REVIEW ARTICLE

METHOD DECISION FOR DETERMINING SPECIFIC MICROORGANISMS IN PHARMACEUTICAL PRODUCTS: AN OVERVIEW

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Article Info:

Abstract

In the direction of microbiological quality control analysis in pharmaceutical products, determining the microbiological load of the product at the end-use stage is very important for human health. Quality control parameters in pharmaceutical products vary according to the structure of the type of product and administration route. In this context, according to the pharmacopoeias, parenteral products and eye drops are classified as sterile products and the other group of pharmaceuticals are classified as non-sterile products. However, non-sterile pharmaceuticals also must have a certain microbiological quality. For this reason, the pharmaceuticals should have a certain microbiological load and should not contain defined microorganisms specified to its type. Since the control of the microbiological quality of the products is important for safety, it should be determined by quality control analysis. In this study, standard methods used to detect specific microorganism in pharmaceutical products were compared. Application steps in standard methods and identification tests of specific microorganisms were examined. In addition, studies that are alternative to standard methods were evaluated.
Keywords: ISO standards, method suitability study, microbiological quality control, pharmaceutical products, pharmacopoeial methods.

INTRODUCTION

Microbiological quality control analysis in pharmaceutical products is one of the most fundamental steps in determining the quality of the pharmaceuticals. The microbiological burden of the final pharmaceutical product is important in terms of safe use and product effectiveness. In addition to the microbiological controls carried out during the manufacturing stages, ensuring the microbiological quality of the final product is the most fundamental issue in determining the quality of both the manufacturing system and the pharmaceutical product. Microbiological quality is one of the main issues of Good Manufacturing Practices and quality assurance of pharmaceutical products. Since the efficacy and safety of a product depends on its pharmaceutical quality, it is necessary to reach the quality target both in the related manufacturing stages and in the finished product. Control and proving of microbial quality depends on the application of defined tests. Microbiological quality control tests for raw materials and finished products; involves microbial enumeration tests for total aerobic microbial counts (TAMC), total yeast and mold counts (TYMC), and detection of specific microorganism. Specific microorganisms; *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella spp., Candida albicans* and bile-tolerant Gram negative bacteria. Ensuring microbiological quality is very important in the use of the product due to its toxic or infectious effect. Low levels of pathogenic microorganisms can cause serious effects[1]. Many requirements and analysis methods have been defined in pharmacopoeias for microbiological analysis of pharmaceutical products. United States Pharmacopoeia (USP) and European Pharmacopoeia (EP) can be utilized to determine the microbiological quality of pharmacological products. According to the pharmacopoeia requirements, the products should have a certain bioburden and should not contain specific microorganisms. The microbiological quality of the products varies according to the type of the product[2]. In this review, microbiological quality control of pharmaceutical products is given in terms of method decision and determination of specific microorganisms, which is necessary for a comprehensive microbiological quality evaluation of pharmaceuticals.

Microbiological quality control methods in Pharmacopoieas

Internationally accepted pharmacopoeia methods are used in determining microbiological quality control. According to the EP and USP, which specifies the analysis methods and requirements of internationally accepted pharmaceutical products, the evaluation of microbiological quality in pharmaceutical products varies according to their type and route of administration. According to EP and USP, pharmaceutical products are examined in two groups as sterile and non-sterile products. Ocular and injectable products which must be sterile are subject to the microbiological quality control tests that are specified in USP 71 and EP 2.6.1 Sterility Test Section. The quality control tests of products that are not necessarily sterile are specified in USP 1111 and EP 5.1.4 Microbial Quality of Non-Sterile Pharmaceutical Preparations and Substances for Pharmaceutical Use. Quality control tests of non-sterile pharmaceutical products are subject to USP 1111 and EP 5.1.4. Bioburden is determined according to USP 61 and EP. Microbial Enumeration Tests section; specific microorganisms in pharmaceutical products are determined according to the section of USP 62 and EP **Tests For Specified Microorganisms section**

Microbiological quality control parameters and limits according to the administration route of pharmaceuticals are shown in Table 1[2,3].

Microbiological bioburden and specific micro-organisms that should not be present in the product vary according to the method of use and target. Microbiological bioburden is determined quantitatively by determining TAMC and TMYC. In addition to the microbiological bioburden, the product should not contain specific microorganisms according to the method and target of the pharmaceutical product. Specific microorganism detection is a qualitative test. While determining the microbiological quality of the final product and raw materials, the parameters specified in Table 1 should be analyzed and should be within the specified limits. While determining the microbiological quality of the product, both the microbiological and the specific microorganism quality in grams or ml of the pharmaceutical preparation are evaluated[2,3].

