

PROTOCOLS
**MICROBIOLOGICAL
ANALYSIS IN
PHARMACEUTICAL
INDUSTRY**

Inspired by knowledge

ALL PROCEDURES ACCORDING TO EUROPEAN PHARMACOPEIA

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The pharmaceutical industry is one of the world's most influential business sectors, comprised of public and private organizations dedicated exclusively to the research, manufacture, marketing and distribution of pharmaceutical chemicals for human and animal health.

Regulating the analysis and control of medications in this sector is extremely important both in the development of new drugs and in the control of existing products in order to ensure their quality, effectiveness and safety.

The European Pharmacopoeia is the official legal instrument in the EU established **to ensure the quality of this type of products**. It includes quality standards for active principles, general standards for formulation, and general standards for manufacturing medications. It is made up by Expert Committees of the countries involved and other countries, as well as the WHO, participate as observers.

This standard considers the **microbial analysis** of pharmaceutical products, raw materials used, water, air, equipment or packaging material, among others, which are vital to **minimize the type and number of microorganisms present**. Those products of parenteral administration or for use in eyes must be sterile.

Drugs are considered contaminated when it exceeds a limit of opportunistic or objectionable pathogenic microorganisms, which have the capacity to limit the effectiveness of the product, when they present toxic metabolites or physical or chemical deterioration. The ineffective dose varies between species and even between individuals.



This guide will describe the culture media and the procedures formulated according to the Pharmacopoeia required to perform:

- **Sterility testing**
- **Non-sterile product testing**
- **Microbiological count testing**
- **Microbiological water testing**



Microbiological Water Testing

Water is the most commonly used substance in the pharmaceutical industry, both for production, as an ingredient in the product or in one of the manufacturing stages; and for washing equipment. Microbiological control of water is of vital importance at entrance to the treatment plant, purification, storage, and distribution stages since microorganisms can proliferate throughout any stage described above, affecting its quality.

Water samples should be analyzed to avoid changes in the microbial count process. Once the sample has been collected, it must be stored at a temperature between 2 and 10°C. The count must be performed within 8 hours and the analysis of coliform bacteria within 30 hours. Sampling, reception and analysis records must demonstrate that the established timelines are met.

Water for pharmaceutical use can be classified according to use and method of production.

- **Water for injections:** Used as a vehicle (bulk injection water) or as a substances/preparations diluent for preparing medications to be administered parenterally.
- **Purified water:** Used for preparing medications that require sterility and apyrogenicity, except those authorized.
- **Water for preparation of extracts:** For preparing medication extracts.
- **Highly purified water:** Its purpose is the preparation of medicinal products, for which a high biological quality is required (except those cases in which water is used for injectables).

Bibliography

American Public Health Association (1985) Standard Method for the Enumeration of Water and Wasterwater. European Pharmacopeia 9.3.

PURIFIED WATER: 100 CFU/ml

HIGHLY PURIFIED WATER: 10 CFU/100 ml

Use at least 200 ml of sample

WATER FOR INJECTIONS: 10 CFU/ 100 ml

Use at least 200 ml of sample

**WATER FOR PREPARATION OF EXTRACTS:
100 CFU/100 ml**

**STORED PURIFIED WATER:
100 CFU/ 100 ml**

Determine by means of a filter membrane with a pore size not exceeding 0.45 µm. Culture in R2A Agar (**CAT. 1071**)

Incubation at 30-35 °C // 5 days

Determine by means of a filter membrane with a pore size not exceeding 0.45 µm. Culture in Trypticasein Soy Agar (TSA) (**CAT. 1068**)

Incubation at 30-35 °C // 5 days

Sterility Testing



Introduction


The purpose of the sterility test is to verify the absence of contamination by microorganisms in products that, according to pharmacopoeias, are required to be sterile, whether they have been sterilized or prepared aseptically. A satisfactory result indicates the absence of contaminating microorganisms in the sample examined under test conditions. To achieve such conditions, the test environment must be adapted to the way in which the sterility test is performed. These working conditions are regularly monitored by means of an appropriate sampling of the working area.

This test appeared for the first time in the British Pharmacopoeia in 1932, and later in the United States Pharmacopoeia in 1936 for sterile solutions. Over the years, constant changes have been made, which have led to the improvement of techniques for detecting contamination in this type of products.

