Pharmacopoeia	From an endotoxin reference	From an endotoxin reference	From an endotoxin reference
	Endotoxin	Standard for endotoxin or an	Reference Standard that has	Reference Standard Endotoxin	standard that has been	standard that has been	standard that has been
	Stock	endotoxin reference standard	been calibrated to the	that has been calibrated to the	calibrated against the	calibrated against the	calibrated against the
	Solution	that has been calibrated	current WHO International	current WHO International	International Standard, for	International Standard, for	International Standard, for
		against the WHO	Standard for Endotoxin.	Standard for Endotoxin, using	example endotoxin standard	example endotoxin standard	example endotoxin standard
		International Standard for		water for BET.	BRP.	BRP.	BRP.
Test		endotoxin.					
solutions	Standard						
Solutions	Endotoxin		Seria	l dilutions of the standard endotox	kin stock solution using water fo	r BET	
	Solution						
	Sample	Dissolve or dilute the	Dissolve or dilute drugs	Dissolve or dilute drugs	Dissolve or dilute active	Dissolve or dilute active	Dissolve or dilute active
	Solutions	pharmaceutical substance or	using water for BET	using water for BET	substances or medicinal	substances or medicinal	substances or medicinal
		the finished preparation	• Adjust pH (6.0-8.0)	• Adjust pH (6.0-8.0)	products using water for	products using water for	products using water for
		using water BET.			BET	BET	BET
		• Adjust pH (6.0-8.0)				• Adjust pH (6.0-8.0)	• Adjust pH (6.0-8.0)

					• Adjust pH (6.0-8.0)			
Determination  Maximum Va  Dilution (MV	alid	$MVD = rac{endotoxin\ limit\ x\ concentration\ of\ test\ solution}{\lambda}$						
		• <i>Endotoxin limit:</i> for parenteral preparations, defined on the basis of dose = $\frac{K}{M}$						
		K: threshold pyrogenic dose of	endotoxin per kilogram of bod	y mass				
		M: maximum recommended bo	olus dose of product per kilogra	nm of body mass				
		<ul> <li>Concentration of test solution:         <ul> <li>mg/mL if the endotoxin limit is specified by mass (IU/mg),</li> <li>Units/mL if the endotoxin limit is specified by unit of biological activity (IU/Unit),</li> <li>ml/mL if the endotoxin limit is specified by volume (IU/mL).</li> </ul> </li> <li> <ul> <li>the labelled lysate sensitivity in the gel-clot technique (IU/mL) or the lowest concentration used in the standard curve of the turbidimetric or chromogenic techniques.</li> </ul> </li> </ul>						

Table S2: Comparative analysis of the Gel-clot techniques between IP, USP, JP, EP and also BP and RFE