Table 1: Microbiological quality control parameters and limits for non-sterile pharmaceutical[2,3].

Route of Administration	TAMC (cfu/g or cfu/ml)	TMYC (cfu/g or cfu/ml)	Specified Microorganism(s)
Nonaqueous preparations for oral administration	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 ml)
Aqueous preparations for oral administration	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 ml)
Rectal administration	10^3	10^2	-
Oromucosal administration	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
Gingival administarion	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
Cutaneous administration	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
Nasal administration	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
Auricular administration	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
Vaginal administration	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) Absence of *Candida albicans* (1 g or 1 ml)
Transdermal administration (Transdermal patches)	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
Inhaler administration	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) Absence of bile-tolerant Gram-negative bacteria (1 g or 1 ml)

Specific microorganism detection in pharmaceuti-cals- Specific microorganisms are investigated in order to determine the microbiological quality of pharmaceutical products. Recovery of media used for specific microorganism detection is very low. For this reason, the pre-enrichment process facilitates the detection of the sought microorganism to examine the specific microorganisms. In internationally used methods, pre-enrichment is routinely applied. Pre-enrichment is the process that increasing the level of the specific microorganism. However, in addition to the microorganism sought in this process, unwanted microorganisms are also enriched. This may cause a false positive result. Bioburden, whose level is increased by the pre-enrichment process, is reduced to the sought microorganism by selective enrichment and

selective subculture. Pharmacopoeia methods are generally used in the analysis of pharmaceutical products. However, the International Organization for Standardization (ISO) methods can also be used because the specific microorganisms to be analyzed are similar in cosmetic products[4,5].

Specific microorganisms are generally detected in four steps;

- Non-selective pre-enrichment
- Selective pre-enrichment
- Selective subculture
- Identification

The pharmaceutical product is included in the analysis of specific microorganism detection, according to the groups specified in Table 1. The requirements specified in internationally applied standards meet these working steps. The media and analysis steps used during the studies are similar to the pharmacopoeia methods. Dissolve process is applied to prepare the pharmaceutical flour for the analysis steps. Generally, Buffered Sodium Chloride Peptone Solution pH 7.0, Phosphate Buffer Solution pH 7.2, or Soybean–Casein Digest Broth is used in the dissolve process. If necessary, adjust to pH of 6 to 8. In the dissolve process, 10 g or ml of product is transferred in 90 ml of solvent. A surface-active agent such as 1 g per L of polysorbate 80 may be added to assist in the suspension of poorly wettable substances. For fatty products, the dissolution process is accelerated with polysorbate 80 added to the solvent. Also, after the sample is added to the solvent, if necessary, the solution is heated in a water bath and mixed (not more than 40°C).When a homogeneous solution is obtained, non-selective enrichment can be started. 10 ml sample taken from the obtained solution is used in a non-selective pre-enrichment process[4-7].

Non-selective Pre-enrichment
The non-selective pre-enrichment process is applied to enrich the specific microorganism sought in the analyzed pharmaceutical product. Pre-enrichment is usually done in a medium that can produce all microorganisms. In general, Tryptic Soy Broth (TSB) medium is used for pre-enrichment. Since this medium is a general productive medium, it can produce all microorganisms. In case of an antimicrobial active substance in the content of the analyzed product, the additional chemicals can be added to the medium. 1 g or ml of product analyzed in the pre-enrichment process is incubated in TSB medium at 32.5±2.5°C for 18-24 hours. As a result of incubation, microorganisms in 1 gram of product will develop and be easier to detect. In the detection of bile-tolerant Gram-negative bacteria indicated in the Table 1, 1 gram or ml of the analyzed product is transferred to TSB medium and incubated at 22.5±2.5°C for 2-5 hours. In the detection of *C. albicans* indicated in Table 1, 1 gram or ml of the analyzed product is transferred to Sabouraud Dextrose Broth (SDB) medium and incubated at 32,5±2,5°C for 3-5 days. TSB media can be used as an alternative for *C. albicans* detection[4-7].