Bibliography

Brewer. JAMA, 115. 1940. Vera. J. Bact. 47:59, 1944.
Pittman. J. Bact. 51:19, 1946.
European Pharmacopoeia 9.3.

Aerobic, anaerobes, and fungus growth promotion test

 AEROBIC BACTERIA <i>(Staphylococcus aureus, Pseudomonas aeruginosa)</i>	 ANAEROBIC BACTERIA <i>(Clostridium sporogenes)</i>
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Inoculate in containers separated by microorganism Thioglycolate Liquid Medium (**CAT. 1508**) with < 100 CFU of each species described above.

 AEROBIC BACTERIA <i>(Bacillus subtilis)</i>	 FUNGUS <i>(Candida albicans, Aspergillus brasiliensis)</i>
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Inoculate in containers separated by microorganism Trypticasein Soy Broth (TSB) (**CAT. 1224**) with < 100 CFU of each species described above.

Incubate bacteria for no more than 3 days, and fungi and yeasts for no more than 5 days.

Method Suitability Test

This test must be carried out simultaneously with the sterility testing of the examined product. It is carried out when a sterility testing of a new product needs to be carried out, or when the experimental conditions of the test change.

Use exactly the same procedure selected in the Product Sterility Testing described below except for the following modifications.

A

MEMBRANE FILTRATION METHOD

After transferring the contents on the container(s) of the products to be examined to the filter membrane, inoculate a small amount of viable microorganisms (<100 CFU) to the final sterile diluent amount used to rinse the filter.

B

DIRECT INOCULATION METHOD

After transferring the contents on container(s) of the products to be examined to the culture medium, inoculate a small amount of viable microorganisms (< 100 CFU).

In both cases, use the same microorganisms described in the test for the growth promotion of aerobic, anaerobes and fungus. Carry out a growth promotion test as a positive control. Incubate containers for a period not exceeding 5 days.

Sterility testing of the product to be examined

A

MEMBRANE FILTRATION METHOD

Product to be analyzed:

- Aqueous solutions
- Soluble solids
- Oils and oily solutions
- Semi-solids (creams and ointments)

B

DIRECT INOCULATION METHOD

Product to be analyzed:

- Oily liquids
- Semi-solids (creams and ointments)
- Suture thread and related products for use in animals

Incubate inoculated media for 14 days.

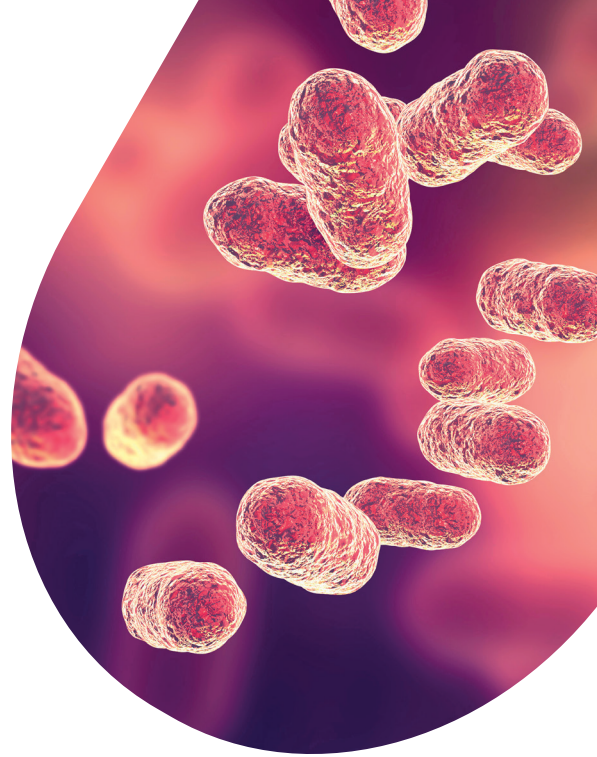
Observe during the incubation time and, once period has elapsed, observe the macroscopic evidence of microbial growth. If the medium becomes too turbid to read the presence/absence of microbial growth, transfer 1 ml to a new medium, and incubate it for not less than 4 days.

If there is no evidence of microbial growth, the product complies with the sterility testing.

