

Sterility Test Validation of Gentamicin Injection: Overcoming Antimicrobial Activity *Via* Membrane Filtration with Magnesium Chloride

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ABSTRACT

Introduction: Gentamicin may inhibit microbial growth during sterility testing, potentially interfering with the detection of microorganisms. This study evaluated the suitability of a membrane filtration procedure designed to overcome the antimicrobial activity of gentamicin injection.

Materials and Methods: Gentamicin injection was tested by membrane filtration using a 0.45 µm low-binding PVDF membrane. The procedure included pre-wetting with Fluid A, treatment with 0.5 M magnesium chloride, and subsequent rinsing with Fluid D and Fluid A. Method suitability was assessed using low levels of specified challenge microorganisms, together with positive and negative controls and growth promotion testing.

Results: Growth of the specified challenge microorganisms was observed in the presence of the product following magnesium chloride treatment and rinsing. Positive controls demonstrated growth, while negative controls remained free from microbial growth.

Conclusion: Under the evaluated conditions, the procedure sufficiently reduced gentamicin-associated antimicrobial inhibition to permit microbial detection. The findings support the suitability of the optimized membrane filtration procedure for sterility testing of the investigated gentamicin injection formulation.

Keywords: Gentamicin, Sterility Testing, Membrane Filtration, Magnesium Chloride, Method Suitability

INTRODUCTION

Sterility is a critical quality attribute of parenteral pharmaceutical products because these preparations are administered by routes that bypass several of the body's natural protective barriers. Consequently, sterility testing forms an essential component of the microbiological quality control of injectable medicinal products [1–3].

Gentamicin is an aminoglycoside antibiotic with potent antibacterial activity and is widely used in the treatment of serious infections

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caused by susceptible microorganisms [4]. However, its antimicrobial activity presents a particular challenge during sterility testing. Residual gentamicin associated with the test sample or membrane may inhibit the growth of challenge microorganisms, potentially resulting in false-negative results during method suitability testing. Therefore, the antimicrobial activity of the product must be adequately removed or sufficiently inactivated to demonstrate that the sterility test method is capable of detecting microbial contamination [1-3].

Pharmacopoeial sterility tests are principally performed by membrane filtration or direct inoculation [1,2]. For filterable aqueous products with antimicrobial properties, membrane filtration is generally preferred because the product can be removed from the membrane by suitable rinsing before incubation in the required culture media [1,2]. For strongly antimicrobial products such as gentamicin injection, an appropriate rinsing and or inactivation strategy may be required to sufficiently overcome inhibitory activity [1-3].

The use of an appropriate inactivating or antagonizing agent may therefore be necessary during method development and suitability testing [1-3]. Previous studies have demonstrated that divalent cations, including Mg^{2+} , can influence the antibacterial activity of aminoglycosides by interfering with their interaction with bacterial cells [5,6]. Based on these observations, magnesium chloride was investigated in the present study as part of the membrane-filtration strategy to overcome the residual antimicrobial activity of gentamicin and permit recovery of the pharmacopoeial challenge microorganisms.

The aim of this study was to develop and to demonstrate the suitability of a sterility testing procedure for gentamicin injection using membrane filtration combined with magnesium chloride to overcome residual antimicrobial activity. The suitability of the method was assessed by demonstrating recovery of the specified challenge microorganisms in the presence of the product under the established test conditions [1-3].

MATERIALS AND METHODS

Reagents and Instrumentations

In this study, the following were used: Analytical balance Explorer OHAUS, Euroclone Top safe microbiology safety cabinet, Petri plates Isolab, IKA MS 3 Basic Vortex, Tuttinauer

model 3850 MLV, INE 800 Memmert incubator, UM 800 Memmert incubator, pH meter Ultrabasic UB-10, Denver Instrument, Millipore Steritest Compact Pump (Merck), Steritest Devices for antibiotics (Merck Millipore);