Selective Pre-enrichment
Selective pre-enrichment process is the reduction of the microorganism obtained in the non-selective pre

enrichment process. This process is not applied in all microorganism groups as specified in standard methods. It is applied in searching for *E. coli* and bile-tolerant Gram-negative bacteria from specific microorganisms specified in Table 1. For *E. coli*, 1 ml sample taken from the TSB obtained in the non-selective pre-enrichment process is transferred to the MacConkey Broth (MCB) medium. For bile-tolerant Gram-negative bacteria, 1 ml sample taken from the TSB obtained in the non-selective pre-enrichment process is transferred to the Enterobacter Enrichment Broth (EEB) medium. MCB medium is incubated at 43±1°C for 24-48 hours, EEB medium at 32.5±2.5°C for 24-48 hours. Selective subculture for other specific microorganisms is sufficient to detect the microorganism sought. For this reason, there is no need for selective pre-enrichment[4-7].

Selective Subculture
Selective subculture is transferring only the sought microorganism to a medium where it can grow. With this process, only the sought microorganisms are produced and detected. In the selective subculture process, MacConkey Agar (MCA) medium is used for *E. coli* detection and the MCA medium is incubated at 32.5±2.5°C for 18-72 hours; Mannitol Salt Agar (MSA) medium is used for *S. aureus* detection and the MSA medium is incubated at 32.5±2.5°C for 18-72 hours; Cetrimide Agar (CA) medium is used for *P. aeruginosa* detection and the CA medium is incubated at 32.5±2.5°C for 18-72 hours; Sabouraud Dextorose Agar (SDA) medium is used for *C. albicans* detection and the SDA medium is incubated at 32.5±2.5°C for 24-48 hours; Violet Red Bile Glucose Agar (VRBGA) medium is used for bile-tolerant Gram-negative bacteria detection and the VRBGA medium is incubated at 32.5±2.5°C for 18-24 hours. Colonies with typical signs of growth on the plates after incubation are taken for an identification test[4-7]. The above analysis steps have been evaluated in terms of international standards. The comparison is given in Table 2[4-11].

Identification
Identification tests are the process of verifying the positive result obtained. In this process, the colonies detected as positive on the medium are identified biologically, morphologically or biochemically. Identification tests are determination of colony morphology, determination of cellular morphology, Gram staining or other differential staining techniques, and basic biochemical reactions. For the identification test, positive results specified in the standards must be observed. Identification test should be applied to detect *E. coli*, *S. aureus, C. albicans* and *P. aeruginosa* according to EP and USP.

However, the details of the identification tests are not specified. For other specific microorganisms, the presence of colonies during the sub-culturing process is sufficient for detection and typical colonies that should be seen on the media as a result of sub-culturing were not specified. In addition, identification tests in USP are defined by USP 1113 Microbial Characterization, Identification and Strain Typing.

Table 2: Specific microorganism detection assessment according to standards[4-11].

Specific Microorganism	Non-selective pre-enrichment			Selective pre-enrichment			Selective subculture		
	EP 2.6.13	USP 62	ISO 21150 ISO 22718 ISO 22717 ISO 18416	EP 2.6.13	USP 62	ISO 21150, ISO 22718, ISO 22717, ISO 18416	EP 2.6.13	USP 62	ISO 21150, ISO 22718, ISO 22717, ISO 18416
Escherichia coli	TSB 30-35°C for 18-24 hours	TSB 30-35°C for 18-24 hours	Eugon LT 100 Broth[1] 30-35°C for 18-72 hours	MCB 42-44°C for 24-48 hours	MCB 42-44°C for 24-48 hours	N/A	MCA 30-35°C for 18-72 hours	MCA 30-35°C for 18-72 hours	MCA 30-35°C for 18-24 hours
Staphylococcus aureus	TSB 30-35°C for 18-24 hours	TSB 30-35°C for 18-24 hours	Eugon LT 100 Broth[2] 30-35°C for 18-72 hours	N/A	N/A	N/A	MSA 30-35°C for 18-72 hours	MSA 30-35°C for 18-72 hours	Baird Parker Agar[3] 30-35°C for 18-24 hours
Pseudomonas aeruginosa	TSB 30-35°C for 18-24 hours	TSB 30-35°C for 18-24 hours	Eugon LT 100 Broth[4] 30-35°C for 18-72 hours	N/A	N/A	N/A	CA 30-35°C for 18-72 hours	CA 30-35°C for 18-72 hours	Pseudomonas Agar P[5] 30-35°C for 18-24 hours
Candida albicans	SDB 30-35°C for 3-5 days	SDB 30-35°C for 3-5 days	Eugon LT 100 Broth[6] 30-35°C for 18-72 hours	N/A	N/A	N/A	SDA 30-35°C for 24-48 hours	SDA 30-35°C for 24-48 hours	SDA 30-35°C for 18-24 hours
Bile-tolerant Gram-negative bacteria	TSB 20-25°C for 2-5 hours	TSB 20-25°C for 2-5 hours	Out of scope	EEB 30-35°C for 24-48 hours	EEB 30-35°C for 24-48 hours	Out of scope	VRBGA 30-35°C for 18-24 hours	VRBGA 30-35°C for 18-24 hours	Out of scope