Microbiological I count test

TAMC (Total Aerobic Microbial Count) & TYMC (Total Yeast and Mold Counts)

The tests described in this section allow quantitative enumeration of mesophilic bacteria, total aerobic microbial (TAMC) and fungus count, total combined yeasts and mold count (TYMC), which can grow under aerobic conditions



Bibliography

Standard Methods for the Examination of Dairy Products.
11th Edition. APHA., Inc. New York, 1960.

European Pharmacopoeia 9.3.

A - MEMBRANE FILTRATION

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample in Buffered Peptone Water (**CAT. 1401**) at pH 7.0 and homogenize.

This stock solution can be supplemented with neutralizers and surfactants

MICROORGANISMS AEROBIC TOTALS

Transfer 10 ml of the solution of stock to the filter membrane (pore size 0.45 µm)

MOLDS AND YEASTS TOTAL

Transfer 10 ml of the solution of stock to the filter membrane (pore size 0.45 µm)

Wash each filter membrane 3x100 ml of Buffered Peptone Water (**CAT. 1401**) at pH 7.0

MICROORGANISMS AEROBIC TOTALS

Transfer the membrane to a Trypticasein Soy Agar (TSA) (**CAT. 1068**)
Incubation at 30-35 °C // 5 days

MOLDS AND YEASTS TOTALS

Transfer the membrane to Sabouraud Dextrose Agar Plates (**CAT. 1024**)
Incubation at 20-25 °C // 5-7 days

Calculate the number of CFU per gram or milliliter of product

B - PLATE COUNT METHODS

B.1 POUR PLATE METHOD

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample in Buffered Peptone Water (**CAT. 1401**) at pH 7.0 and homogenize.

This stock solution can be supplemented with neutralizers and surfactants

MICROORGANISMS AEROBIC TOTALS

Transfer 1 ml of the stock solution to at least one empty and sterilized Petri dish.
Add 15-20 ml of Trypticasein Soy Agar (TSA) (**CAT. 1068**) at < 45 °C
Incubation at 30-35 °C // 5 days

MOLDS AND YEASTS TOTAL

Transfer 1 ml of the stock solution to at least one empty and sterilized Petri dish. Add 15-20 ml of Sabouraud Dextrose Agar (**CAT. 1024**) at < 45 °C
Incubation at 20-25 °C // 5-7 days

Select the plates corresponding to the given dilution and presenting the highest number of colonies lower than 250 for TAMC and 50 for TYMC.

Find the arithmetic mean of the counts by culture medium.

Calculate the number of CFU per gram or milliliter of product

B - PLATE COUNT METHODS

B.2 SURFACE METHOD

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample in Buffered Peptone Water (CAT. 1401) at pH 7.0 and homogenize.

This stock solution can be supplemented with neutralizers and surfactants

MICROORGANISMS AEROBIC TOTALS

Transfer at least 0.1 ml of the stock solution to a plate of Trypticasein Soy Agar (TSA) (CAT. 1068) Incubation at 30-35 °C // 120 hours

TOTAL MOLDS AND YEASTS

Transfer at least 0.1 ml of the stock solution to a Sabouraud Dextrose Agar plate (CAT.1024) Incubation at 20-25 °C // 5-7 days

Calculate the number of CFU per gram or milliliter of product

C - MOST LIKELY NUMBER METHOD (TAMPC)

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample in Buffered Peptone Water (CAT. 1401) at pH 7.0 and homogenize.

This stock solution can be supplemented with neutralizers and surfactants

Make two serial dilutions from the stock solution of 1 ml / dilution in two tubes of 9 ml each of Trypticasein Soy Broth (TSB) (CAT. 1224)

Using the initial three dilutions, carry out three further dilutions with at least 3 tubes/dilution in a concentration of 1:10 in Trypticasein Soy Broth (TSB) (CAT. 1224) Incubation at 30-35 °C/120 hours.

Using the initial three dilutions, carry out three further dilutions with at least 3 tubes / dilution in a concentration of 1:10 in Trypticasein Soy Broth (TSB) (CAT. 1224) Incubation at 30-35 °C/ max. 3 days.