Gel-clot tec	hniques	IP	USP	JP	EP	BP	RFE
Limit test		Method A			Method A	Method A	Method A
Quantitativ	Quantitative test				Method B	Method B	Method B
Preparatory	testing						
Confirmati	on of the labelled	lysate sensitivity (λ)					
Replicates		4	4	-	4	4	4
Standard so	lutions	2λ, λ, 0.5λ, 0.25λ	2λ, λ, 0.5λ, 0.25λ	2λ, λ, 0.5λ, 0.25λ	2λ, λ, 0.5λ, 0.25λ	2λ, λ, 0.5λ, 0.25λ	2λ, λ, 0.5λ, 0.25λ
(concentrati	ons equivalent)						
Procedure		Mix 1 volume Lysate	Mix 1 volume Lysate TS + 1	Mix 1 volume Lysate TS + 1	Mix 1 volume Lysate	Mix 1 volume Lysate	Mix 1 volume Lysate
		solution + 1 volume	volume standard solution	volume standard solution	solution + 1 volume	solution + 1 volume	solution + 1 volume
		standard solution			standard solution	standard solution	standard solution
Conditions:							
Temperatur	re	37 ± 19C	37 ± 19C	37 ± 19C	37 ± 19C	37 ± 19C	37 ± 19C
Time		60 ± 2 minutes	60 ± 2 minutes	60 ± 2 minutes	60 ± 2 minutes	60 ± 2 minutes	60 ± 2 minutes
Results	Gel	+	+	+	+	+	+
(Invert	No gel	-	-	-	-	-	-
each tube	Valid test	The lowest concentration	The lowest concentration of	When $0.25\lambda$ of the standard	The lowest concentration	The lowest concentration	The lowest concentration
180°)		of the standard solutions	the standard solutions	solution shows a (-) result	of the standard solutions	of the standard solutions	of the standard solutions
		shows a (-) result	shows a (-) result		shows a (-) result	shows a (-) result	shows a (-) result
	Endpoint	The lowest concentration	The smallest concentration	The last (+) test	The lowest concentration	The lowest concentration	The lowest concentration
		that clots the lysate	that clots the lysate		that clots the lysate	that clots the lysate	that clots the lysate
Geometric r	nean end-point						
concentration	on			Geometric mean end-point	concentration = antilog $\frac{\Sigma e}{f}$		

	$\Sigma$ e= The sum of the log end	lpoint concentrations of the dilu	tion series used					
	<b>f:</b> The number of replicates							
	If the geometric mean endpoint concentration is $>0.5\lambda$ and $<2\lambda$ , the labeled sensitivity is confirmed, and is used in tests performed with this lysate.							
Test for interfering factors	Prepare the solutions (A-	Prepare the solutions (A- Prepare the solutions (A-D) Prepare the solutions A, B, Prep						
	D) and perform the	and perform the inhibition/	C and D	C and D and use the test	C and D and use the test	C and D and use the test		
	inhibition/ enhancement	enhancement test on the		solutions at a dilution less	solutions at a dilution less	solutions at a dilution less		
	test on the sample	sample solutions at a		than the MVD not	than the MVD not	than the MVD not		
	solutions at a dilution	dilution less than the MVD		containing any detectable	containing any detectable	containing any detectable		
	less than the MVD not	not containing any		endotoxins	endotoxins	endotoxins		
	containing any	detectable endotoxins						
	detectable endotoxins							
Solution A:								
Endotoxin concentration /	None / test solution	None / sample solution	0 / sample solution	None / test solution	None / test solution	None / test solution		
Solution to which endotoxin								
is added								
Diluent	-	-	-	-	-	-		
Dilution factor	-	-	-	-	-	-		
Endotoxin concentration	-	-	-	-	-	-		
• Replicates	4	4	4	4	4	4		
Solution B:								
Endotoxin concentration /	2λ / test solution	2λ / sample solution	2λ / sample solution	2λ / test solution	2λ / test solution	2λ / test solution		
Solution to which endotoxin								
is added								
Diluent	Test solution	Sample solution	Sample solution	Test solution	Test solution	Test solution		

Dilution factors	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8
Endotoxin concentrations	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0,5λ-0,25λ
• Replicates	4	4	4	4	4	4
Solution C:						
• Endotoxin concentration /	2λ / water for BET	2λ / water for BET	2λ / water for BET	2λ / water for BET	2λ / water for BET	2λ / water for BET
Solution to which endotoxin						
is added						
• Diluent	Water for BET	Water for BET	Water for BET	Water for BET	Water for BET	Water for BET
Dilution factors	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8
Endotoxin concentrations	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0,5λ-0,25λ
• Replicates	2	2	2	2	2	2
Solution D:						
• Endotoxin concentration /	None / water for BET	None / water for BET	0 / water for BET	None / water for BET	None /water for BET	None / water for BET
Solution to which endotoxin						
is added						
• Diluent	-	-	-	-	-	-
Dilution factor	-	-	-	-	-	-
Endotoxin concentration	-	-	-	-	-	-
• Replicates	2	2	2	2	2	2