[1]Alternatively, a medium can be used Soybean Casein Lecithin Poysorbate 80 medium. [2]Alternatively, a medium can be used TSB medium.
[3]Alternatively, a medium can be used MSA medium. [4]Alternatively, a medium can be used Soybean Casein Lecithin Poysorbate 80 medium.
[5]Alternatively, a medium can be used CA medium. [6]Alternatively, a medium can be used TSB medium

Table 3: According to international standards, results considered positive in the selective subculture procedure[8-12].

Specific Microorganism	EP 2.6.13	USP 62	ISO 21150 ISO 22718 ISO 22717 ISO 18416
Escherichia coli	Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification tests.	Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification tests.	Brick-red; may have surrounding zone of precipitated bile
Staphylococcus aureus	The possible presence of *S. aureus* is indicated by the growth of yellow or white colonies surrounded by a yellow zone. This is confirmed by identification tests.	The possible presence of *S. aureus* is indicated by the growth of yellow or white colonies surrounded by a yellow zone. This is confirmed by identification tests.	Black, shiny colonies, surrounded by clear zones (2 mm to 5 mm)
Pseudomonas aeruginosa	Growth of colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification tests.	Growth of colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification tests.	Yellow-green pigment (pyocyanin), which fluoresces under UV light.
Candida albicans	Growth of white colonies may indicate the presence of *C. albicans*. This is confirmed by identification tests.	Growth of white colonies may indicate the presence of *C. albicans*. This is confirmed by identification tests.	White to beige, creamy and convex
bile-tolerant Gram-negative bacteria	All detected colonies are considered positive.	All detected colonies are considered positive.	Out of scope

However, the test and positive results to be performed were not specified. For this reason, general standards can be used for identification tests. In the ISO standards, which include cosmetic products, typical colony characteristics seen with the subculture process are defined[12]. According to USP 1113, the first step of identification is to obtain a pure culture. This process ensures that sufficient culture is formed in the subsequent identification process. Test details are given in Table 4[12].

Table 4: Identification tests according to USP[11,12,13].

Categories	Characteristics
Culture	Colony morphology, colony color, shape and size, pigment production
Morphological	Cellular morphology, cell size, cell shape, flagella type, reserve material, Gram reaction, spore and acid-fast staining, mode of sporulation
Physiological	Oxygen tolerance, pH range, temperature optimum and range, salinity tolerance
Biochemical	Carbon utilization, carbohydrate oxidation or fermentation, enzyme patterns
Inhibition	Bile salt-tolerance, antibiotic susceptibility, dye tolerance
Serological	Agglutination, fluorescent antibody
Chemo-taxonomic	Fatty acid profile, microbial toxins, whole cell composition
Ecological	Origin of the organism

Identification tests according to USP 1113
Gram and Spore Staining: Gram staining is a 4-step identification procedure; crystal violet (primary stain), iodine (mordant), alcohol or alcohol– acetone (decolorizer), and safranin (counterstain). Dye removal is applied at each staining step and Gram-positive organisms retain the purple-blue color, Gram-negative organisms appear red. Malachite green stain can be used in the spore staining process[12].
Biochemical Screening: Basically, oxidase test, catalase test and coagulase test can be used in biochemical identification tests. Gram negative rods into non-fermenters bacteria oxidase positive; enteric bacteria are oxidase negative. *Staphylococci* and *Streptococci* are separatedfrom the calatase test. *Staphylococci* catalase negative, *Streptococci* catalase positive. Coagulase testing can be used to separate *Staphylococci* as coagulase negative and coagulase positive *Staphylococci*[12].
Identification tests according to ISO standards
Identification tests for determination of below given specific microorganisms are briefly explained according to ISO standards.
Identification of *Escerichia coli*; Gram staining Gram-negative rods, metallic sheen under reflected light and a blue-black appearance under transmitted light colonies should be seen by subculture on Eosin-

