BACTERIAL CONTAMINANTS OF BIOLOGICAL PRODUCTS PRODUCED LOCALLY

A Thesis Presented

By

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جامعة الاسكندرية

كلية الطب البيطرى بادفينا

قسم الميكروبيولوجيـــــــا

" بسم الله الرحمن الرحيم "

قـــرار لجنـــة الحكـم والمناقشـــــــــة

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CONTENTS

1. INTRODUCTION

Until the recent past, sterility of an injectable product was only discussed in absolute terms. Any description of sterility other than as an absolute could simply not be envisioned. While dealing in absolute yes/no statements is philosophically satisfying, these yes/no statements can't accommodate all real world scientific problems. Among these problems is the sterility problems faced in the mass production of injectable compounds.

Sterility is defined as the absence of living organisms. It is achieved by heating, by filtration, by treatment with ethylene oxide or by ionizing irradiation, and by conducting any subsequent processes aseptically.

Freedom from contaminants is defined as the absence of specified living organisms. This may be achieved by selecting materials from source shown to be free from the specified organisms and by conducting subsequent procedures aseptically. Adequate assurance of sterility and freedom from contaminates can only be achieved by proper control of the primary materials used and their subsequent processing and storage. Tests on the product are necessary to check that this control has been achieved.

Many descriptions of procedures employed to achieve sterility in parenteral production batches were reported in the literature. The theoretical framework that could unite the widespread observations and practices into practical methodology was missing until recently. Production line control of the sterility of injectable products was essentially based on gut evaluations.

The present achievement of rational, production line control of product sterility is based on the recognition that product sterility could not be simply regarded as a sharply edged yes/no affair. The present rational control is based on the fact that the sterility of a product is determined by the degree of contamination in the product prior to sterilization and to the parameters of the sterilization process.

The end result of the sterilization process is now described as a probabilistic reduction of the initial contamination. The end result of the sterilization process is now described as a probabilistic reduction of the initial contamination.

As in many disciplines, the ability to achieve an objective evaluation of this important attribute provided the basis for scientific analysis, improved control and thus improved production and reduced cost. An equivalent framework is essential for the communication and standardization of the results of a visual inspection for contaminating particles. The control of particle contamination in injectable products is a two-fold problem for the pharmaceutical industry. The two parts of the problem are:

1) Achieving contamination free product

2) Achieving this contamination free quality at an economic cost acceptable to the user. Today, there is no commonly accepted framework for the definition or analysis of the results of a manual inspection for "visible" particles. Any progress toward global harmonization of the results of a visual particle inspection must commence with the

development of a common scientific language with which inspection security and economic effectiveness of an inspection can be discussed and rationally evaluated.

In the case of particle contamination, the final product quality depends on product quality prior to inspection and to the parameters of the inspection process.

The aim of this work is to detect the possibility of presence of various bacteria and fungi contamination in the human and veterinary biological products. For attaining this aim, the following items were undertaken :

- Examination of human biologicals bacteriologically and mycologically for the isolation and identification of different contaminants,
- Examination of veterinary biologicals bacteriologically and mycologically for the isolation and identification of different contaminants.

2. REVIEW OF LITERATURES

2.1. Aim of sterility test:-

The Untied States of America Pharmacopeia (1960) Sterility tests are highly exacting and should be conducted by personnel having expert training and experience in rigid aseptic techniques. Testing should not be conducted under direct exposure to ultraviolet light or in areas under aerosol treatment. Suitable environmental control tests, including plate counts, should be performed at regular intervals.

The Untied States of America Pharmacopeia (1965) Transfer specified amounts of the product under test to suitable amounts of medium. After appropriate incubation, examine visually for the presence of viable micro-organisms.

If on the first test no growth is found, the material under examination meets the requirements of the sterility. If growth is found, the test may be repeated to rule out laboratory contamination which may be introduced during the test, using twice the number of samples.

The Untied States of America Pharmacopeia (1970) Sterility tests is conducted for revealing the presence of viable forms of bacteria and fungi and are applicable to most types of pharmaceutical products and devices, the variety of which creates the need for an array of media and test procedures. For a discussion of the factors that bear upon

the conduct of the sterility tests and their interpretation.
The sterility testing of the human and veterinary biologics is conducted by specific procedures set forth in the regulations applicable to their manufacture or distribution.

The European Pharmacopeia (1971) The test for sterility is carried out under aseptic conditions to avoid contamination of the product to be examined but without the direct action of any sterilising agent (for example, ultra-violet light). The method to be used should be suitable for the product to be examined, having regard to the presence of any substances capable of inhibiting the growth of micro-organism.

For the purpose of the test, a batch is defined as homogeneous collection of sealed containers prepared in such a manner that the risk of contamination is the same for each of the units in it.
The containers whose contents are to be examined are taken in a random manner. This does not include the containers necessary to establish the effectiveness of the medium in the presence of the preparation to be examined.

World Health Organization (1973) Sterility test should be performed whenever specified in the requirements for individual products. Tests for sterility are often performed advantageously at various stages of manufacture, in addition to those given in requirements.

Detailed rules and precautions for the sterility test procedures should be written and carefully followed.

The staff performing the sterility tests should have experience in the duties assigned. Supervision of the work and interpretation of sterility tests should be undertaken by an individual with training in scientific subjects, who should also be trained in microbiology or be given training in that subject.