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Results	Test valid when A and	Test valid when A and D	Test valid when A and D	Test valid when both	• Test valid when A and D	• Test valid when A and D
	D show no reaction and	show no reaction and C	show no reaction and C	replicates of B and C are	show no reaction and C	show no reaction and C
	C confirms the labelled	confirms the labelled	confirms the labelled	(+) and those of D are (-).	confirms the labelled	confirms the labelled
	sensitivity	sensitivity	sensitivity	• When a (-) result is found	sensitivity	sensitivity
	• Sol. B: if the sensitivity	• Sol. B: if the sensitivity of	• Sol. B: if the sensitivity of	for both replicates of A,	• Sol. B: if the sensitivity of	• Sol. B: if the sensitivity of
	of the lysate is >0.5λ and	the lysate is >0.5λ and <2λ	the lysate is $>0.5\lambda$ and $<2\lambda$	the preparation complies	the lysate is $>0.5\lambda$ and $<2\lambda$	the lysate is $>0.5\lambda$ and $<2\lambda$
	<2λ the sample solution	the sample solution does not	the sample solution does	with the test. When a (+)	the sample solution does	the sample solution does
	does not contain	contain interfering factors	not contain interfering	result is found the	not contain interfering	not contain interfering
	interfering factors	• If the sample under test	factors	preparation does not	factors	factors
	• If the sample under test	does not comply with the	• If the sample under test	comply with the test.	• If the sample under test	• If the sample under test
	does not comply with the	test at a dilution less than	does not comply with the	• When a (+) result is	does not comply with the	does not comply with the
	test at a dilution less	the MVD, repeat the test	test at a dilution less than	found for one replicate of	test at a dilution less than	test at a dilution less than
	than the MVD, repeat the u		the MVD, repeat the test	A and a (-) result is found	the MVD, repeat the test	the MVD, repeat the test
	test using a greater di-	not exceeding the MVD.	using a greater di- lution,	for the other, repeat the	using a greater dilution,	using a greater dilution,
	lution, not exceeding the		not exceeding the MVD.	test. In the repeat test, the	not exceeding the MVD.	not exceeding the MVD.
	MVD.			preparation complies with		
				the test if a (-) result is		
				found for both replicates		
				of solution A.		
Limit test	IP	USP	JP	EP	BP	RFE
Procedure	Prepare solutions A, B, C	Prepare solutions A, B, C	Prepare solutions A, B, C	Prepare solutions A, B, C	Prepare solutions A, B, C	Prepare solutions A, B, C
	and D.	and D.	and D.	and D.	and D.	and D.
	Follow the procedures:	Follow the procedures:	Follow the procedures:	Follow the procedures:	Follow the procedures:	Follow the procedures:
	<ul> <li>Confirmation of the</li> </ul>	Confirmation of the	<ul> <li>Confirmation of the</li> </ul>	Confirmation of the	<ul> <li>Confirmation of the</li> </ul>	Confirmation of the
	labelled lysate sensitivity	labelled lysate sensitivity	labelled lysate sensitivity	labelled lysate sensitivity	labelled lysate sensitivity	labelled lysate sensitivity
	<ul> <li>Test for interfering factors</li> </ul>	Test for interfering factors	<ul> <li>Test for interfering factors</li> </ul>	Test for interfering	• Test for interfering factors	Test for interfering
				factors		factors