Methylene Blue Agar Medium (EMB) medium (30-35°C for 24-48 h)[8].
Identification of *S. aureus;* Gram staining Gram-positive cocci, catalase positive test, coagulase positive test[9].
Identification of *P. aeruginosa*; Gram staining Gram-negative rods, oxidase positive test. Inoculate the surface of the Pseudomonas agar medium for the detection of pyocyanin with suspect isolated colonies grown on cetrimide agar medium, so that individual colonies develop. Incubate at 30-35°C. Check for bacterial growth after 24 h, 48 h and 72 h. *P. aeruginosa* forms colonies with a red to dark brown zone due to pyorubin production[10].
Identification of *C. albicans*; Gram staining, the microscopic observation shall reveal a violet colour, short ovoid or elongated cells, sometimes with budding cells. Germ tube production; the formation of germ tubes characterizes the presence of *C. albicans*[11].
Method Suitability and Growth Promotion Tests
In the method suitability study, it is aimed to detect the target microorganism group. The analyzed pharmaceutical product has no negative effect on the detection of specific microorganisms. In method, suitability studies, non-selective pre-enrichment process should be performed by inoculating the target and inhibited microorganism specified in Table 5 to the sample prepared by dissolving process.

Table 5: Method suitability test target and inhibitory microorganisms[4-7].

Specific Microorganisms	Target Microorganism	Inhibitory Microorganism
Escherichia coli	*Escherichia coli* ATCC 8739	*Staphylococcus aureus* ATCC 6538
Staphylococcus aureus	*Staphylococcus aureus* ATCC 6538	*Escherichia coli* ATCC 8739
Pseudomonas aeruginosa	*Pseudomonas aeruginosa* ATCC 9027	*Escherichia coli* ATCC 8739
Candida albicans	*Candida albicans* ATCC 10231	-
bile-tolerant Gram-negative bacteria	*Escherichia coli* ATCC 8739 *Pseudomonas aeruginosa* ATCC 9027	*Staphylococcus aureus* ATCC 6538

As a result of the procedures performed, the tests in which the target microorganisms were inoculated should be positive, and the tests in which the inhibited microorganisms were inoculated should be negative. Microorganism inoculation amounts in all test groups should be 10-100 colony forming units (cfu)[4-7].

In the growth promotion test, all the media used in the analysis should be analyzed with the microorganisms specified in Table 5. In the growth promotion test, target microorganisms should be visible signs of growth as a result of incubation, and inhibitory microorganisms should not be a growth sign. The TSB medium used in the non-selective pre-enrichment

process should be analyzed with 5 different microorganisms in the growth promotion test. Since the medium used is the general producer medium, it must be controlled with 5 different microorganisms. Control microorganisms for TSB are, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *C. albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404. Microorganism inoculation amounts in all test groups should be 10-100 cfu/mL. Incubation temperatures and times in method suitability and growth promotion tests are shown in Table 2[4-7].

Alternative Identification Test Methods for Dedection of Specific Microorganisms

Alternative methods can be used besides traditional methods for the detection of specific microorganisms in pharmaceutical products. All analysis steps are similar for the detection of specific microorganisms. However, fast alternative methods can be used for the identification test. All analysis steps should be applied prior to the identification test. These alternative methods can only be applied at the identification test step. Because pure culture is required for the identification step. Traditional methods specified in standard methods or alternative methods can be used for the identification of pure culture. Microorganisms in pure culture obtained by alternative methods can be detected by biochemical, fatty acid methyl ester analysis, MALDI-TOF mass-spectrometry, Fourier transform–Infrared (FT-IR) spectroscopy, nucleic acid extraction, PCR amplification and rRNA base sequencing and ribotyping methods. Many test kits or identification analysis systems have been developed for these methods. Traditional methods used for diagnosis may vary in the phenotypic properties of microorganism. Identification tests performed with phenotypic methods may cause false results due to the influence of microorganisms from environmental conditions. For this reason, alternative identification methods can be used. Generally, phenotypic methods require more pure microorganism cultures than other methods. In addition, the microorganism phenotype may change due to the medium used in the detection of specific microorganisms, incubation conditions, and the antimicrobial substances in the medium. For this reason, it is necessary to use alternative methods to phenotypic analysis methods[13,14]. All the alternative identification test methods should be confirmed by validation procedures.

