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Professional paper

Determination of bacterial endotoxins in parenteral solutions produced on a small scale in a clinical hospital

Renata Bozhinovska Popovska[1], Tatjana Gjurichikj[2], Branislava Janeva[3], Jelena Acevska[4], Aneta Dimitrovska[4]

[1]*Clinic Hospital "dr. Trifun Panovski", Partizanska bb, 7000 Bitola, R. North Macedonia*
[2]*Alkaloid Ad, Blvd Aleksandar Makedonski No 12, 1000 Skopje, R. North Macedonia*
[3]*IPH – Institute for public health, 50 th division No 6, 1000 Skopje,R. North Macedonia*
[4]*Faculty of Pharmacy, Ss Cyril Methodius University in Skopje, Majka Thereza 47, 1000 Skopje, R. North Macedonia*

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Abstract

Two different limulus amoebocyte lysate assays of bacterial endotoxins in parenteral solutions produced on a small scale in a clinical hospital are evaluated. The gel-clot LAL method and turbidimetric kinetic method were applied as techniques for determination of endotoxin from Gram – negative bacteria in a large volume of 0.9% NaCl solution for infusion. The same principle is applied but using different official methods for determination of bacterial endotoxin′s concertation. An overview of the advantages and disadvantages to the implementation of both methods is provided, with a detailed description of the procedures, comparison of the suitability of each method and recommendations based on the findings. The conventional gel-clot LAL method as a simple method can be recommended due to its economic sustainability in terms of equipment. On the other hand, the turbidimetric kinetic assay offers distinct advantages such as enhanced sensitivity, quantitative analysis capability, and instrumental reading. Furthermore, this method helps eliminate potential human variability in endpoint assessments.

Therefore, the obtained results and critical aspects of the applied methods: the gel-clot LAL method and the turbidimetric kinetic method indicate that both LAL methods can be used for the determination of bacterial endotoxins in parenteral solutions, produced on a small scale and provide an opportunity for small laboratories to facilitate the decision – making process for choosing a method that best meets their requirements and resources.

Key words: determination of bacterial endotoxins, gel-clot LAL method, turbidimetric kinetic method, small scale production

Introduction

Pyrogenic contamination of parenteral dosage forms, such as the infusion preparations, is very important in clinical medicine. The most common and most potent cause of toxic reactions, due to contamination of pharmaceutical preparations with pyrogenic substances, are bacterial endotoxins (BE) producing from Gram-negative bacteria, which are chemically classified as lipopolysaharide (LPS) and are common constituents of the cell walls of gram-negative bacteria (Baggerman et al., 1985; Oishi et al., 1985). Intravenous (IV) injected bacterial endotoxins in humans even in quantity as 0.01 μg can cause endotoxin shock with variety of pathophysiological effects such as fever, headache, chest pain, nausea, vomiting, diarrhea, hyperglycemia, as well as tissue injury and lethality (Ochiai et al., 2003; Yamamoto

*email: andi@ff.ukim.edu.mk

et al., 2000). Therefore, because of the already mentioned high level of risk, pharmaceutical parenteral products are tested for pyrogens including, or exclusively, bacterial endotoxins (Danner et al., 1991).

The pyrogenicity of parenteral solutions as a particular problem for solutions, which are applied in an amount greater than 10 mL, is solved by ensuring that they are strictly non-pyrogenic. Therefore, the monographs of pharmaceutical preparations intended for parenteral use in the European Pharmacopoeia prescribe two tests for evaluation of pyrogenicity of pharmaceutical preparations: Rabbit Pyrogen Test, RPT (European Pharmacopoeia 01/2020:20608 Pyrogens, 2024) and Bacterial Endotoxins Test, BET (European Pharmacopoeia Monograph 01/2018:20614 Bacterial Endotoxins, 2024). BET predominates over the RPT and it is based on reactions of endotoxins with lysate of amebocytes of the horseshoe crab (Limulus Polyphemus) (Levin, 1985; Ochiai et al., 2003).