CONFIRMATION Tubes with turbidity

Perform a subculture in the same broth or on a plate of Trypticasein Soy Agar (TSA) (CAT.1068). Determine the Most Probable Number of microorganisms by gram or milliliter of product

CONFIRMATION Tubes without turbidity

Determine the Most Probable Number of microorganisms per gram or milliliter of product



Escherichia coli

Introduction

This microorganism belongs to the Enterobacteriaceae family; it is a gram-negative, mobile, non-sporulating bacillus. In addition, it is lactose-positive and negative oxidase. Escherichia coli can be distinguished from other coliforms by its ability to produce indole from tryptophan or by the production of the β -glucuronidase enzyme. These characteristics are used for selective isolation and confirmation in various analysis processes.

These ubiquitous bacteria are located in the intestine of humans and warm-blooded animals. Their high presence in the intestinal tract and feces make it a marker that indicates bad hygienic practices or fecal contamination during the manufacture or handling of drugs. Their presence is used as a marker that other pathogenic organisms of fecal origin may be present, especially in products meant for oral consumption and natural raw materials.

Today, the tests also contemplate the concentration of Escherichia coli according to the European Pharmacopoeia.

Bibliography

MacConkey J. H. 5:33. 1905. Joseph Md. State. Dept. Health. Procedures, 1960. *European Pharmacopoeia* 9.3.

Chils, E., and L. A. Allen. 1953. *Improved methods for determining the most probable number of Bacterium coli and of Enterococcus faecalis*. J. Hyg.Camb. 51:468-477.



Method

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample using at least 1 g/ 1 ml of the product to be examined in Buffered Peptone Water (**CAT. 1401**) and homogenize.

This stock solution can be supplemented with neutralizers and surfactants.

PRIMARY ENRICHMENT

Spread 10 ml of the stock solution or the quantity corresponding to 1 g/1 ml of the product in 100 ml of Trypticasein Soy Broth (TSB) (**CAT. 1224**) and homogenize. Incubation at 30-35 °C // 18-24 hours

SELECTIVE ENRICHMENT

Spread again 1 ml of Trypticasein Soy Broth in 100 ml of MacConkey Broth (**CAT.1210**)
Incubation at 42-44 °C // 24-48 hours

SELECTIVE ISOLATION

Perform subcultures on Agar MacConkey (**CAT. 1052**). Incubation at 30-35 °C // 18-72 hours

RESULTS INTERPRETATION

The growth of colonies in the medium indicates the possible presence of *E. coli*. Confirmation by identification test. The product passes the test if no colonies are observed or if identification tests return negative

Bile-tolerant gram-negative bacteria

Introduction

Infectious diseases have had a decisive influence on the evolution of human history. They are currently one of the main mortality causes in the world, being responsible for a significant number of infections.

Gram-negative bacteria are characterized by a double cell membrane, one external and one cytoplasmic, where a thin wall of peptidoglycan can be found that prevents them from retaining the dye during Gram stain. The external membrane structure comprises an external part consisting of a complex of lipopolysaccharides whose lipidic part acts as an endotoxin and is responsible for the pathogenic capacity of the microorganism. This external membrane protects them from antibiotics such as penicillin, colorants or detergents, a characteristic that makes it possible for them to colonize environments that other microorganisms find difficult to colonize.

Gram-negative bacteria resistant to bile salts include *Escherichia*, *Pseudomonas* and *Aeromonas*.

Bibliography

ISO 21528. *Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of Enterobacteriaceae.*

ISO 8523 *Microbiology – General guidance for the detection of Enterobacteriaceae with pre-enrichment. European Pharmacopoeia 9.0.*

Mossel D.A.A., Visser M. and Cornelissen A.M.R.J App, Bact. 24:444. 1963.

European Pharmacopoeia 9.3.



Method

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample using at least 1 g of the product to be examined in Trypticasein Soy Broth (TSB) (CAT.1224) and homogenize. Incubation at 20-25 °C // 2-5 hours

This stock solution can be supplemented with neutralizers and surfactants.