1. Sampling

The specified number of suitable samples shall be taken from the product. Such samples should be taken at least from each final bulk, as well as from each final lot.

1.1 Sampling from bulk

A sample shall be taken from each bulk to be tested in such a manner as to be representative of the material to be tested. The amount taken shall be sufficient to perform the tests and any repeat tests that may be required. Such samples should be taken so as to maintain intact the level of sterility of the material, or, if this is not possible, the samples shall be taken at the stage of further processing.

Since any microbial contaminants in a liquid may settle out, through mixing is required before the sample is taken.

1.2 Sampling from final lots

Samples of final containers from each final lot to be tested shall be taken in such a manner as to be preventative of the lot to be tested. Appropriate periodic samples shall be taken, including samples at the beginning and the end of the filling operation.

If a product lot is filled through several outlets from a single bulk, samples should be taken from each outlet (filling lot) so as to be representative of the filling assembly.

2. Culture media

The culture media used for sterility tests for bacteria and fungi shall be those approved by the national control authority. Such media shall have been shown to be capable of supporting the growth of a wide variety of micro-organisms, with both aerobic and anaerobic growth characteristics, including the types found in the environment of the manufacturing operations.

3. Performance of the test

Prior to conducting a sterility test on any product, it shall have been determined whether or not the material to be tested itself has the property of killing or inhibiting the growth of micro-organisms, or contains preservatives or other substances that have this effect. If such an effect is shown, the sterility shall be made using a suitable procedure to contract the effect.

The inhibitory effect in a preparation to be tested for sterility may be overcome by increasing the volume of the culture medium used, so that the inhibitors are rendered ineffective, or if the preparation can be filtered, the inhibitors removed by membrane filtration. The volume of the medium required to overcome the inhibitory effect should be determined. Once established, these quantities may be used for subsequent sterility tests unless a change is made in the composition of the product.

The Untied States of America Pharmacopeia (1975) The sterility tests presented are suitable for revealing the presence of viable forms of bacteria, fungi, and yeasts in or on pharmaceutical products and devices. Alternative procedures or procedural details may be employed to demonstrate that an article is sterile, provided the results obtained are of equivalent reliability. Where a difference appears, or in the event of a dispute, only the result obtained by the procedure given in this pharmacopoeia is conclusive.

Adventitious microbial contamination that is transmitted to an article from the environment during the course of a sterility test invalidates the results of the test. It is necessary to demonstrate rigorously, by the use of appropriate monitoring techniques, that the extraneous micro-organisms have been excluded throughout the test period.

Where a sterility test is applied to discrete units drawn from a group of similar units, the results obtained cannot be extrapolated with certainty to characterize the sterility status of the units that remain untested.

Seyfarth 1975 The USP XIX is to be published in the summer 1975. In this paper the most important passages of its sterility test are compared with the regulations of the EP 1. So a test should be rendered possible to come up to both Pharmacopoeia.

a) Both the membrane filtration method and the direct inoculation of media may be used as the test methods. The membrane filtration method is the method of choice. But the mentioned pore diameter of

0.45 +/- 0.02 µm is too large. Those filters with a pore diameter of 0.2 +/- 0.02 µm are preferable.

b) While EP 1 does not mention the culture media, USP XIX prescribes Fluid Thioglycollate Medium and Soybean-Casein Digest Medium. These culture media are not sufficient. The use of Fluid Thioglycollate Medium, Soybean-Casein Digest Medium and Fluid Sabouraud Medium is suggested.

c) The same organisms are recommended for the control of the culture medium as well as for the determination of the minimal inhibiton concentration (***Clostridium sporogenes***, ***Bacillus subtilis***, ***Staphylococcus aureus***, ***Candida albicans*** and perhaps a mould fungus).

d) In the USP the number of samples is decided by the risk of contamination. This is preferable to the EP 1, where the number of samples is fixed by the size of the charge.

e) While EP 1 lays down 7 days as an incubation time for bacteria as well as fungi, the USP XIX prescribes a time between 7 and 14 days, depending on the contamination risk and on the method used. As a compromise 10 days for bacteria and 14 days for fungi are recommended.

Christianson and Koski (1983) Stated that a direct inoculation method for sterility testing veterinary biologics was compared with a closed membrane filtration method. The filtration method detected extraneous contamination in 29% more batches of biologics than the direct inoculation method. Live viral biologics produced in cell culture were found to be the only product type that could be filtered; hence the

filtration method cannot be recommended universally as a replacement for the direct inoculation method.

Pappalardo etal (1988) noticed that for the sterility testing of intravenous solutions, the Pharmacopeia recommends the use of liquid media. However, contamination by a single micro-organism during the test may lead to the inappropriate blocking of the release of the batch.
An alternative method using solid media for culturing the membranes used in the filtration was therefore sought. With an artificial contamination with 10-100 Colony-Forming-Units (CFU) of nine different micro-organisms, including those advocated by the Pharmacopeia, the feasibility of two different methods was assessed using two different intravenous solutions manufactured in our hospital. The results show that recoveries of minute amounts of bacteria using solid media and liquid media were similar. In all experiments there was a concordance in both positive and negative results. However, the batches of solutions have to be tested as quickly as possible after their preparation, whatever the method used, to avoid false-negative tests.