Three batches of gentamicin injection Batch nos. 2406; 2504; 2513 were manufactured by ProfarmaSh.a), Trypticasein Soy Agar EP/USP/ISO (medium Cat No.1068 by Condalab, Spain), Sabouraud Dextrose Agar 4% medium (Cat. No.1024 by Condalab, Spain); Fluid A (Cat. No. 638042) and Fluid D (Cat. no. 638043), Thioglycolate Medium (FTM, Cat. No. 638040 and Soybean-Casein Digest Medium (SBCDM, Cat. No.622787) were purchased from Merck Millipore, USA); Lyophilized bacterial cultures (containing 10-100 CFU/mL) of *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), *Clostridium sporogenes* (ATCC 19404), and fungal strains *Candida albicans* (ATCC 10231) and *Aspergillus brasiliensis* (ATCC 16404) were obtained as EZ- Accu Shot™ quantitative microorganism preparation (Microbiologics, Inc., St. Cloud, MN, USA) and were prepared according to the manufacturer's instructions to obtain the challenge inoculum. The microorganism *Micrococcus luteus*, an environmental isolate recovered from the cleanroom environment, was also included in the study.

Sterilization

All sterility tests were performed aseptically in an ISO Class 5 biological safety cabinet (BSC) to minimize the risk of environmental contamination during sample handling and filtration [7]. Trypticasein Soy Agar and Sabouraud Dextrose Agar were sterilized at 121 °C for 15 minutes using a steam autoclave. Sterility testing of gentamicin injection was performed using the membrane filtration method in accordance with USP <71> and Ph. Eur. 2.6.1 [1,2]. The procedure was carried out under aseptic conditions using sterile membrane filters with a nominal pore size of 0.45 µm. A defined volume of sample was filtered, followed by appropriate rinsing of the membrane to reduce residual antimicrobial activity [1-3]. The membranes were transferred to Fluid Thioglycolate Medium (FTM) and Soybean-Casein Digest Medium (SCDM) and incubated under the specified compendial conditions for not less than 14 days [1,2]. The media were examined for evidence of microbial growth. Appropriate controls and growth promotion testing were included to demonstrate the suitability of the test procedure and culture media [1-3].

Growth Media Control

Sterility testing was performed using compendial culture media suitable for detecting a broad range of microorganisms [1,2]. Before method suitability testing, growth promotion and sterility controls were performed to verify the suitability of the culture media [1–3]. Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) were used for growth promotion, isolation, and confirmation of the viability of the challenge microorganisms. The media were examined for sterility and growth-promoting properties before use.

Growth promotion testing was performed using appropriate reference microorganisms at low inoculum levels to demonstrate the ability of the media to support microbial growth [1,2]. TSA was used to support bacterial growth, whereas SDA was used for yeast and mold growth. The media were incubated under appropriate conditions according to the microorganisms being evaluated.

Fluid Thioglycollate Medium (FTM) and Soybean–Casein Digest Medium (SCDM) were used as the sterility-test media. FTM, intended primarily for the detection of anaerobic bacteria but also capable of supporting aerobic bacteria, was incubated at 30–35 °C. SCDM, suitable for the detection of aerobic bacteria and fungi, was incubated at 20–25 °C [1,2]. Both media underwent growth promotion testing before use to confirm their ability to support the growth of the specified microorganisms [1,2].

Media sterility controls were included to verify the absence of contamination before testing. The appearance and pH of the media were also checked against the applicable specifications, and the batch numbers and shelf-life information were documented. Tables 1 and 2 summarize the culture media and microorganisms used in the study.