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Solution A:						
Endotoxin concentration/	None / Diluted test solution	None / Diluted sample		None / Diluted test	None / Diluted test	None / Diluted test
Solution to which		solution	0 / Sample solution	solution	solution	solution
endotoxin is added	2					
Replicates		2	2	2	2	2
Solution B:						
Endotoxin concentration/	2λ / Diluted test solution	2λ / Diluted sample solution		2λ / Diluted test solution	2λ / Diluted test solution	2λ / Diluted test solution
Solution to which			2λ / sample solution			
endotoxin is added	2	2		2	2	2
Replicates			2			
Solution C:						
Endotoxin concentration/						
Solution to which	2λ / water for BET	2λ / water for BET	2λ / water for BET	2λ / water for BET	2λ / water for BET	2λ / water for BET
endotoxin is added						
Replicates	2	2	2	2	2	2
Solution D:						
Endotoxin concentration/						
Solution to which	None / water for BET	None / water for BET	0 / water for BET	None / water for BET	None / water for BET	None / water for BET
endotoxin is added						
Replicates	2	2	2	2	2	2
Interpretation	Test valid:	Test valid:	Test valid:	Test valid:	Test valid:	Test valid:
	• both replicates of B and C	• both replicates of B and C	• both replicates of B and C	• both replicates of B and	• both replicates of B and C	• both replicates of B and
	are (+) and those of D are	are (+) and those of D are	are (+) and those of D are (-).	C are (+) and those of D	are (+) and those of D are	C are (+) and those of D
	(-).	(-).	• (-) result for both replicates	are (-).	(-).	are (-).
	• (-) result for both	• (-) result for both	of sol. A.	• (-) result for both	• (-) result for both	• (-) result for both
	replicates of sol. A	replicates of sol. A	A (+) result for one replicate	replicates of sol. A	replicates of sol. A	replicates of sol. A

	A (+) result for one replicate	A (+) result for one replicate	of sol. A and a (-) result for	A (+) result for one	A (+) result for one	A (+) result for one
	of A and a (-) result for the	of A and a (-) result for the	the other, repeat the test.	replicate of A and a (-)	replicate of A and a (-)	replicate of A and a (-)
	other, repeat the test.	other, repeat the test.		result for the other, repeat	result for the other, repeat	result for the other, repeat
				the test.	the test.	the test.
Quantitative test	IP	USP	JP	EP	BP	RFE
Procedure	Prepare solutions A, B, C	Prepare solutions A, B, C	Prepare solutions A, B, C	Prepare solutions A, B, C	Prepare solutions A, B, C	Prepare solutions A, B, C
	and D.	and D.	and D.	and D.	and D.	and D.
	Test these solutions	Test these solutions	When preparing solutions A	Test these solutions	Test these solutions	Test these solutions
	according to Confirmation	following the test	and B, use sample solutions	according to Confirmation	according to Confirmation	according to Confirmation
	of the labelled lysate	Confirmation of the labelled	complying with the test for	of the labelled lysate	of the labelled lysate	of the labelled lysate
	sensitivity.	lysate sensitivity.	interfering factors.	sensitivity.	sensitivity.	sensitivity.
			Concerning the test			
			conditions, follow the			
			procedure described in the			
			confirmation of the labelled			
			lysate sensitivity.			
Solution A:						
Endotoxin	None / sample solution	None / sample solution	0 / sample solution	None / test solution	None / test solution	None / test solution
concentration / Solution						
to which endotoxin is						
added	Water for BET	Water for BET	Water for BET	Water for BET	Water for BET	Water for BET
Diluent	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8
Dilution factor	-	-	-	-	-	-
• Endotoxin	2	2	2	2	2	2
concentration						
Replicates						