Winkler and Lukaszewicz (1989) summarized the results of sterility testing after 10-16 years and found that there was no differences in between and assumed a fixed methodological, technological and biological balance. The necessity of changes in these methods and the achieved balance is discussed.

The United States of America Pharmacopeia (1990) the sterility tests are applicable for determining whether a pharmacopeial article purporting to be sterile complies with the requirements set forth in the individual monograph with respect to the test for sterility. The sterility test procedures should be a part of the quality control in the manufacture. In view of the possibility that positive results may be due to faulty aseptic techniques or environmental contamination in testing, two stages of testing should be included.

Alternative procedures may be employed to demonstrate that an article is sterile, provided the results obtained were at least of equivalent reliability. Where a difference appears, or in the event of a dispute, when evidence of microbial contamination is obtained by the procedure given in this pharmacopeia, the result so obtained was conclusive of failure of the article to meet the requirements of the test. Similarly, failure to demonstrate microbial contamination by the procedure given in this pharmacopeia is evidence that the article meets the requirements of the test.

Akers etal.(1991) the need for sterility testing of antimicrobial-containing injectable solutions is discussed and specific testing methods are described. Despite their antimicrobial activity, antimicrobial-containing injectable drug products are not necessarily self-sterilizing and can become contaminated. In addition to practicing aseptic technique,

pharmacists must perform end-product sterility testing on intravenous solutions to ensure their sterility.

The United States Pharmacopoeia provides guidelines for the performance and validation of two sterility test methods: membrane filtration and direct transfer to culture media. Membrane filtration is the method of choice for sterility testing of many antimicrobial-containing injectable solutions. After the test article is filtered, the membrane is rinsed with sterile fluid to remove residual antimicrobial agent, cut into two portions, and immersed in two types of culture medium.

Visible turbidity of a sample within the appropriate incubation period indicates the presence of a contaminating micro-organism. Closed filtration systems minimize false-positive results.

In the direct transfer method, samples of the test article are directly inoculated into vessels of culture media, and antimicrobial activity is eliminated by dilution or by deactivation with chemical or enzymatic agents. Sterility testing as well as aseptic technique is needed to ensure the sterility of antimicrobial-containing injectable solutions.

Bathgate etal. (1993) stated that, sterility test results gathered over a ten year period have been analysed to determine the effects of the incubation period. Overall there was no difference between the membrane filtration test and direct inoculation in the time required for visible growth of contaminants.

Growth occurred earlier if products had no preservative or antimicrobial substances.

However use of the membrane filtration method did not significantly enhance the efficiency of detection at seven days incubation. Regardless of the nature of the product or the method of test an unacceptable proportion of contaminants would be missed by limiting incubation to seven days.

Van Doorne etal. (1998) sterility testing and media fills are essential requirements in the pharmaceutical industry. With the results obtained the manufacturer must ensure that the aseptic filling process is under control. In an eight year (1988-1995) retrospective survey of three major Dutch pharmaceutical companies the performance of the sterility test, of media fills and their relationship have been statistically evaluated. The products included human and veterinary pharmaceuticals and biologicals, and were divided into six different groups according to their production process and primary containers. A distinction was made between the results from the period 1985-1991 and the period 1992-1995, because this made the statistical analyses of a number of types of products possible, and because some significant changes in the production process were made in 1991 at some of the production sites. The results of the evaluation of the sterility test show that the frequency of false positives has not changed significantly.

For all product groups the frequency of positive sterility tests has decreased during the period of investigation.

During the period 1992-1995 there was no significant difference between the results of product sterility tests and the negative controls for any of

the product groups. This indicates that given the present state-of-the-art production the sterility test offers little or no additional security.

In the more recent period there is a good agreement between the observed positive rate of the sterility tests and the positive rate that had been estimated from the results of the media fills. This indicates that despite some shortcomings, media fills are an adequate simulation of the production process and can be used to give an estimate of the rejection rate for the various product groups.

The overall conclusion is that the production conditions of the participating Dutch pharmaceutical companies comply with the current international guidelines for aseptic production and sterility testing.

The European Pharmacopoeia, Third Edition (1998) the test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined. The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily preparations and for preparations miscible with or soluble in aqueous or oily solvents which do not have an antimicrobial effect in the conditions of the test.

Membrane filtration - Use membrane filters having a nominal pore size not greater than 0.45 μm whose effectiveness to retain micro-organisms has been established. Cellulose nitrate filters, foe examples, are used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters, for example, for strongly alcoholic solutions. Specially adapted filters

may be needed for certain products. If filters of different diameter are used the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane are sterilised by appropriate means. The apparatus is so designed that the solutions to be examined can be introduced and filtered under aseptic conditions; it permits the aseptic removal of the membrane for transfer to the medium or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

2.2. Preparation for conducting sterility test:-

Center for Veterinary Biologics and National Veterinary Services Laboratories (1999) stated that this Supplemental Assay Method describes the test procedure used to detect viable bacteria and fungi in all live viral vaccines and Master Seed Virus samples as prescribed in the Code of Federal Regulations, Title 9 (9 CFR), Part 113.27. In the presence of these contaminating extraneous agents, the medium will be rendered turbid by macroscopic examination.