Historically speaking, Levin and Bang (1964) discovered that the BE can activate intravascular coagulation in the horseshoe crab (Limulus Polyphemus). This discovery became the proposition or principle for all endotoxin tests with Limulus Amoebocyte Lysates (LAL test) or BET, and it is presented for the first time formally in the 21st edition of the U.S Pharmacopoeia (<85> Bacterial Endotoxins Test) as the most sensitive method for detection of bacterial endotoxins (Cooper et al., 1971). It`s important to note that BET or LAL assays carry certain limitations; mainly: the possibility of interference with some drugs or other substances, as well as their dependence on a relatively narrow pH range and a specific temperature range (Cooper, 1988; Ronneberger, 1977).

BET or LAL assays is completely harmonized according to the ICH guideline Q4B annex 14: Evaluation and Recommendation of Pharmacopeial Texts for Use in the ICH Regions on Bacterial Endotoxins Test (including text from European Pharmacopoeia; US Pharmacopoeia; Japanese Pharmacopoeia and The Internacional Pharmacopoeia). Currently, there are predominantly three variations of the LAL test technique:

- Gel-clot method, which includes gel formation and enables detection or determination of the maximum allowed concentration of BE. It is based on gelation of the lysate in the presence of bacterial endotoxins;
- Turbidimetric method, which is based on the development of turbidity after cleavage of an endogenous substrate. The time required to produce a specified level of turbidity is proportional to the amount of BE in a sample; and
- Chromogenic technique, which is based on development of color after cleavage of synthetic peptide – chromogen complex.

According to European pharmacopoeia there are six methods for LAL test, and each of them can be used for determination of bacterial endotoxins in parenteral solutions: (1) Method A Gel-clot Method: limit test (semi-quantitative); (2) Method B Gel-clot Method: quantitative test; (3) Method C Turbidimetric kinetic method; (4) Method D Chrmogenic kinetic method; (5) Method E Chromogenic end-point method; and (6) Method F Turbidimetric end point method.

Thus, the aim of this study was to give an overview of the two LAL assays, among the six previously mentioned methods: conventional gel-clot (Ph. Eur LAL Method A test) and turbidimetric kinetic (Ph. Eur LAL Method C test) as the simplest and most suitable LAL methods for determination of BE in small scale production of parenteral solutions. In this study we also present comparative analyses of the results obtained using these two methods. Suitability of each method is already defined in the monograph for parenteral solutions, but this study examines the factors that are essential for deciding between gel-clot LAL method and turbidimetric kinetic method, as more suitable for the determination of bacterial endotoxins in parenteral solution produced on a small scale in a clinical hospital.

Material and methods

A small - scale production batches of 0.9% NaCl parenteral solution for intravenous infusion, 500 mL, produced by infusion department, clinical hospital - Bitola, Republic of North Macedonia, were used as samples for determination of endotoxins, using the two methods.

One batch was used in the gel-clot LAL method (batch No. 27) and three batches (No. 11, No. 12, No. 27) were used in the turbidimetric kinetic LAL method (one of the batches is used in both methods, batch No. 27) with the representative samples obtained from the beginning and the end of production.

Reagents used (in gel-clot LAL method manufactured by FUJIFILM WAKO Chemicals USA Corporation, and in turbidimetric kinetic LAL method manufactured by LONZA Walkersville MD USA):

- Limulus amebocyte lysate (LAL reagent) prepared and stored according to the manufacturer's instructions.
- Control Standard Endotoxin (CSE) is standardized against the Reference Standard Endotoxin (RSE) which is an analogue of BRP (Biological Reference Preparation). CSE is prepared according to the certificate from the manufacturer - Stock solution.
- LAL water – endotoxin free water, highly purified water that does not contain detectable endotoxins.
- LAL 0.1 M HCL and LAL 0.1M NaOH were used to adjust the pH of the mixture to 6.0-8.0 to obtain optimal gel formation. The ideal pH of the mixture was 7.3±0.5. For the turbidimetric kinetic method HCL and NaOH were not used; instead LAL reagent was reconstituted using LAL reconstitution buffer, according to the LONZA manufacturer's instructions.