A - TEST FOR THE ABSENCE OF ENTEROBACTERIACEAE AND OTHER GRAM-NEGATIVE BACTERIA

PRIMARY ENRICHMENT

Unless otherwise indicated, spread the volume corresponding to 1 g of product in 100 ml of Mossel EE Broth (CAT. 1202). Incubation at 30-35 °C // 24-48 hours

SELECTIVE ISOLATION

Subcultivate in Violet Red Bile Agar With Glucose (VRBG) (CAT. 1092) plates Incubation at 30-35 °C // 18-24 hours

RESULTS INTERPRETATION

The product passes the test if no colony growth is observed

B - QUANTITATIVE TEST OF ENTEROBACTERIACEAE AND OTHER GRAM-NEGATIVE BACTERIA

PRIMARY ENRICHMENT

Prepare three tubes with dilutions containing 0.1 g, 0.01 g and 0.001 g of the product to be examined in 10 ml of Mossel EE Broth (CAT. 1202). Incubation at 30-35 °C // 24-48 hours

SELECTIVE ISOLATION

Perform subcultures in Violet Red Bile Agar With Glucose (VRBG) (CAT. 1092). Incubation at 30-35 °C // 18-24 hours

RESULTS INTERPRETATION

The growth of colonies indicates a positive result. Write down the smallest amount of the product that gives a positive result and the largest amount that gives a negative result. Determine from Table 2.6.13.2 of European Pharmacopoeia

Salmonella

Introduction

Salmonella is a very diverse family of microorganisms, including some 2,300 distinct serotypes. It is one of the four main causes of diarrheal diseases. This group of bacteria occurs in the form of gram-negative bacillus (as they belong to the enormous group of Enterobacteriaceae), they are facultative anaerobic and mobile by means of flagella. In addition, they are glucose fermenters but not lactose fermenters and do not produce urease.

It is estimated that salmonellosis affects tens of millions of people worldwide every year and causes more than 100,000 deaths. This type of bacteria is the reason for millions of hospitalizations worldwide, and this number may increase up to 30 times if infections that are not diagnosed are included, as they can usually cause a normal diarrhea process. It behaves as a facultative intracellular pathogen that, depending on the serotype, inoculum, virulence factors expressed by the strain, the host involved, and the immune status of the patient can cause from a medium to severe gastrointestinal infection to a systemic infection.

It is widely distributed in nature and as normal flora of the intestinal tract in animals and humans. Unlike other microorganisms that cause gastrointestinal diseases, it may be commonly found in natural raw materials, making it vitally important to research them, especially those of animal origin.

Bibliography

Rollender, W. U. Beckford; R.D. Belsky, B. Krostoff (1969) *Comparison of Xylose Lysine desoxycholate agar and MacConkey agar for the isolation of Salmonella and Shigella from clinical specimens* (tech. Bull. Reg. Med. Tech, 39 (1) 8-p).
European Pharmacopoeia. 9.3.



Method

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample using at least 10 g / 10 ml of the product to be examined in Trypticasein Soy Broth (TSB) (CAT. 1224) and homogenize. Incubation at 30-35 °C // 18-24 hours

Trypticasein Soy Broth can be supplemented with neutralizers and surfactants.

SELECTIVE ENRICHMENT

Spread 0.1 ml of Trypticasein Soy Broth in 10 ml of Rappaport Vassidialis Broth (CAT. 1414) Incubation at 30-35 °C // 18-24 hours

SELECTIVE ISOLATION

Perform subcultures on XLD Agar (Xylose Lysine Desoxycholate Agar) (CAT. 1080) Incubation at 30-35 °C // 18-48 hours

RESULTS INTERPRETATION

All red colonies with/without black nucleus are susceptible of *Salmonella* Confirmation by means of an identification test. The product passes the test if no colonies of the types described are observed or if the confirmation tests are negative