Table 1. Recommended microbial cultures for TSA and SDA

Name of the Media	Microbial Cultures Recommended	Incubation Temperature (°C)
Aerobic Bacteria:		
Tryptic Soy Agar (TSA)	Staphylococcus aureus ATCC 6538	30-35
	Bacillus subtilis ATCC 6633	
	Pseudomonas aeruginosa ATCC 9027	
Fungi:		
Tryptic Soy Agar (TSA)	Candida albicans ATCC 10231	30-35
	Aspergillus brasiliensis ATCC 16404	
Fungi:		
Sabouraud Dextrose Agar (SDA)	Candida albicans ATCC 10231	20-25
	Aspergillus brasiliensis ATCC 16404	

Table 2. Media and recommended microbial cultures for sterility testing

Name of the Media	Microbial Cultures Recommended	Incubation Temperature (°C)
	Aerobic Bacteria:	
Fluid Thioglycolate Medium (FTG medium/FTM)	<i>Staphylococcus aureus</i> ATCC 6538	30-35
	<i>Bacillus subtilis</i> ATCC 6633	
	<i>Pseudomonas aeruginosa</i> ATCC 9027	
	Anaerobic Bacteria:	
	<i>Clostridium sporogenes</i> ATCC 19404	
	Aerobic Bacteria:	
Soybean-Casein Digest Medium (SCDM)	<i>Bacillus subtilis</i> ATCC 6633	20-25
	Fungi:	
	<i>Candida albicans</i> ATCC 10231	20-25
	<i>Aspergillus brasiliensis</i> ATCC 16404	
Soybean-Casein Digest Medium (SCDM)	Environment Isolate	20-25
	<i>Micrococcus luteus</i>	
	Environment Isolate	
Fluid Thioglycolate Medium (FTG medium /FTM)	<i>Micrococcus luteus</i>	30-35

Tested Samples

Sixty ampoules of gentamicin injection (80 mg/2 mL) from three different batches (20 ampoules per batch) were included in the study. Before testing, the ampoules were visually inspected to confirm container-closure integrity and the absence of visible particulate matter. The external surfaces of the ampoules were appropriately disinfected, and all subsequent manipulations, including opening the ampoules and transferring their contents, were performed aseptically in an ISO Class 5 environment using sterile equipment and appropriate aseptic techniques [1,2,7].

Method Suitability

Method suitability testing was performed to demonstrate that the antimicrobial activity of gentamicin did not interfere with the detection of the specified challenge microorganisms under the established test conditions [1–3]. The product was challenged separately with low levels (10–100 CFU) of specified microorganisms representing aerobic bacteria,

anaerobic bacteria, yeasts, and molds, and was subsequently subjected to the membrane filtration procedure [1–3].

Micrococcus luteus, an environmental isolate recovered from the cleanroom environment, was included as an additional challenge organism to assess recovery of a representative environmental microorganism during method suitability testing. Recovery was considered acceptable when visible growth was observed in the positive product control (PPC) within the specified observation period, comparable with the corresponding positive control (PC).

A 0.45 µm low-binding polyvinylidene difluoride (PVDF) membrane was used for filtration. The membrane was pre-wetted with 50 mL of Fluid A, followed by filtration of the gentamicin injection. The membrane was then treated with 200 mL of 0.5 M magnesium chloride, followed by rinsing with 200 mL of Fluid D and 100 mL of Fluid A. Thus, the magnesium chloride treatment and subsequent rinsing steps comprised a total volume of 500 mL.

Growth of the challenge microorganisms in the presence of the product was compared with the corresponding positive controls to determine whether residual antimicrobial activity interfered with microbial detection. Following filtration and rinsing, the membranes were transferred to the appropriate culture media and incubated under the specified compendial conditions [1–3].

Validation Parameters

Method suitability was assessed using predefined parameters to demonstrate the suitability of the sterility testing procedure for gentamicin injection [1–3]. The assessment included recovery of the specified challenge microorganisms, effectiveness of the procedure in overcoming gentamicin-associated antimicrobial inhibition, membrane integrity, sterility controls, and growth promotion of the culture media.

The effectiveness of the inactivation and rinsing procedure was assessed by comparing growth of the challenge microorganisms in the presence of the product with the corresponding positive controls [1–3]. Membrane integrity and sterility controls were included to verify the integrity of the filtration system and to ensure that the media and diluents remained sterile throughout the procedure. Growth promotion testing was performed to confirm that the culture

media supported the growth of the specified microorganisms [1,2].