As we have already maintained, the same reagent was used in both methods, but from different manufacturers. However, the LAL reagent that is used in gel-clot LAL method has a different sensitivity than the one in the turbidimetric kinetic method. The CSE has a different endotoxin concentration in both methods.

Equipment used: Dragonlab pipettor (5-50 microliters), Eppendorf pipettor (10-100 microliters), Linear pipettor (100-1000 microliters), vortex mixer, stopwatch. All test tubes, dilution tubes and pipette tips that are used must be endotoxin free (for both of the two different methods) .

The difference in the equipment is that in the gel-clot method a heating block for incubation (37±1 ºC) was used, where the test sample mixed with LAL reagent is placed. For the turbidimetric kinetic method, the instrumental determination is preformed using a spectrophotometer PYROGENT®-5000 Kinetic Turbidimetric Assay which offers a quantitative LAL method with a sensitivity range from 0.01 to 100 EU/mL. The test samples with LAL reagent are placed in an incubating absorbance plate reader. They are then monitored over time for the appearance of turbidity (measured at the 340 nm wavelength). This assay is performed in a 96-well plate, which allows many samples to be processed at one time.

Results and discussion

The principle of performing the test for bacterial endotoxin in both the gel clot method and the turbidimetric kinetic method is the same. The main differences are the performance procedure, required material and necessary equipment. However, the essential difference in the detection of BE is in the visual inspection of the formation of a gel in the gel clot method, while in the turbidimetric kinetic method the turbidity is read by instrumental means. With both methods, the test allows the detection of BE based on the coagulation (formation of a gel) of the lysate, in form of turbidity which occurs after the enzymatic cascade in the lysate by the endotoxin. The minimum concentration of endotoxin required to clot the lysate under standard conditions is the stated sensitivity of the lysate, which must be re-verified in the preparatory test and during the method validation of both methods. The product needs to be diluted to prevent interference, but to allow detection of the endotoxin limit at the same time. Maximum Valid Dilution (MVD) is the maximum allowable dilution of the sample, at which the BE limit can be determined, MVD = EL / ʎ (for the preparations where the endotoxin limit is expressed in EU/mL). So, for this study for the gel-clot LAL method MVD = 1:16, and for the turbidimetric kinetic method MVD = 1:25 were prepared (EL is endotoxin limit, i.e. maximal allowable of endotoxin in a given sample or product and according to regulatory guidelines for 0.9% NaCl is 0.25 EU/mL).

The essential point regarding both the gel clot method and the turbidimetric kinetic method is that before doing the main test, the method must be validated first, in order to confirm that the analytical procedure is safe, reproducible and suitable. Therefore, preparatory testing for both methods include: confirmation of the labelled lysate sensitivity and a test for interfering factors (according to European Pharmacopoeia 11.0, 2.6.14 and ICH guideline Q4B annex 14). In this study, confirmation of the labelled lysate sensitivity (the minimum endotoxin concentration which causes gelation of the lysate) with gel-clot method are described in Table 1 and Table 2.

Table 1. Test for confirmation of the labelled lysate sensitivity (gel-clot LAL method)

Vial	2λ 0,03125 EU/mL	Λ 0,015625 EU/mL	λ/2 0,0075 EU/mL	λ/4 0,00375 EU/mL	LAL Reagent Water Blink
1	100 µL of 2λ + 100 µL of lysate	100 µL of λ + 100 µL of lysate	100 µL of λ/2 + 100 µL of lysate	100 µL of λ/4 + 100 µL of lysate	100 µL of LAL water + 100 µL of lysate
2	100 µL of 2λ + 100 µL of lysate	100 µL of λ + 100 µL of lysate	100 µL of λ/2 + 100 µL of lysate	100 µL of λ/4 + 100 µL of lysate	100 µL of LAL water + 100 µL of lysate
3	100 µL of 2λ + 100 µL of lysate	100 µL of λ + 100 µL of lysate	100 µL of λ/2 + 100 µL of lysate	100 µL of λ/4 + 100 µL of lysate	100 µL of LAL water + 100 µL of lysate
4	100 µL of 2λ + 100 µL of lysate	100 µL of λ + 100 µL of lysate	100 µL of λ/2 + 100 µL of lysate	100 µL of λ/4 + 100 µL of lysate	100 µL of LAL water + 100 µL of lysate

It is incubated for 60±2 min at 37±1 C˚

The lysate sensitivity is calculated by determining the geometric mean of the endpoint of the last dilutions, in log 10, which give positive results - endpoint.