Solution B:						
Endotoxin	2λ / sample solution	$2\lambda$ / sample solution	$2\lambda$ / sample solution	2λ / test solution	2λ / test solution	$2\lambda$ / test solution
concentration / Solution						
to which endotoxin is						
added	-	-	-	-	-	-
• Diluent	1	1	1	1	1	1
Dilution factors	2λ	2λ	2λ	2λ	2λ	2λ
• Endotoxin	2	2	2	2	2	2
concentrations						
• Replicates						
Solution C:						
• Endotoxin	2λ / water for BET	2λ / water for BET	2λ / water for BET	2λ / water for BET	2λ / water for BET	2λ / water for BET
concentration / Solution						
to which endotoxin is						
added	Water for BET	Water for BET	Water for BET	Water for BET	Water for BET	Water for BET
• Diluent	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8	1-2-4-8
Dilution factors	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ	2λ-1λ-0.5λ-0.25λ
• Endotoxin	2	2	2	2	2	2
concentrations						
• Replicates						
Solution D:						
• Endotoxin	None / water for BET	None / water for BET	0 / water for BET	None /water for BET	None /water for BET	None /water for BET
concentration / Solution						
to which endotoxin is						
added	-	-	-	-	-	-
• Diluent	-	-	-	-	-	-
Dilution factor	-	-	-	-	-	-

• Endotoxin	2	2	2	2	2	2
concentration						
<ul> <li>Replicates</li> </ul>						
Calculation and	Test is valid if:	Test is valid if:	Test is valid if:	Test is valid if:	Test is valid if:	Test is valid if:
interpretation	• Both replicates of sol. D are • Both replicates of sol. D		Both replicates of sol. D are	Both replicates of sol. D	Both replicates of sol. D	Both replicates of sol. D
	(-)	are (-)	(-)	are (-)	are (-)	are (-)
	Both replicates of sol. B are	Both replicates of sol. B are	Both replicates of sol. B are	Both replicates of sol. B	Both replicates of sol. B	Both replicates of sol. B
	(+)	(+)	(+)	are (+)	are (+)	are (+)
	Geometric mean end-point	Geometric mean end-point	Geometric mean end-point	Geometric mean end-	Geometric mean end-	Geometric mean end-
	concentration of sol. C is in	concentration of sol. C is in	concentration of sol. C is in	point concentration of sol.	point concentration of sol.	point concentration of sol.
	the range of $0.5\lambda$ to $2\lambda$	the range of $0.5\lambda$ to $2\lambda$	the range of $0.5\lambda$ to $2\lambda$	C is in the range of $0.5\lambda$ to	C is in the range of $0.5\lambda$ to	C is in the range of $0.5\lambda$ to
				2λ	2λ	2λ
Endotoxin concentration	Calculate the end-point	Calculate the end-point	The endpoint is defined as	Calculate the end-point	Calculate the end-point	Calculate the end-point
of sol. A:	concentration for each	concentration for each	the maximum dilution	concentration for each	concentration for each	concentration for each
	replicate by multiplying	replicate by multiplying	showing the last positive test	replicate by multiplying	replicate by multiplying	replicate by multiplying
	each end-point dilution	each end-point dilution	in the dilution series of	each end-point dilution	each end-point dilution	each end-point dilution
	factor by $\lambda$ .	factor by $\lambda$ .	solution A	factor by $\lambda$ .	factor by $\lambda$ .	factor by $\lambda$ .
Endotoxin concentration	It is the end-point	It is the end-point	It is calculated by	It is the end-point	It is the end-point	It is the end-point
in the sample solution	concentration of the	concentration of the	multiplying the endpoint	concentration of the	concentration of the	concentration of the
	replicates. If the test is	replicates. If the test is	dilution factor by $\lambda$ .	replicates. If the test is	replicates. If the test is	replicates. If the test is
	conducted with a diluted	conducted with a diluted		conducted with a diluted	conducted with a diluted	conducted with a diluted
	sample solution, calculate	sample solution, calculate		sample solution, calculate	sample solution, calculate	sample solution, calculate
	the concentration of	the concentration of		the concentration of	the concentration of	the concentration of
	endotoxin in the original	endotoxin in the original		endotoxin in the original	endotoxin in the original	endotoxin in the original
	sample solution by	sample solution by		sample solution by	sample solution by	sample solution by
	multiplying by the dilution	multiplying by the dilution		multiplying by the	multiplying by the	multiplying by the