The method was considered suitable when growth of the specified challenge microorganisms was observed in the presence of the product, the corresponding positive controls demonstrated growth, the negative controls remained free from microbial growth, and the culture media fulfilled the applicable growth-promotion requirements [1–3].

RESULTS

Growth promotion testing of TSA and SDA

Growth promotion testing confirmed that Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) supported growth of the specified challenge microorganisms. Each microorganism was inoculated at a concentration of 10-100 cfu/ml. All test microorganisms showed visible growth after incubation, with colony morphology consistent with each organism's expected characteristics (Tables 3 and 4). No microbial growth was detected in the uninoculated control media (negative control, NC), confirming the sterility of the prepared media. These findings show that both TSA and SDA met the acceptance criteria for growth promotion testing and confirm the viability of the challenge organisms used in the validation.

Table 3: Observations of microbial growth of challenged microorganisms on TSA at 35°C

Culture/media	Description	Day of Observation		
		Day 1	Day 2	Day 3
<i>S. aureus</i> /TSA	PC	-	+	+
<i>P. aeruginosa</i> /TSA	PC	-	+	+
<i>B. subtilis</i> /TSA	PC	+	+	+
<i>Candida albicans</i> /TSA	PC	-	-	+
<i>Aspergillus brasiliensis</i> / TSA	PC	-	+	+
Negative control NC		-	-	-

Keys: PC-Positive Control; +: Growth observed; -: Growth not observed

Table 4: Observations of microbial growth of challenged microorganisms on SDA at 25°C

Culture/media	Description	Date of Observation				
		Day 1	Day 2	Day 3	Day 4	Day 5
<i>Candida albicans</i> /SDA	PC	-	-	+	+	+
<i>Aspergillus brasiliensis</i> /SDA	PC	-	-	+	+	+
Negative control NC		-	-	-	-	-

Keys: PC-Positive Control; +: Growth observed; -: Growth not observed

Results of bacteriostasis and fungistasis studies

Bacteriostasis and fungistasis testings demonstrated the successful recovery of all specified bacterial and fungal test organisms in the presence of the product following membrane filtration and appropriate neutralization. Visible bacterial growth was observed within the prescribed incubation period in Fluid Thioglycolate Medium (Table 5), whereas characteristic fungal growth was detected in Soybean-Casein Digest Medium (Table 6). No inhibition of microbial growth was observed compared to the positive controls, and no growth was detected in the negative controls.

For *Micrococcus luteus*, visible growth was observed in both the positive control (PC) and positive product control (PPC) from Day 2, demonstrating successful recovery of the environmental isolate under the method suitability conditions (Table 5). These results confirm the absence of bacteriostatic and fungistatic effects and validate the sterility testing method for the reliable detection of microbial contamination.

Table 5: Bacteriostasis Study Results

Culture	Description	Day of Observation		
		1st	2nd	3rd
<i>S. aureus</i>	PC	-	+	+
	PPC	-	+	+
<i>B. subtilis</i>	PC	-	+	+
	PPC	-	+	+
<i>P. aeruginosa</i>	PC	-	+	+
	PPC	-	+	+
<i>C. sporogenes</i>	PC	-	+	+
	PPC	-	+	+
<i>Micrococcus luteus</i>	PC	-	+	+
	PPC	-	+	+
Negative control NC		-	-	-

Keys: PC - Positive Control; PPC – Positive Product Control
 +: Growth observed; - : Growth not observed; NC-negative control

Table 6: Fungistasis Study Results

Culture	Description	Day of Observation				
		1st	2nd	3rd	4th	5th
<i>C. albicans</i>	PC	-	-	+	+	+
	PPC	-	-	+	+	+
<i>A. brasiliensis</i>	PC	-	+	+	+	+
	PPC	-	-	+	+	+
Negative control NC		-	-	-	-	-

Keys: PC - Positive Control; PPC - Positive Product Control
 +: Growth observed; - : Growth not observed; NC- negative control

Visible microbial growth was observed in all inoculated test samples (PPC) and corresponding positive controls (PC) within the specified observation periods. The PPC showed comparable visible growth (Figure 1) to the Positive Control (PC) within 5 days, while no growth was detected in the negative controls, demonstrating the absence of inhibitory effects and confirming the suitability of the membrane filtration method for routine sterility testing of gentamicin injection.