Staphylococcus aureus

Introduction

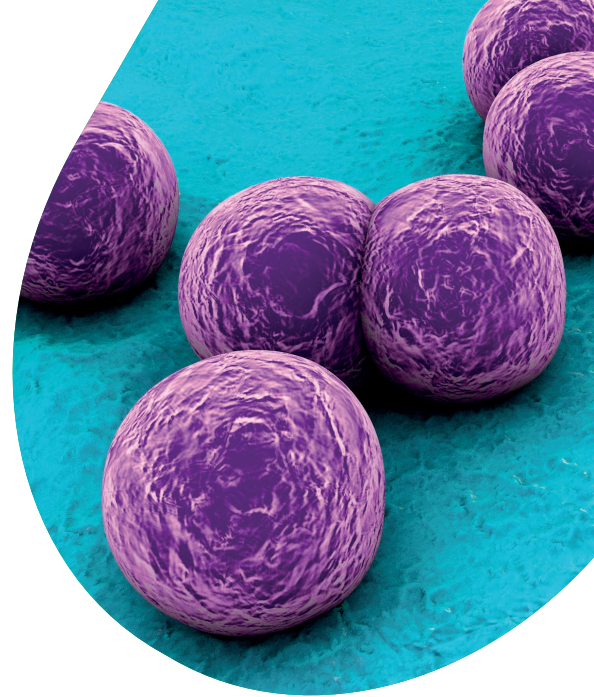
It's a bacterium that belongs to the *Staphylococcaceae* family. They are facultative anaerobic cocci grouped in clusters, gram-positive, immobile and not sporulating. Among its most distinctive characteristics is the production of virulence factors such as coagulases and catalases that are used for confirmatory tests after isolation. It is a microorganism widely distributed worldwide and acts as a commensal in the human epithelium and mucous membranes. It is often found in the mouth, blood, mammary glands, intestine, genitourinary tract, and airways.

It is also an opportunistic pathogen, causing acute and pyogenic infections which, if untreated, can spread to the surrounding tissue or to other organs via bacteremia. Among the types of infections caused by this microorganism are pneumonia, osteomyelitis, and acute endocarditis, among others. The presence of *Staphylococcus* and, particularly, *S. aureus* genus in raw material or pharmaceutical or cosmetic product, indicates that the source of contamination may be human, i.e. operators. These microorganisms can be transported through dust, skin, clothing, and moisture droplets generated by moving, talking, and sneezing.

Bibliography

McColloch Am. J. Vet. Research, 8:173. 1947. Velilla, Faber, and Pelczar Am. J. Vet. Research, 8:275. 1947. Chapman, G.H. 1945 J. Bact. 50:201-203.

European Pharmacopoeia. 9.3.



Method

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample using at least 1 g/ 1 ml of the product to be examined in Trypticasein Soy Broth (TSB) (**CAT.1224**) / Buffered Peptone Water (**CAT.1401**) at pH 7.2 and homogenize.

This stock solution can be supplemented with neutralizers and surfactants.

PRIMARY ENRICHMENT

Spread 10 ml of the stock solution or the quantity corresponding to 1 g/1 ml of the product in 100 ml of Trypticasein Soy Broth (TSB) (**CAT. 1224**) and homogenise.
Incubation at 30-35 °C // 18-24 hours

SELECTIVE ISOLATION

Perform subcultures in Mannitol Salt Agar (MSA) (Chapman Medium) (**CAT. 1062**). Incubation at 30-35 °C // 18-72 hours

RESULTS INTERPRETATION

The growth of white/white colonies surrounded by a yellow zone indicates the possible presence of *Staphylococcus aureus*. Confirmation by means of an identification test. The product passes the test if no colonies of the types described are observed or if the confirmation tests are negative.

Pseudomonas aeruginosa

Introduction

It's a bacterium that belongs to the *Pseudomonadaceae* family. They are straight or curved bacilli, strictly aerobic, gram-negative, mobile and non-sporulating. In addition, they are positive catalase, using this characteristic for later identification. This group of bacteria is capable of producing different pigments such as pyocyanin and uorescein. Therefore, different culture media have been developed to promote the production of these pigments and thus be able to identify the different species.

This microorganism combines its adaptability to different ecosystems with a wide variety of virulence factors, and the spectrum of diseases it can cause ranges from mild infection to sepsis. During its mechanism of action it attaches to epithelial cells by means of polar pili and, once attached, it produces proteases, hemolysins, exotoxins and endotoxins that damage tissues.