Preparation for the sterility test:-

1. Personnel qualifications/training

The personnel performing the test must have experience or training in this protocol. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling, and disposal of biological agents, reagents, tissue culture samples, and chemicals. The personnel must also have knowledge of safe operating procedures and policies and Quality Assurance (QA) guidelines of the Center for Veterinary

Biologics-Laboratory (CVB-L) or equivalent, as well as training in the operation of the necessary laboratory equipments.

2. Preparation of equipments/instrumentation

2.1 Turn the biosafety cabinets on at the beginning of the work week and leave them on all week.

2.2 Monitor the incubators daily for temperature.

2.3 Monitor freezers and coolers used for the storage of biologicals for temperature daily.

3. Preparation of the samples

3.1 Receive the biological samples to be tested from the Biological Materials Processing Section (BMPS).

3.2 Log in the biological samples by comparing the serial numbers of all vials, recording the diluent numbers, assigning a test number, and completing the testing log book.

3.3 Determine the volume of test media needed for each serial to be tested. Record volumes used in the log book.

3.4 Order sufficient SCDM from the media preparation department to be delivered 1 day before the biological samples are to be tested. Order sufficient media to include testing for positive, negative, and tech controls.

3.5 Order sterile purified water in serum vials from the media preparation department in sufficient volumes, as stated on the label or in the outline, for those serials without accompanying diluents. Order enough sterile water for the tech controls.

The United States of America Pharmacopeia (2000)

Pharmacopeial articles are to be tested by the Membrane Filtration Method where the nature of the products permits. If the membrane filtration technique is unsuitable, use the Direct Transfer Method.

Because sterility testing is very exacting procedure, where asepsis of the procedure must be ensured for a correct interpretation of results, it was important that personnel be properly trained and qualified.

These pharmacopeial procedures are not by themselves designed to ensure that a batch of product is sterile or has been sterilized. This was accomplished primarily by validation of the sterilization process or of the aseptic processing procedures. When evidence of microbial contamination in the article is obtained by the appropriate pharmacopeial method, the result so obtained was conclusive evidence of failure of the article to meet the requirements of the test for sterility, even a different result is obtained by an alternative procedure.

The European Pharmacopoeia (2000) the sterility test is applied to substances, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating micro-organism has been found in the sample examined in the conditions of the test.

Precaution against microbial contamination

The test for sterility is carried out under aseptic conditions using, for example, a class A laminar-air-flow cabinet located within a class B clean-room, or an isolator. The precautions taken to avoid contamination

are such that they do not affect any micro-organisms which should be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate controls.

Diachenko and Levitskii (2001) noticed that agar bacteriological nutrient media are suggested, based on soybean extract subjected to enzymatic hydrolysis and lyophilized native. The growth of test strains, number of grown colonies and their size were virtually the same as after inoculation of these strains in control nutrient media.
Soybean salt medium with yolk suspension was tried with good results as elective medium for isolation of staphylococci from clinical material. Commercial manufacture of these media mainly from native soybean extract is proposed.

Boll Chim Farm (2002) throughout the evolution of sterility test methodology, different periods of incubation were adopted depending on both the type of micro-organism to be detected and the employed inoculation methodology. Some official compendiums, such as Brazilian and Mexican pharmacopoeias, recommend different incubation periods, according to the inoculation methodology employed for the sterility test, whereas the United States Pharmacopeia, in its last edition, started to adopt an incubation period of 14 days, independently from the methodology employed. The aim of this work was the evaluation of the influence of the incubation time on the efficiency of different methodologies for sterility test, as well as the benefits that could be

achieved with the incubation time extension. The experiments led to the conclusion that an incubation period of 14 days is enough for the detection of microbiological contaminants in pharmaceutical products submitted to sterility tests, independently from the methodology employed, what values the alteration introduced in the USP XXIV, to the detriment of other pharmacopoeias which maintain different periods of time in accordance with the inoculation method employed.

The sterility test aims at the contact of a product with a culture medium, as a way of detecting the possible presence of viable micro-organisms in products which have been submitted to a sterilization process and/or to aseptic processing. Since the official introduction of the methodology, in 1932, several culture media have been proposed and adopted, in a constant attempt to offer conditions which support the growth of as many contaminants as possible. This work aimed at a comparative evaluation of the efficiency of microbial contaminants detection in the different types of culture media employed in sterility tests. The study led to the conclusion that the culture media recommended by the pharmacopoeia compendia, soybean casein digest and fluid thioglycollate, present the best results in micro-organisms detection. Besides, the microbial strains, as well as microbial suspension density, recommended by the main pharmacopoeias to verify culture media growth promoting capacity, have also proved suitable for use.

Pharmaceutical Inspection Convention (2002) although "sterility" is an absolute term, the assurance that any given item is sterile is a probability function, commonly expressed as a negative power to the base ten. The minimum acceptable Sterility Assurance Level (SAL) for terminally sterilised drugs was generally based on the probability of a non-sterile unit of 10^{-6}.

In practice, the sterility of a product is defined by the absence of viable and actively multiplying micro-organisms when tested in specified culture media.

Turbidity in the broth media usually indicates contamination. This test is performed on the end-product and is one of the quality control tests specified for release of a batch of sterile product. The sterility test cannot be used to demonstrate the sterility of the entire batch but it may assist in identifying a non sterile batch of product.

It is acknowledged that the sterility test suffers from significant statistical limitations and this contributes to the low probability of detecting anything less than gross contamination. However, these limitations can be reduced considerably by performance of the test under conditions that optimise the recovery of micro-organisms.