The results demonstrated that 0.5M magnesium chloride and Fluid D successfully neutralized the antimicrobial activity of gentamicin and cleared all antimicrobial residues from the membrane, allowing recovery and robust growth of all test organisms within acceptable timeframes. No inhibition of microbial growth was observed in the presence of the drug-inactivator mixture. Negative controls of each medium and test specimens from three batches were examined after 14 days and showed no microbial growth (Table 7). These results confirmed that the three batches of gentamicin injection were sterile after 14 days of incubation.

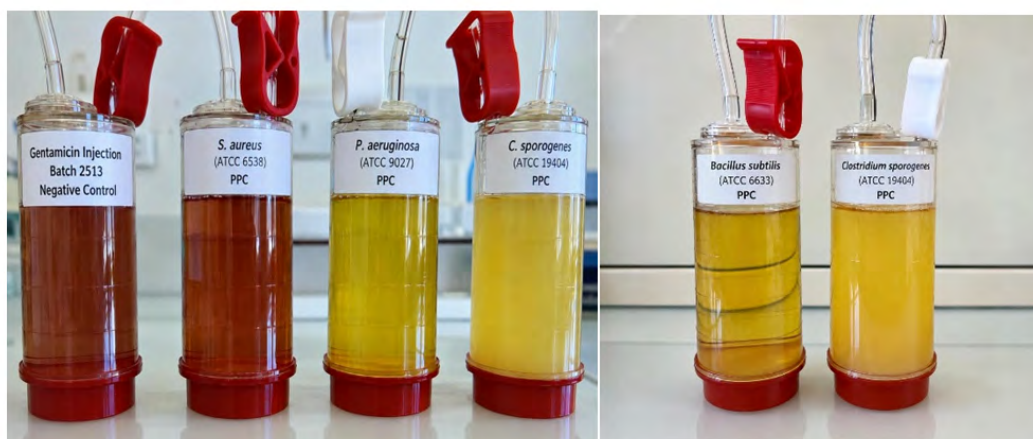


Figure 1: Growth of bacteria, mold, and fungi on Positive Product Control (PPC) after 3 and 5 days.

Table 7: Sterility Test Report

Day	FTM (Incubation at 30-35°C)				SCDM (Incubation at 20-25°C)			
	Positive Control	Negative control	Positive Product Control	Test specimen	Positive Control	Negative control	Positive Product Control	Test specimen
1	+	-	-	-	-	-	-	-
2	+	-	+	-	+	-	-	-
3	+	-	+	-	+	-	+	-
4	NA	-	NA	-	+	-	+	-
5	NA	-	NA	-	+	-	+	-
6	NA	-	NA	-	NA	-	NA	-
7	NA	-	NA	-	NA	-	NA	-
8	NA	-	NA	-	NA	-	NA	-
9	NA	-	NA	-	NA	-	NA	-
10	NA	-	NA	-	NA	-	NA	-
11	NA	-	NA	-	NA	-	NA	-
12	NA	-	NA	-	NA	-	NA	-
13	NA	-	NA	-	NA	-	NA	-
14	NA	-	NA	-	NA	-	NA	-

Note: +=Growth observed;- =Growth not observed; NA=Not applicable after confirmation of adequate microbial growth in the control cultures.

Following visual confirmation of adequate growth of each microorganism (Day 3 for bacteria and Day 5 for fungi/mold), the corresponding canisters were removed from the incubators. Negative controls for each medium and test specimens from three lots were examined for 14 days.

The study confirmed that magnesium chloride is an effective and reliable inactivator for the sterility testing of gentamicin injection. The validated method ensures accuracy, sensitivity, and compliance with regulatory sterility requirements, supporting its routine use for product quality control.