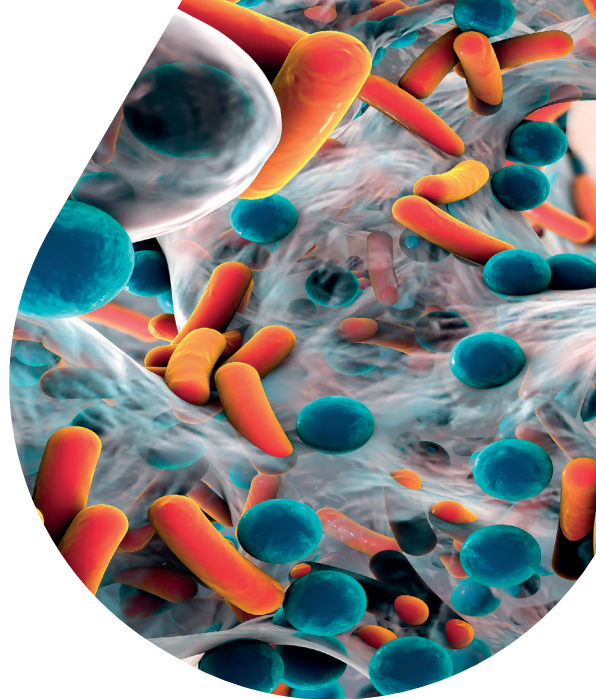
The *P. aeruginosa* infection is often related to water and aqueous solutions contamination. One of the most severe infections caused by this microorganism is endocarditis due to intravenous administration of medications, when injected into the patient and dissolved or suspended in watery vehicles. Hence, the importance of detecting its presence in pharmaceutical products that are to be administered by inhalation and ocular route or in aqueous vehicles. Cosmetics may also be susceptible to this type of contamination, especially liquids and creams.

In addition, it can colonize water purification systems by the formation of biofilms, structures very difficult to eliminate with the use of salinizing agents. Its remarkable resistance to antimicrobials is one of the world's greatest concerns.

Bibliography

King, Ward and Raney. J. Lab. and Clin. Med. 44:301. 1954. Brown and Lowbury. J. Clin. Path. 18:752. 1965. Lowbury. J. Clin. Path. 4:66. 1951. Lowbury and Collins. J. Clin. Path. 8:47. 1955.

European Pharmacopoeia. 9.3.



Method

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample using at least 1 g of the product to be examined in Buffered Peptone Water (CAT. 1401) at pH 7 and homogenize

This stock solution can be supplemented with neutralizers and surfactants.

PRIMARY ENRICHMENT

Spread 10 ml of the stock solution or the quantity corresponding to 1 g/1 ml of the product in 100 ml of Trypticasein Soy Broth (TSB) (CAT. 1224) and homogenise. Incubation at 30-35 °C // 18-24 hours

SELECTIVE ISOLATION

Perform subcultures in Cetrimide Agar (CAT. 1102) Incubation at 30-35 °C for 18-72 hours

RESULTS INTERPRETATION

The growth of colonies in the medium indicates the possible presence of *Pseudomonas aeruginosa*. Confirmation by means of an identification test. The product passes the test if no colonies are observed or if the confirmation tests are negative

Clostridia

Introduction

They are a class of bacteria belonging to the Firmicutes phylum class. They are characterized by being mostly gram-positive anaerobes bacilli. Most genres do not form spores, but some do, such as *Clostridium*, one of the best known.

These bacteria are widely distributed in nature, mainly in the soil and in the intestinal tract of many animal species, including man, where they may also be found as normal flora on the skin and mucous membranes of the nasopharynx, pharynx, mouth, urethra, and vagina. They can cause both endogenous and exogenous infections, affecting any region of the body, provided the conditions in the tissues are favorable. They are fundamentally involved in abscesses of any organ, colitis, appendicitis, bacteremia, chronic otitis media, crackling cellulitis, dental and oral infections, endocarditis and endometritis.

The presence of anaerobic microorganisms, especially those belonging to the *Clostridium* genus is usually found in natural raw materials, powder, starch, etc. And also in medicinal herbs and phytotherapeutic medications.

Bibliography

Ellner, Stossel, Drakeford and Vasi. AM J. Clin. Path. 45:502-504. 1966.

European Pharmacopoeia. 9.3.



Method

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample using at least 2 g/2 ml of the product to be examined in Trypticasein Soy Broth (TSB) (CAT. 1224) / Buffered Peptone Water (CAT. 1401) at pH 7.2 and homogenize

This stock solution can be supplemented with neutralizers and surfactants.