Table 2. Results of test for confirmation of the labelled lysate sensitivity (gel-clot method)

Vial	2λ 0,03125 EU/mL	Λ 0,015625 EU/mL	λ/2 0,0075 EU/mL	λ/4 0,00375 EU/mL	LAL Reagent Water Blink
1	+	+	-	-	-
2	+	+	-	-	-
3	+	+	-	-	-
4	+	+	-	-	-

Allowed: ʎ/2 (0.0075 EU/mL) ≤ 0.015 EU/mL ≤ 2ʎ (0.03125 EU/mL)

After having the results for the geometric mean end - point concentration of 0.015 EU/mL, the labeled sensitivity was confirmed.

Test for interfering factors (gel-clot LAL method)

The presence of BE in a product may be masked by factors interfering with the reaction between the endotoxins and amoebocyte lysate. Interference does not exist if null hypothesis is confirmed, i.e. the lysate sensitivity in the presence of the tested preparation does not differ significantly from the lysate sensitivity in the absence of the preparation. The null hypothesis is acceptable in the case when the lysate sensitivity in the presence of the preparation is within the limits of ʎ/2 - 2ʎ (where ʎ is the labeled lysate sensitivity). The preliminary test for the presence of inhibitory factors is performed by making a series of double dilutions of the tested sample in water. In this study, the procedure and the results of the preliminary test for interfering factors are described in Table 3.

PPC is positive product control, so in this case there is no interference in any dilution. The validation will be performed on a 1:8 scale.

Calibration curve of the control standard endotoxins (CSE) and diluted test solution (1:8) – concentration equivalent to 2ʎ, ʎ; ʎ/2; ʎ/4

The standard endotoxin is prepared using the diluted test solution 1:8 as a diluent. The testing is performed in quadruplicate. At the end of the incubation period, the presence or absence of gel is registered and the geometric mean is calculated from the endpoint of the entire series. The results are presented in Table 4.

Table 3. Preliminary test for interfering factors (gel-clot LAL Method)

Batch 27	SOLUTION A		SOLUTION B	
Dilution	Results			
	1 100µL preparation + 100 µL lysate	2 100µL preparation + 100 µL lysate	3 (PPC) 100µL preparation + 100 µL lysate + 10µL CSE (2λ)	4 (PPC) 100µL preparation + 100 µL lysate + 10µL CSE (2λ)
LAL water	-	-	+	+
1: 2	-	-	+	+
1: 4	-	-	+	+
1: 8	-	-	+	+
1: 16	-	-	+	+

Table 4. Results for interfering factors and diluted test solution (1:8) - (gel-clot LAL Method)

Vial	2λ 0,03125 EU/mL	Λ 0,015625 EU/mL	λ/2 0,0075 EU/mL	λ/4 0,00375 EU/mL	Negative Control LAL water
1	+	+	-	-	-
2	+	+	-	-	-
3	+	+	-	-	-
4	+	+	-	-	-

Table 5. Procedure for main test (gel-clot LAL method

1 (solution A)	100 μL tested preparation with dilution not exceeding MVD+ 100 μL lysate	100 μL tested preparation with dilution not exceeding MVD+ 100 μL lysate
2 (solution B)	100 μL tested preparation with dilution not exceeding MVD + 100 μL lysate+ 10 μL CSE 2λ	100 μL tested preparation with dilution not exceeding MVD + 100 μL lysate+ 10 μL CSE 2λ
3 (solution C)	100 μL LAL water + 100 μL lysate+ 10 μL CSE 2λ	100 μL LAL water + 100 μL lysate+ 10 μL CSE 2λ
4 (solution D)	100 μL LAL water + 100 μL lysate	100 μL LAL water + 100 μL lysate