	factor.	factor.		dilution factor.	dilution factor.	dilution factor.
If none of dilutions is (+)	Report the endotoxin	Report the endotoxin	Report the endotoxin	Report the endotoxin	Report the endotoxin	Report the endotoxin
	concentration as less than $\lambda$	concentration as less than $\lambda$	concentration as less than $\lambda$ ×	concentration as less than	concentration as less than	concentration as less than
			the lowest dilution factor of	λ	λ	λ
			the sample solution			
If all dilutions are (+)	Reported as equal to or	Reported as equal to or	reported as equal to or	Reported as equal to or	Reported as equal to or	Reported as equal to or
	greater than the largest	greater than the largest	greater than the greatest	greater than the largest	greater than the largest	greater than the largest
	dilution factor multiplied by	dilution factor multiplied by	dilution factor of solution A	dilution factor multiplied	dilution factor multiplied	dilution factor multiplied
	$\lambda$ $\lambda$ multiple		multiplied by $\lambda$ .	by λ	by λ	by λ
	The preparation under test	The preparation under test	The sample com- plies with	The preparation under test	The preparation under test	The preparation under test
	meets the requirements of	meets the requirements of	the Bacterial Endotoxins	meets the requirements of	meets the requirements of	meets the requirements of
	the test if the concentration	the test if the concentration	Test if the endotoxin	the test if the	the test if the	the test if the
	of endotoxin in both	of endotoxin in both	concentration of the sample	concentration of endotoxin	concentration of endotoxin	concentration of endotoxin
	replicates is less than that	replicates is less than that	in both replicates meets the	in both replicates is less	in both replicates is less	in both replicates is less
	specified in the individual	specified in the individual	requirement for the	than that specified in the	than that specified in the	than that specified in the
	monograph.	monograph.	endotoxin limit specified in	individual monograph.	individual monograph.	individual monograph.
			the individual monograph.			

Table S3: Comparative analysis of the Photometric quantitative techniques between IP, USP, JP, EP and also BP and RFE

Photometric quantitative		IP	USP	JP	EP	BP	RFE
techniques							
Turbidimetric	End-point	Mathad D			Method F	Method F	Method F
	Kinetic	Method B			Method C	Method C	Method C
Chromogenic	End-point	Mathad C			Method E	Method E	Method E
	Kinetic	Method C			Method D	Method D	Method D
Temperature		37 ± 1 ℃					

Criteria for the standard	IP	USP	JP	EP	BP	RFE
curve						
Endotoxin concentrations	3	3	3	3	3	3
within the range						
Replicates of each standard	3	3	3	3	3	3
endotoxin solution						
r	≥ 0,980	≥ 0,980	≥ 0,980	≥0,980	≥ 0,980	≥ 0,980
Test for interfering factors	Select an endotoxin			Select an endotoxin concentration at or	Select an endotoxin	Select an endotoxin
	concentration at or			near the middle of the endotoxin	concentration at or near	concentration at or near the
	near the middle of			standard curve	the middle of the	middle of the endotoxin
	the endotoxin				endotoxin standard curve	standard curve
	standard curve					
Solution A:						
Endotoxin concentration	(-)	(-)	0	(-)	(-)	(-)
• Solution	Sample solution	Sample solution	Sample solution	Test solution	Test solution	Test solution
Replicates	≥ 2	≥ 2	≥ 2	≥2	≥2	≥2
Solution B:						