INACTIVATION SPORES

Divide the sample into 2 tubes of at least 10 ml. Heat 1 portion to 80 °C/10 min and cool quickly. Do not heat the other portion

PRIMARY ENRICHMENT

Spread 10 ml, separately, of each portion of the stock solution in 100 ml of Reinforced Clostridial Medium (CAT. 1007). Incubation at 30-35 °C // 48 hours

SELECTIVE ISOLATION

Perform subcultures in Columbia Agar (CAT. 1104) Incubation under anaerobic conditions at 30-35 °C for 48-72 hours

RESULTS INTERPRETATION

The growth of colonies (with/without spores) gives a negative reaction to catalase indicating the presence of Clostridia. Confirmation by identification test

Candida albicans

Introduction

These are yeasts belonging to the *Saccharomycetaceae* family and the *Candida* genus. These fungi develop in a unicellular form and are human saprophytic. It is very common to find them in oral cavities, gastrointestinal tract and vaginal area. Although they have a very important function in the fermentation of certain sugars for the body, there are times that this microorganism acts as an opportunistic pathogen, being able to cause superficial or systemic candidiasis in weakened people, in newborns and in old people with a deficient immune system.

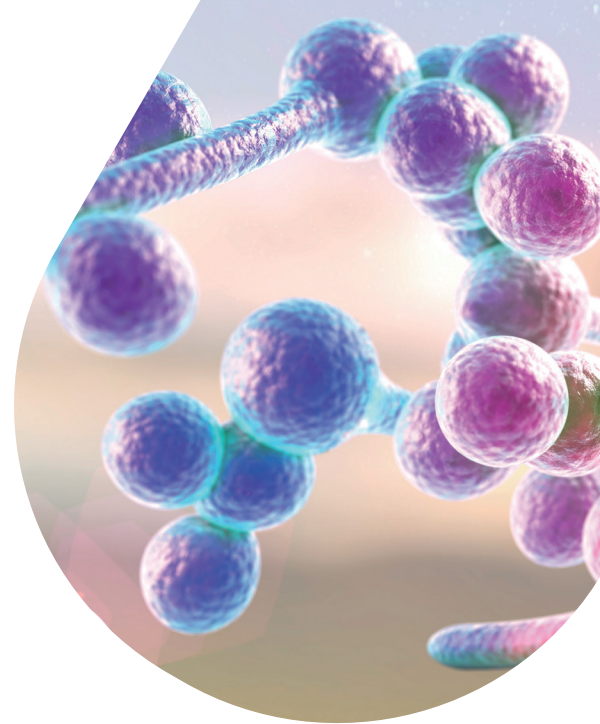
The products with the highest susceptibility to fungal contamination are ophthalmic solutions, ointments, suppositories, salves and in cosmetics, soaps, powders, and others that contain nutrients rich in carbohydrates and fatty acids.

It is therefore necessary to analyze these pharmacological products in order to identify the presence of this microorganism and thus avoid the consequences of a possible infection by *C. albicans* in the consumer.

Bibliography

Beuchat, L.R., J.E Corry, A.D King, Jr. And J.I Pitt (ed) 1986) *Methods for the mycological examination of food*. Plenum Pres, New York *European Pharmacopoeia*. 9.3.

Sabouraud, Ann. *Dermat and Syphilol* 1892-3. Gerog J. Lab.Clin. Med. 67;355 1953.



Method

SAMPLE PREPARATION

At a concentration of 1:10, prepare the sample in Trypticasein Soy Broth (TSB) (CAT.1224) / Buffered Peptone Water (CAT. 1401) at pH 7.2 and homogenise

This stock solution can be supplemented with neutralizers and surfactants

PRIMARY ENRICHMENT

Spread 10 ml of the stock solution or the quantity corresponding to 1 g/1 ml of the product in 100 ml of Sabouraud Dextrose Broth (CAT. 1205) and homogenize. Incubation at 30-35 °C // 3-5 days

SELECTIVE ISOLATION

Perform subcultures in Sabouraud Dextrose Agar plates (CAT.1024). Incubation at 30-35 °C // 24-48 hours

RESULTS INTERPRETATION

The growth of white colonies in the medium indicates the possible presence of *Candida albicans*. Confirmation by means of an identification test. The product passes the test if these colonies are not observed or if confirmatory tests return negative



Condalab

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