2.3. Precautions against microbial contamination:

Therapeutic Goods Administration Guidelines for Sterility Testing of Therapeutic Goods (2002)

1. Sterility cannot be guaranteed by the quality control laboratory. It must be built into the product during processing. Experience in many countries over the years has confirmed that greater reliance must be placed on appropriate techniques and procedures throughout the manufacture of the product (including in-process sterility testing at various stages) rather than simply depending on sterility tests made on a number of samples of the final batch as the sole criterion of sterility.

2. Permission to delete the sterility test from batch release specifications may be granted by the national authority where manufacturing standards are of a high order and the product is terminally sterilised in its final container.

3. In all other cases, sterility testing is necessary to detect contamination arising from technical malfunction, human error or mix-up between sterilised and non-sterilised goods. It must be performed as part of batch release specifications for product that is not terminally sterilised in the final container. It is also the only analytical method available for finished product testing by the national authority.

4. It is important to realise that background contamination as detected by negative controls can obscure low levels of product contamination and for this reason every effort should be made to ensure that background contamination is kept as low as possible. Statistics compiled by the TGA Laboratories show that skilled operators working under the prescribed

conditions can achieve a level as low as one contamination in five thousand control inoculations (0.02%).

Precaution against microbial contamination.

1. Tests for sterility are to be carried out by trained personnel using techniques and equipment which minimise the risks of accidental microbial contamination of the tests and of the testing environment.
2. Tests for sterility should be conducted in a clean-room environment that is equivalent to the standard of clean-room required for the aseptic manufacture of pharmaceutical products.
3. Personnel occupying the aseptic testing area during sterility testing or associated aseptic manipulations should wear sterilised over garments.
The use of sanitised garments may be acceptable under certain conditions.
4. All equipment, vessels and materials with which the sterile test media or the goods under test may come into contact in the course of the testing should be sterilized prior to use. Preferred methods are heating in an autoclave, so that all surfaces are held at a temperature of 121°C and exposed to saturated steam for at least 15 minutes, or by heating to, and holding for at least 2 hours at a temperature of 160°C in a hot air oven, or by exposure to a minimum absorbed radiation dose of 25 kGy.
5. All substances added to the goods tested and all substances added to sterile media or introduced into sterile membrane filtration units (other than the preparations under test) should be sterilized prior to use by heat. If this reduces the effectiveness of the test, the most effective alternative method should be used.

6. Prior to sterilization all vessels, substances or outer clothing to be used for the performance of tests for sterility or introduced into the testing area should be appropriately packaged or closed to prevent the access of micro-organisms. Each package or item being sterilised should bear a visual indicator appropriate to the method of sterilisation to indicate that it has been processed but the appropriate change in the appearance of the indicator should not be taken as a guarantee of the sterility of the contents. Each package or item should be dated with the date of sterilisation to assist in correct stock rotation.
7. The outer surfaces of all packages of equipment, vessels, etc., which are introduced into the aseptic testing environment (including vessels of media and packages or containers of goods to be tested) should be free of contamination immediately prior to their introduction into the aseptic environment: they should be sterilised or disinfected by an appropriate method which does not prejudice the viability of micro- organisms which may be present in the preparations to be tested. A pass-through hatch (or transfer box) is considered part of the testing environment. If the packages are double-wrapped and sterilised the outer wrapping should be removed just prior to the introduction of the package into the testing environment.

Bungo and Pinto Tele (2003) their studies were conducted to evaluate the effects of media, incubation temperature and duration on the detection of bacteria and fungal growth using British, United States and Brazilian compendial sterility test methods. 5 to 50 CFU of nine different

micro-organisms (including both anaerobic and aerobic bacteria and moulds) were used to contaminate test units containing various growth media (soybean-casein digest, thioglycollate, Sabourand and Causen broths). Test units were incubated at temperatures ranging from 12 to 42°C for 1 to 28 days. Inoculations were conducted according to compendial procedures. Optimal detection conditions were obtained at 22 to 32°C over 14 days using soybean casein digest and thioglycollate broths.

2.4. Methods of inspection of biological products for microbiological contaminations:

As ***The United States of America Pharmacopeia (1970)*** the media used for isolation prepared or dehydrated mixtures may be used provided that, when reconstituted as directed by the manufacturer or distributor, they have growth-promoting properties equal or superior to those obtained from the given formulas.

Confirm the sterility of each lot of medium used by incubation of samples of it at the temperature and for the length of time specified for it, where its use is called for in the tests.

In the sterilization of filled vessels of media, the desired temperature of sterilization should be reached as rapidly as possible, and the medium cooled quickly after sterilization to preserve its growth-promoting qualities. Close the test vessels in such manner as to exclude airborne and other contaminants, and to reduce evaporation during incubation.

Store the sterilized media at a temperature preferably between 20°C and 30°C, protected from light.

Fluid Thioglycollate Medium is intended for use with clear, fluid products. Alternative Thioglycollate Medium is acceptable in place of Fluid Thioglycollate Medium for the turbid and viscid products, provided it is freshly prepared or is boiled and cooled just prior to use. Soybean Casein Digest Medium is particularly useful for the detection of contamination with a low incidence of aerobic bacteria and fungi.