Table 6: Validation of alternative qualitative tests for the presence or absence of microorganisms[15].

Criterion	Qualitative test	Identification test
Accuracy	+	+
Precision	-	-
Specificity	+	+
Detection limit	+	-
Quantitation limit	-	-
Linearity	-	-
Range	-	-
Robustness	+	+
Suitability testing	+	-
Equivalence testing	+	-

Alternative microbiological methods are listed below[15]:

A. Growth-Based Methods
1. General critical aspects of methods based on early detection of growth
2. Electrochemical methods
3. Measurement of consumption or production of gas
4. Bioluminescence
5. Turbidimetry
6. Growth detection using selective and/or indicative media

B. Direct Measurement
1. Solid phase cytometry
2. Flow cytometry
3. Direct epifluorescent filtration technique (DEFT)
4. Autofluorescence

C. Cell Component Analysis
1. Phenotypic techniques
1.1. Immunological methods
1.2. Fatty acid profiles
1.3. Fourier transform infrared (FTIR) spectroscopy
1.4. Mass spectrometry
1.5. Biochemical assays based on physiological reactions
2. Genotypic techniques
2-1. Direct hybridisation

2-2. Nucleic acid amplification techniques (NAT)
2-3. Genetic fingerprinting

Validation of Alternative Microbiological Methods

Microbiological methods to be used as an alternative need to be validated in order to be applied in quality control analysis. Specific microorganism detection is a qualitative test. For this reason, the accuracy of the studies should be confirmed with certain analyzes in the validation study. This is also the case for identification tests. Alternative fast test systems are frequently used. For this reason, the validation of the systems has gained importance. The validation criteria of alternative methods for the detection of specific microorganisms are shown in Table 6[15].

Critical validation stages are briefly explained below.

Specificity: In an alternative qualitative test, specificity is the ability to detect only desired microorganisms, i.e., the test will not give a false positive result. This can be done using a suitable microorganism table.

Detection Limit: The detection limit of an alternative qualitative method is equal to the lowest number of microorganisms that can be detected in a sample under specified analytical conditions. The microbiological limit test determines the presence or absence of

microorganisms in a defined amount of the sample tested.

Robustness: The robustness of an alternative method is a measure of its capacity to remain unaffected by small but intentional changes in method parameters (e.g. incubation time or incubation temperature range).

Suitability testing: The alternative method should be applied in accordance with the specified application and with the samples to be analyzed under the responsibility of the users. It should show that the test sample does not interfere with the detection capability of the system or microbial recovery.

Equivalence testing: The equivalence testing of two qualitative methods can be done directly through the validation parameters. With this approach, testing microorganisms must be performed in sufficient replicates with low levels of inoculation (eg, less than 5 cfu).

CONCLUSIONS

In microbiological quality control analysis of pharmaceutical products, specific microorganism detection is very important. Detailed methods for the detection of specific microorganisms are described in the pharmacopoeias. In pharmacopoeia methods, the preparation of the product, media and incubation conditions are described in detail. In pharmaceutical products, neutralization methods of cosmetic products can be used to eliminate the antimicrobial effect. When standard methods are compared, they are mostly similar. However, the media used vary. Identification tests of microorganisms are specified in ISO standards. However, there is no definition for identification tests in pharmacopoeia methods. It is seen that general identification tests are specified only in USP. Especially in identification tests, there are deficiencies about how to determine positive results and there are deficiencies about how to determine positive results. Nowadays, alternative rapid identification tests have been developed for identification tests. However, validation is a must in order to use these systems in quality control analysis. Validation of alternative rapid test systems complicates the process. However, it shortens the analysis time. Alternative rapid test systems provide convenience in the identification phase, but these systems may also require the desired microorganism culture. For this reason, alternative rapid test systems also require traditional microbiological methods.

ACKNOWLEDGEMENTS

AUTHOR'S CONTRIBUTIONS

CENGİZ G: conception and design, writing original draft, literature survey. **ALGIN YAPAR E:** methodology, formal analysis, conceptualization. Both authors revised the article and approved the final version.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICTS OF INTEREST

The authors have no conflicts to report.

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