Table 6. Results for main test (gel-clot LAL method)

Vial	Result	
1 (solution A)	-	-
2 (solution B)	+	+
3 (solution C)	+	+
4 (solution D)	-	-

Solution A: negative control of the product not exceeding MVD
Solution B: positive control of the product not exceeding MVD
Solution C: positive control of water
Solution D: negative water control

Table 7. Curve acceptability parameters

Concentartion, EU/mL	0.01	0.1	1	10	100
CV [%]	3.94	0.97	0.97	1.73	1.82
Correlation coefficient	0.993	0.993	0.993	0.993	0.993

Main test (gel-clot LAL method)

For the gel-clot LAL method, the procedure and the obtained results are shown in Table 5 and Table 6.

The analyzed test preparation, infusion solution 0.9% NaCl 500 mL, is non-pyrogenic, because a negative result was obtained from both samples of the test preparation, i.e. the presence of an intact gel formation in both replicas of the test sample. Hence, the sample meets the requirements for the absence of bacterial endotoxins since in both preparations, examined at the same time and under the same conditions, a negative result was confirmed.

The following was concluded:

1. The lysate sensitivity determined in the presence of product diluted 1:8 is not lower than λ/2 (0.0075 EU/mL) and not higher than 2λ (0.03125 EU/mL).
2. During routine testing, the preparation should be tested at a dilution of 1:8.
3. If, during a routine test, the results are positive or the positive product control (PPC) is negative, the test should be performed at a greater dilution (MVD).
4. The purpose of the negative control is to confirm the absence of detectable endotoxin concentration in LAL water.

Turbidimetric kinetic LAL method

The test for assurance of criteria for the standard curve (which confirms lysate sensitivity) and the test for interfering factors with the turbidimetric kinetic method, are with same procedure as with gel-clot method, the only difference is that in this method the test samples with the lysate are placed in a microplate and after filling the microplate wells, the microplate is placed in the spectrophotometer.

The conditions for a status pass or curve acceptability are as follows:

1. The absolute value of the correlation coefficient must be greater than or equal to 0.9.

2. The Correlation Coefficient (CV) expressed as a percentage should be lower than 10%.

The software results in this study have the status 'pass', as shown in Table 7.

The selected endotoxin concentration at or near the middle of the endotoxin standard curve, needed for the test for interfering factors was: 1; 0.1 and 0.01 EU/mL.

Criteria for the interference test are met in the following conditions:

1. Negative controls should be negative;
2. The correlation coefficient must be ≥0.980;

The percentage of spike recovery should be within the limits of 50-200% (if it is lower than 50%, the product inhibits the reaction, if it is higher than 200%, it stimulates the reaction).

In this study, the software results have the status 'pass', with results for working dilution of 1:10. A set of interfering factors was determined, which helped us obtain results for PPC recovery of 80% for dilution of 1:8 and PPC recovery of 103% for dilution of 1:16. A greater dilution of 1:10 was made to determine if similar results for PPC recovery could be obtained (even with a dilution of 1:8 and 1:16, it was within acceptable limits of 50-200%). For the 1:10 dilution, PPC recovery of 100% was generated and it was chosen as a working concentration for future templates.

Main test (Turbidimetric kinetic LAL method)

The test for confirming lysate sensitivity (standard curve) and absence of interfering factors is done once and it is suitable for the entire batch of lysate i.e. for all the batches of the product.

In this study, three batches of 0.9% NaCl solution for infusion were used as test preparations. There are two test samples for each batch, one from the beginning and one from the end of batch production. Thus, accordingly from the working dilution of 1:10, four wells in the template were filled (two with spikes, two without spikes) from the beginning and four wells (two with spikes, two without spikes) from the end of production of each individual batch, as shown in Table 8.

Criteria for acceptance of the main test:

1. Negative controls must be negative;
2. The correlation coefficient of the standard curve must be ≥ 0.980;
3. The percentage of spike recovery (parallel mean value) must be within the limits of 50-200%;
4. The mean value of endotoxin concentration of product parallels must be lower than the endotoxin limit confirmed for that product.