DISCUSSION

Gentamicin presents a particular challenge during sterility testing because its intrinsic antibacterial activity may inhibit microbial growth and consequently interfere with the demonstration of method suitability. For antimicrobial pharmaceutical products, the suitability of the compendial method should be demonstrated by showing that the specified challenge microorganisms can grow in the presence of the product. Where antimicrobial activity interferes with microbial recovery, appropriate neutralization, membrane filtration, dilution, rinsing, or a combination of these approaches may be applied [1–3].

Membrane filtration was selected for the present study because gentamicin injection is a filterable aqueous preparation and because this approach facilitates separation of microorganisms from the antimicrobial product. USP <71> and Ph. Eur. 2.6.1 describe membrane filtration as an established approach for products that can be filtered and provide for appropriate measures to overcome antimicrobial activity [1,2]. USP <1227> also provides principles for demonstrating microbial recovery from products possessing antimicrobial activity [3]. Previous research has demonstrated the use of membrane filtration for sterility testing of a gentamicin-containing pharmaceutical preparation, although the formulation and antimicrobial-inactivation procedure differed from those used in the present study [10]. Swenson et al. [10] studied gentamicin-containing liposomes and used dilution with Triton X-100 followed by USP membrane filtration, making it relevant supporting evidence without implying that the procedure was identical to the present method.

In the present study, the optimized procedure incorporated a 0.45 µm low-binding PVDF membrane, pre-wetting with

Fluid A, treatment with 0.5 M magnesium chloride (200 mL), followed by rinsing with Fluid D (200 mL) and Fluid A (100 mL), giving a total treatment and rinsing volume of 500 mL. Under these experimental conditions, growth of the specified challenge microorganisms was observed in the presence of the product. These findings indicate that gentamicin-associated antimicrobial inhibition was sufficiently reduced to permit microbial detection and support the suitability of the procedure for the tested gentamicin injection formulation.

The use of magnesium chloride has a scientific rationale because divalent cations can influence the interaction and antibacterial activity of aminoglycoside antibiotics. Previous studies have reported effects of divalent cations and ionic conditions on aminoglycoside activity, including gentamicin [5,6]. The need to remove or inactivate residual aminoglycoside activity during microbiological procedures has also been demonstrated in previous experimental work involving gentamicin [11]. Den Hollander et al. [11] developed an enzymatic method for removing the activity of gentamicin and tobramycin and compared it with dilution, illustrating the importance of controlling residual aminoglycoside activity in microbiological measurements. However, these findings should not be interpreted as demonstrating that 0.5 M magnesium chloride universally neutralizes gentamicin. The effectiveness observed in the present study should be considered specific to the formulation, membrane system, magnesium chloride concentration, rinse volumes, microorganisms, and experimental conditions evaluated.

Method suitability was further supported by growth of the specified challenge microorganisms at low inoculum levels (≤ 100 CFU) and comparison with the corresponding positive controls. The absence of growth in negative controls indicated that contamination was not introduced by the test system or materials, while growth promotion testing demonstrated the ability of the culture media to support the specified microorganisms. These findings are consistent with the principles of compendial sterility testing and microbial method suitability [1–3].

An important limitation of the conventional compendial sterility test is its relatively long incubation period. USP <71> and Ph. Eur. 2.6.1 require incubation for not less than 14 days, meaning that final sterility results are not immediately available [1,2]. Rapid microbiological methods (RMMs)

may shorten the time required for microbial detection and provide earlier results. Nevertheless, alternative microbiological methods require appropriate validation and demonstration that their performance is suitable for the intended application [8,9].

Despite this limitation, the conventional membrane filtration method was selected for the present study because it is an established pharmacopoeial approach, was applicable to the gentamicin injection under investigation, and could be performed using the laboratory's existing equipment and infrastructure. Implementation of some RMM technologies may require specialized instrumentation, additional method development, qualification, and validation [8,9]. Therefore, the conventional method represented a practical and cost-conscious approach for the present study, although its longer time to result remains an important limitation. Future studies could evaluate validated RMMs as potential alternatives for reducing detection time while maintaining appropriate microbial detection capability.