The European Pharmacopeia (1971)

The tests for bacterial and fungal contamination are carried out on the same sample of the preparation or the substances to be examined. When the volume or the quantity in a single container is insufficient to carry out the tests, the contents of two or more containers are used to inoculate the different media.

Culture media - Any culture media suitable for aerobic or anaerobic bacteria or both and any media suitable for the lower fungi may be used provided that they have been recently prepared and their nutritive properties demonstrate in fertility tests using an adequate range and suitable strains of an aerobic organism such as ***Staphylococcus aureus***, an anaerobic organism such as ***Plectridium sphenoides*** and a yeast such as ***Candida albicans***.

Pharmaceutical inspection Convention (2004)

1. SAMPLING

1.1 The number of containers tested per batch and quantity tested from each container should be, as a minimum, in accordance with the pharmacopoeial method followed.

1.2 Samples from aseptic fills should be selected from at least the beginning, middle and end of the batch fill. Additionally, SOPs should define criteria for inclusion and collection of samples immediately after interruptions and operator interventions during the filling process.

1.3 Samples from terminal sterilisation cycles should be selected from at least the potentially coolest part of the load if such a location was identified during validation studies, and from every load sterilised.

1.4 If an original test is declared invalid, then any samples used for the repeat sterility test should reflect the original samples in terms of sampling locations or aseptic processing times.

2. TEST METHODOLOGY

2.1 The test methodology should be in accordance with the pharmacopoeial method used. Membrane filtration of the product, with either an open or a closed system, is the preferred sterility test methodology. The filter should be pre-wetted, particularly when small volumes and antibiotics are tested. Filtration of the product should be followed by the minimum number of washes of the membrane with a suitable rinsing fluid established during validation studies. The membrane should not be permitted to dry out between filtration steps.

2.2 If the product cannot be filtered, then direct inoculation, immersion, in-situ incubation or combination methods as appropriate are acceptable.

3. MEDIA TYPES AND MANUFACTURE

3.1 The media used should be in accordance with the pharmacopoeial method followed. Soya-bean casein digest (SCD) and fluid thioglycollate media (FTM) should normally be used. Alternative media are permitted and may be appropriate if the nature of the product or method of manufacture could result in the presence of fastidious organisms (e.g. vaccines, blood products, etc). Validation studies should demonstrate that alternative media are capable of supporting the growth of a wide range of micro-organisms. Inactivators of antimicrobials may be incorporated into growth media or rinse solutions as indicated by validation studies.

3.2 Media should be purchased from an approved supplier, or prepared in-house according to standard operating procedures that are based on validated sterilisation processes. pH checks of media should be included in these procedures to ensure that the pH is within specifications at the time of use.

3.3 A batch number and a shelf-life should be assigned to all media and batch manufacturing documentation should be maintained.

FDA Guide to inspections of microbiological pharmaceutical quality control laboratories (1991)

The agency published a proposed rule regarding the manufacture of drug products by aseptic processing and terminal sterilization. A list of

contaminated or potentially contaminated drug products made by aseptic processing and later recalled was also made available. Many of the investigations/inspections of the recalled products started with a list of initial sterility test failures. FDA review of the manufacturer's production, controls, investigations and their inadequacies, coupled with the evidence of product failure (initial sterility test failure) ultimately led to the action.The USP points out that the facilities used to conduct sterility tests should be similar to those used for manufacturing product. The USP states, "The facility for sterility testing should be such as to offer no greater a microbial challenge to the articles being tested than that of an aseptic processing production facility". Proper design would, therefore, include a gowning area and pass-through airlock. Environmental monitoring and gowning should be equivalent to that used for manufacturing product.

Since a number of product and media manipulations are involved in conducting a sterility test, it is recommended that the inspection include actual observation of the sterility test even though some companies have tried to discourage inspection on the grounds that it may make the firm's analyst nervous.

The inspection team is expected to be sensitive to this concern and make the observations in a manner that will create the least amount of disruption in the normal operating environment. Nevertheless, such concerns are not sufficient cause for you to suspend this portion of the inspection.

One of the most important aspects of the inspection of a sterility analytical program is to review records of initial positive sterility test results. Request lists of test failures to facilitate review of production and control records and investigation reports. Particularly, for the high risk aseptically filled product, initial positive sterility test results and investigations should be reviewed. It is difficult for the manufacturer to justify the release of a product filled aseptically that fails an initial sterility test without identifying specific problems associated with the controls used for the sterility test.

Examine the use of negative controls. They are particularly important to a high quality sterility test. Good practice for such testing includes the use of known terminally sterilized or irradiated samples as a system control. Alternatively, vials or ampoules filled during media fills have also been used. Be especially concerned about the case where a manufacturer of aseptically filled products has never found an initial positive sterility test. While such situations may occur, they are rare. In one case, a manufacturer's records showed that they had never found a positive result; their records had been falsified. Also, the absence of initial positives may indicate that the test has not been validated to demonstrate that there is no carryover of inhibition from the product or preservative.

Evaluate the time period used for sterility test sample incubation. This issue has been recently clarified. The USP states that samples are to be incubated for at least 7 days, and a proposal has been made to change the USP to require a period of 14 days incubation. You are expected to

evaluate the specific analytical procedure and the product for the proper incubation period. Seven days may be insufficient, particularly when slow growing organisms have been identified. Media fill, environmental, sterility test results and other data should be reviewed to assure the absence of slow growing organisms. Also, you should compare the methods being used for incubation to determine if they conform to those listed in approved or pending applications.