After placing the microplate in the photometer and the software reads the results appropriate for the test samples of three different batches of 0.9% NaCl (No. 11, No. 12, No. 27) a "pass" status was obtained, conforming to the PPC recovery which for all samples was within the range of 50-200% (sequenced spectrophotometer results shown in Table 9).

It is important to notice that when reading the results with the turbidimetric kinetic method, a value for the endotoxin concentration was generated. For the infusion solution 0.9% NaCl 500mL (the same batches No. 27 used in the assay with the gel-clot method) endotoxin recovery of 0.0919 and results for endotoxin concentration < 0.100 EU/mL (permissible limit < 0.25 EU/mL) were obtained, compared to the gel clot method where a numerical value for endotoxin concentration could not be obtained, because the gel formation only confirms that it is lower than the permissible endotoxin limit of 0.25 EU/mL.

Table 8. Template for the main test with turbidimetric kinetic LAL method

	1	2	3	4
A	STD3 1	STD3 1	S3 NaCl inf.0.9% 12	S3 NaCl inf.0.9% 12
B	STD2 0.1	STD2 0.1	S3 + NaCl inf.0.9% 12	S3 + NaCl inf.0.9% 12
C	STD1 0.01	STD1 0.01	S4 NaCl inf.0.9% 12	S4 NaCl inf.0.9% 12
D	Blank	Blank	S4 + NaCl inf.0.9% 12	S4 + NaCl inf.0.9% 12
E	S1 NaCl inf.0.9% 11	S1 NaCl inf.0.9% 11	S5 NaCl inf.0.9% 27	S5 NaCl inf.0.9% 27
F	S1+ NaCl inf.0.9% 11	S1 + NaCl inf.0.9% 11	S5 + NaCl inf.0.9% 27	S5 + NaCl inf.0.9% 27
G	S2 NaCl inf.0.9% 11	S2 NaCl inf.0.9% 11	S6 NaCl inf.0.9% 27	S6 NaCl inf.0.9% 27
H	S2 + NaCl inf.0.9% 11	S2 + NaCl inf.0.9% 11	S6 + NaCl inf.0.9% 27	S6 + NaCl inf.0.9% 27

Table 9. Results for the main test with turbidimetric kinetic LAL method

	Batch 1		Batch 2		Batch 3	
Dilution	PPC Recovery [%]	Raw EU	PPC Recovery [%]	Raw EU	PPC Recovery [%]	Raw EU
10	90	0.0901	87	0.0868	87	0.0873
10	95	0.0948	90	0.0898	92	0.0919

Thus, the obvious advantage of using the turbidimetric kinetic method, where all acceptance criteria are visually obtained with a software reader, is providing an easy and simple way of instrumental reading of the results. This ensures the reliability, simplicity and efficiency of the turbidimetric kinetic assay, eliminating the subjective assessment, as the main point in the gel-clot method. Accordingly, from the examination in this study of the three different batches of 0.9% NaCl (No. 11, No. 12, No. 27) that are analyzed with the turbidimetric kinetic method, as opposed to the one batch (No. 27) with gel-clot method in the same time period (one working day for the main procedure), it is evident that the turbidimetric kinetic method requires less time for the analyses of the samples than the gel-clot method.

Therefore, all aspects of the comparative discussion for the suitability of the two different methods (Ph. Eur method A and Ph. Eur method C) for determination of bacterial endotoxins in parenteral solutions, can indicate that the turbidimetric kinetic LAL method (Ph. Eur method C) produces results that can be generated or computed by software. Instrumental reading of the results makes this method faultless, with automated data collection and reporting processes, exact accuracy and precision of results, and less time needed for analyses compared to the gel-clot LAL method which uses manual handling of the assay and mathematically determined results, without obtaining an exact numerical value. The turbidimetric kinetic LAL method can be suitable in laboratories that frequently process large numbers of samples (allowing for the 96- well plate that the assay is performed in). Additionally, the critical aspects of the applied methods for determination of BE in solutions for infusion indicate that the gel-clot LAL method is less expensive in terms of equipment, although in both methods it is of equal importance to ensure that the analyst is well qualified for carrying out the prescribed procedures.