Endotoxin concentration	Middle	Middle	Middle	Middle concentration of the standard	Middle concentration of	Middle concentration of the
	concentration of the	concentration of	concentration of	curve	the standard curve	standard curve
<ul> <li>Solution</li> </ul>	standard curve	the standard	the standard	Test solution	Test solution	Test solution
<ul> <li>Replicates</li> </ul>	Sample solution	curve	curve	≥ 2	≥2	≥2
	≥2	Sample solution	Sample solution			
		≥2	≥ 2			
Solution C:						
Endotoxin concentration	≥3 concentrations	≥ 3 concentrations	≥3	≥ 3 concentrations (lowest C is	≥ 3 concentrations (lowest	≥3 concentrations (lowest C
	(lowest C is	(lowest C is	concentrations	designated $\lambda$ ). Water for BET Each	C is designated $\lambda$ ). Water	is designated $\lambda$ ). Water for
	designated $\lambda$ ). Water	designated $\lambda$ ).	(lowest C is	concentration ≥ 2	for BET Each	BET Each concentration ≥ 2
• Solution	for BET Each	Water for BET	designated $\lambda$ ).		concentration ≥ 2	
Replicates	concentration ≥ 2	Each	Water for BET			
		concentration ≥ 2	Each			
			concentration ≥ 2			
Solution D:						
Endotoxin concentration	(-)	(-)	0	(-)	(-)	(-)
• Solution	Water for BET	Water for BET	Water for BET	Water for BET	Water for BET	Water for BET
• Replicates	≥2	≥2	≥ 2	≥ 2	≥2	≥2
Result with solution D	• ≤ limit of the blank	•≤limit of the	•≤limit of the	• ≤ limit of the blank value	• ≤ limit of the blank value	• ≤ limit of the blank value
	value	blank value	blank value	<ul> <li>&lt; endotoxin detection limit</li> </ul>	< endotoxin detection	<ul><li>&lt; endotoxin detection</li></ul>
	< endotoxin	< endotoxin	• < endotoxin		limit	limit
	detection limit	detection limit	detection limit			
r  of standard curve	≥ 0,980	≥ 0,980	≥ 0,980	≥ 0,980	≥ 0,980	≥ 0,980
generated using solution C						
Mean recovery of the added	50–200%	50–200%	50–200%	50–200%	50–200%	50–200%
endotoxin						

Procedure	Follow test for interfering factors					
Calculation	Endotoxin	Endotoxin	Endotoxin	Endotoxin concentration of solution A	Endotoxin concentration	Endotoxin concentration of
	concentration of	concentration of	concentration of	using the standard curve generated by	of solution A using the	solution A using the
	solution A using the	solution A using	solution A using	С	standard curve generated	standard curve generated
	standard curve	the standard	the standard		by C	by C
	generated by C	curve generated	curve generated			
		by C	by C			
Requirements	• Sol. C comply	• Sol. C comply	•   r   of sol. C: ≥	Sol. C comply assurance of criteria	• Sol. C comply assurance	• Sol. C comply assurance of
	assurance of criteria	assurance of	0,980	• endotoxin recovery: 50–200%	of criteria	criteria
	• endotoxin	criteria	• endotoxin	Sol. D: ≤ blank value of the lysate	• endotoxin recovery: 50–	• endotoxin recovery: 50–
	recovery: 50–200%	• endotoxin	recovery: 50–	employed or < endotoxin detection	200%	200%
	• Sol. D: ≤ blank	recovery: 50–200%	200%	limit	• Sol. D: ≤ blank value of	• Sol. D: ≤ blank value of the
	value of the	• Sol. D: ≤ blank	• Sol. D: ≤ blank		the lysate employed or <	lysate employed or <
	lysate	value of the lysate	value of the		endotoxin detection limit	endotoxin detection limit
	employed or <	employed or <	lysate employed			
	endotoxin	endotoxin	or < endotoxin			
	detection limit	detection limit	detection limit			
Interpretation	Endotoxin	Endotoxin	Endotoxin	Endotoxin concentration of sol. A <	Endotoxin concentration	Endotoxin concentration of
	concentration of sol.	concentration of	concentration of	endotoxin detection limit	of sol. A < endotoxin	sol. A < endotoxin detection
	A < endotoxin	sol. A < endotoxin	sol. A meets the		detection limit	limit
	detection limit	detection limit	endotoxin			
			detection limit			
			specified in			
			monograph			