Overall, the findings demonstrate that membrane filtration combined with 0.5 M magnesium chloride treatment and appropriate rinsing sufficiently reduced gentamicin-associated antimicrobial inhibition under the evaluated conditions. The results therefore support the suitability of the optimized procedure for sterility testing of the investigated gentamicin injection formulation.

CONCLUSION

This study demonstrated the suitability of a membrane filtration procedure for sterility testing of gentamicin injection. The optimized procedure used a 0.45 μm low-binding PVDF membrane, pre-wetting with Fluid A, treatment with 200 mL of 0.5 M magnesium chloride, followed by rinsing with 200 mL of Fluid D and 100 mL of Fluid A. Method suitability testing demonstrated growth of the specified compendial challenge microorganisms in the presence of the product, while the corresponding positive controls showed growth and the negative controls remained free from contamination. Growth promotion testing further confirmed the suitability of the culture media.

Under the evaluated experimental conditions, the combination of magnesium chloride treatment and membrane rinsing sufficiently reduced gentamicin-associated antimicrobial inhibition to permit microbial

detection. The findings therefore support the suitability of the optimized membrane filtration procedure for sterility testing of the gentamicin injection formulation investigated in this study. The conventional compendial approach remains practical and cost-conscious, although its 14-day incubation period represents an important limitation compared with potentially faster rapid microbiological methods.

Recommendations

Sterility testing of antibiotic-containing injections should use neutralization and rinsing procedures to overcome antimicrobial properties, test method suitability with the selected microorganisms, and regularly test membrane integrity and growth promotion. The neutralizer concentration and rinse volumes should be optimized for maximal efficiency.

STATEMENTS

Conflict of Interest

The authors declare no conflicts of interest regarding this manuscript.

REFERENCES

1. European Directorate for the Quality of Medicines & HealthCare. (1963). European Pharmacopoeia. General chapter 2.6.1: Sterility. Strasbourg: Council of Europe.
2. United States Pharmacopeia. USP-NF. (2002). General chapter <71>: Sterility tests. Rockville (MD): United States Pharmacopeial Convention.
3. United States Pharmacopeia. USP-NF. (2019). General chapter <1227>: Validation of microbial recovery from pharmacopeial articles. Rockville (MD): United States Pharmacopeial Convention.
4. Panpharma UK Ltd. (2025). Gentamicin 40 mg/mL solution for injection/infusion: Summary of Product Characteristics (SmPC) [Internet]. Electronic Medicines Compendium.
5. Ramirez-Ronda CH, Holmes RK, Sanford JP. (1975). Effects of divalent cations on binding of aminoglycoside antibiotics to human serum proteins and to bacteria. *Antimicrob Agents Chemother.* 7(3):239-245.
6. Beggs WH, Andrews FA. (1976). Role of ionic strength in salt antagonism of aminoglycoside action on *Escherichia*

- coli and *Pseudomonas aeruginosa*. *J Infect Dis*. 134(5):500-504.
7. International Organization for Standardization. (2015). ISO 14644-1:2015. Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration. Geneva: International Organization for Standardization.
 8. United States Pharmacopeia. USP–NF. General chapter <1071>: Rapid microbial tests for release of sterile short-life products: A risk-based approach. Rockville (MD): United States Pharmacopeial Convention.
 9. European Directorate for the Quality of Medicines & HealthCare. European Pharmacopoeia. General chapter 5.1.6: Alternative methods for control of microbiological quality. Strasbourg: Council of Europe.
 10. Swenson CE, Reifsnnyder DA, Frazier DK, Stewart KA. (1992). Development and validation of a sterility test method for large, gentamicin-containing liposomes. *J Liposome Res*. 2(1):43-48.
 11. den Hollander JG, Mouton JW, Bakker-Woudenberg IAJM, Vleggaar FP, van Goor MPJ, et al. (1996). Enzymatic method for inactivation of aminoglycosides during measurement of postantibiotic effect. *Antimicrob Agents Chemother*. 40(2):488-490.