Conclusion

In this study, a comparative analysis of the results from two different LAL methods (Ph.Eur LAL Method A and LAL Method C test) for determination of bacterial endotoxins in 0.9% NaCl parenteral solution on a small - scale production in a clinical hospital was performed with the critical review of the suitability of each method.

The conventional gel-clot LAL method can be recommended due to its economic sustainability in terms of equipment, the method is simple to perform and it is suitable for simple products such as infusions, however it is less sensitive and it requires a higher technician involvement compared to the turbidimetric kinetic method. The turbidimetric kinetic method is also suitable for determination of endotoxins in parenteral solutions on a small-scale production because of its advantages such as: less time for analysis, increased sensitivity (0.01 EU/mL - 100 EU/mL), its quantitative character and instrumental reading, which eliminates human variability in endpoint assessments. Therefore, the obtained results and discussion on all critical aspects indicated that both methods can be used for determination of bacterial endotoxins in parenteral solutions, produced on a small scale.

Thus, evaluation of the two different LAL methods - gel-clot and turbidimetric kinetic method in this study supplied with a comprehensive overview of advantages and disadvantages of both methods along with a comparison of their appropriateness, allows small - scale laboratories to streamline the decision- making process for selecting a method founded on their resources and needs.

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Резиме

Определување на бактериски ендотоксини во парентерални раствори произведени во клиничка болница

Рената Божиновска Поповска[1], Татјана Цатевска[2], Бранислава Јанева[3], Јелена Ацевска[4], Анета Димитровска[4]

[1]Клиничка болница "Др. Трифун Пановски", Партизанска бб, 7000 Битола, Р. Северна Македонија
[2]Алкалоид АД, бул. Александар Македонски бр. 12, 1000 Скопје, Р. Северна Македонија
[3]ИЈЗ – Институт за јавно здравје, 50-та Дивизија бр. 6, 1000 Скопје, Р. Северна Македонија
[4]Фармацевтски факултет, Универзитет „Св. Кирил и Методиј", Мајка Тереза 47, 1000 Скопје, Р. Северна Македонија

Клучни зборови: Определување на бактериски ендотоксини, гел-клот ЛАЛ метод, турбидиметриски кинетички метод, производство на мали серии

Во трудот се евалуирани два различни ЛАЛ тестови за определување на бактериски ендотоксини во парентерални раствори произведени како мали серии во клиничка болница. Во студијата се применети гел-клот метод и турбидиметриски кинетички метод, како техники за определување на ендотоксини што потекнуваат од Грам негативни бактерии во инфузионен раствор од 0,9% NaCl. Притоа е користен ист принцип, но различни официјални методи за определување на ендотоксинска концентрација. Даден е посебен акцент и осврт на предностите, односно негативностите кои се јавуваат при имплементација на методите, со даден детален опис на постапките, нивна споредба што ќе помогне при донесувањето на одлука како и препорака кој од дадените методи е попогоден во согласност со направените испитувања. Препораката за користење на конвенционалниот гел-клот ЛАЛ метод, како едноставен метод за определување на ендотоксини, е во согласност со неговата економска погодност во однос на опремата. Но и турбидиметрискиот кинетички метод е погоден за испитување на ендотоксини во мало производство, поради неговите предности: поголема сензитивност, квантитативен карактер, инструментално читање на резултатите, со што се исклучува човечкиот фактор што може да доведе до варијабилни резултати и субјективност во текот или на крајот на испитувањето.

Според тоа, добиените резултати и дискусијата со критичните аспекти околу предложените методи индицира можност за користење и на двата ЛАЛ методи за определување на бакетриски ендотоксини во мали серии на парентерални раствори, но и дава значаен придонес за малите лаборатории при донесувањето на правилна одлука при изборот на методот, согласно нивните ресурси и побарувања.