Code of Federal Regulations (2002)

I- Culture media for detection of bacteria and fungi.

(a) Ingredients for which standards are prescribed in the United States Pharmacopeia, or elsewhere in this part, shall conform to such standards.

In lieu of preparing the media from the individual ingredients, they may be made from dehydrated mixtures which, when rehydrated with purified water, have the same or equivalent composition media and have growth-promoting buffering and oxygen tension- controlling properties equal to or better than such media. The formulas for the composition of the culture media are set forth in the United States Pharmacopeia, 19th Edition.

(b) The licensee shall test each quantity of medium prepared at one time from individual ingredients and the first quantity prepared from each lot of commercial dehydrated medium for growth-promoting qualities.

If any portion of a lot of commercial dehydrated medium is held for 90 days or longer after being so tested, it shall be retested before use. Two or more strains of micro-organisms that are exacting in their nutritive requirements shall be used. More than one dilution shall be used to

demonstrate the adequacy of the medium to support the growth of a minimum number of micro-organisms.

(c) The sterility of the medium shall be confirmed by incubating an adequate number of test vessels and examining each for growth. Additional control may be used by incubation of representative uninoculated test vessels for the required incubation period during each test.

(d) A determination shall be made by the licensee for each biological product of the ratio of inoculum to medium which shall result in sufficient dilution of such product to prevent bacteriostatic and fungistatic activity. The determination may be made by tests on a representative biological product for each group of comparable products containing identical preservatives at equal or lower concentrations.

Inhibitors or neutralizers of preservatives, approved by the administrator, may be considered in determining the proper ratio.

II- Detection of viable bacteria and fungi except in live vaccine.

Each serial and subserial of biological product except live vaccines shall be tested as prescribed in this section unless otherwise specified by the administrator. When cell lines, primary cells, or ingredients of animal origin used in the preparation of a biological product are required to be free of viable bacteria and fungi, they shall also be tested as prescribed in this section.

(a) The media to be used shall be as follows:

(1) Fluid Thioglycollate Medium with 0.5 percent beef extract shall be used to test for bacteria in biological products containing ***clostridial toxoids***, ***bacterins***, and ***bacterin-toxoids***.

(2) Fluid Thioglycollate Medium with or without 0.5% beef extract shall be used to test for bacteria in biological products other than ***clostridial toxoids***, ***bacterins***, and ***bacterin-toxoids***.

(3) Soyabean-Casein Digest Medium shall be used to test biological products for fungi; provided that Fluid Thioglycollate Medium without beef extract shall be substituted when testing biological products containing mercurial preservatives.

(b) Test procedure:

(1) Ten test vessels shall be used for each of two media selected in accordance with paragraph (a) (1), (a) (2), or (a) (3) of this section.
Each test vessel shall contain sufficient medium to negate the bacteriostatic or fungistatic activity in the inoculum as determined in Sec. I(d).

(2) Inoculum:

(i) When completed product is tested, 10 final container samples from each serial and each subserial shall be tested. One ml from each sample shall be inoculated into a corresponding individual test vessel of culture medium: Provided that, if each final container sample contains less than 2 ml, one-half of the contents shall be used as inoculum for each test vessel.
(ii) When cell lines, primary cells, or ingredients of animal origin are tested, at least a 20 ml test sample from each lot shall be tested.

One ml shall be inoculated into each test vessel of medium.

(3) Incubation shall be for an observation period of 14 days at 30 °C to 35 °C to test for bacteria and 14 days at 20 °C to 25 °C to test for fungi.

(4) If the inoculum renders the medium turbid so that the absence of growth cannot be determined by visual examination, subcultures shall be made on the seventh to eleventh day from biological products prepared from ***clostridial toxoids***, ***bacterins***, and ***bacterin-toxoids*** and the third to seventh day for other biological products. Portions of the turbid medium in amounts of not less than 1.0 ml. shall be transferred to 20-25 ml of fresh medium and incubated the balance of the 14-day period.

(c) Examine the contents of all test vessels for macroscopic microbial growth during the incubation period. When demonstrated by adequate controls to be invalid, the test may be repeated. For each set of test vessels representing a serial or subserial in a valid test, the following rules shall apply:

(1) If no growth is found in any test vessel, the serial or subserial meets the requirements of the test.

(2) If growth is found in any test vessel, one retest to rule out faulty technique may be conducted using 20 unopened final container samples.

(3) If growth is found in any test vessel of the final test, the serial, subserial, or ingredients to be used in the preparation of a biological product, as the case may be, is unsatisfactory.

III- Detection of extraneous viable bacteria and fungi in live vaccines.

Unless otherwise specified by the Administrator or elsewhere exempted in this part, each serial and subserial of live vaccine shall be tested for extraneous viable bacteria and fungi as prescribed in this section.

(a) Live viral vaccines. Each serial and subserial of live viral vaccine shall be tested for purity as prescribed in this paragraph. However, products of chicken embryo origin recommended for administration other than by parenteral injection may be tested as provided in paragraph (e) of this section.

(1) Soybean Casein Digest Medium shall be used.

(2) Ten final container samples from each serial and subserial shall be tested.

(3) Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and desiccated vaccine shall be rehydrated as recommended on the label with accompanying diluent or with sterile purified water.

(4) To test for bacteria, place 0.2 ml of vaccine from each final container into a corresponding individual vessel containing at least 120 ml of Soybean Casein Digest Medium. Additional medium shall be used if the determination required in Sec. I (d) indicates the need for a greater dilution of the product. Incubation shall be at 30 °C to 35 °C for 14 days.

(5) To test for fungi, place 0.2 ml of vaccine from each final container sample into a corresponding individual vessel containing at least 40 ml of Soybean Casein Digest Medium. Additional medium shall be used if the determination required in Sec. I (d) indicates the need for a greater dilution of the product. Incubation shall be at 20 °C to 25 °C for 14 days.

(6) Examine the contents of all test vessels macroscopically for microbial growth at the end of the incubation period. If growth in a vessel cannot be reliably determined by visual examination, judgment shall be confirmed by subcultures, microscopic examination, or both.

(7) For each set of test vessels representing a serial or subserial tested according to these procedures, the following rules shall apply:

(i) If growth is found in 2 or 3 test vessels of the initial test, 1 retest to rule out faulty technique may be conducted using 20 unopened final container samples.

(ii) If no growth is found in 9 or 10 of the test vessels in the initial test, or 19 or 20 vessels in the retest, the serial or subserial meets the requirements of the test.

(iii) If growth is found in four or more test vessels in the initial test, or two or more in a retest, the serial or subserial is unsatisfactory.

(b) Live bacterial vaccines. Each serial or subserial of live bacterial vaccine shall be tested for purity as prescribed in this paragraph.

(1) Soybean Casein Digest Medium and Fluid Thioglycollate Medium shall be used.

(2) Ten final container samples from each serial and subserial shall be tested.

(3) Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and desiccated vaccine shall be rehydrated as recommended on the label with accompanying diluent or with sterile purified water. Product recommended for mass vaccination shall be rehydrated at the rate of 30 ml sterile purified water per 1000 doses.

(4) To test for extraneous bacteria, place 0.2 ml of vaccine from each final container into a corresponding individual vessel containing at least 40 ml of Fluid Thioglycollate Medium. Additional medium shall be used if the determination required in Sec. 1 (d) indicates the need for a greater dilution of the product. Incubation shall be at 30 °C to 35 °C for 14 days.

(5) To test for extraneous fungi, place 0.2 ml of vaccine from each final container into a corresponding individual vessel containing at least 40 ml of Soybean Casein Digest Medium. Additional medium shall be used if the determination required in Sec. 1 (d) indicates the need for a greater dilution of the product. Incubation shall be at 20 °C to 25 °C for 14 days.

(6) Examine the contents of all test vessels macroscopically for atypical microbial growth at the end of the incubation period. If growth of extraneous micro-organisms cannot be reliably determined by visual examination, judgment shall be confirmed by subculturing, microscopic examination, or both.

(7) For each set of test vessels representing a serial or subserial tested according to these procedures, the following rules shall apply:

(i) If extraneous growth is found in 2 or 3 test vessels of the initial test, 1 retest to rule out faulty technique may be conducted using 20 unopened final container samples.

(ii) If no extraneous growth is found in 9 or 10 test vessels in the initial test, or 19 or 20 vessels in the retest, the serial or subserial meets the requirements of the test.

(iii) If extraneous growth is found in 4 or more test vessels in the initial test, or 2 or more in a retest, the serial or subserial is unsatisfactory.

3. MATERIAL AND METHODS

3.1. MATERIALS:

3.1.1. Samples:

The samples used in this study are

3.1.1.1. Human Biologics:

3.1.1.1.1. Antisera:

1- Tetanus antitoxin of horse origin

- Vial contains 30 000 IU single dose "curative"
- 40 samples were tested.
- Manufactured by VACSERA*.

2- Diphteria antitoxin of horse origin:

- Vial contains 10 000 IU single dose
- 20 samples were tested.
- Manufactured by VACSERA*.

3- Purified polyvalent antiscorpion serum of horse origin

- Liquid.
- 1 ml ampoule (Single dose)
-20 samples should be tested.
- Manufactured by VACSERA*.

4- Snake venom antiserum of horse origin

-Liquid.

-10 ml vial (single dose).

- 20 samples should be tested.
- Manufactured by VACSERA*.

3.1.1.1.2. Killed Bacterial Vaccine:

1-Adsorbed Diphteria, Tetanus Toxoid & Pertussis (DTP) Vaccine

- 5 ml vial (10 doses)
- 20 samples were tested.
- Manufactured by VACSERA*.

2-Adsorbed Diphteria & Tetanus Toxoid (DT) Vaccine

- 5 ml vial (10 doses)
- 20 samples were tested.
- Manufactured by VACSERA*.

3-Typhoid Vaccine:

(Formaline Killed)

- 10 ml vial (10 doses)
- 20 samples should be tested.
- Manufactured by VACSERA*.

3.1.1.2. Veterinary Vaccine:

• Inactivated Viral Vaccine:

1-Canine Distemper:

- 20 samples should be tested.
- Manufactured by VSVRI**.

2-Paramyxo Vaccine.

- 20 samples were tested.
- Manufactured by VSVRI**.

3-Parvo Vaccine.

- Ten samples were tested.
- Manufactured by VSVRI**.

*VACSERA is the Holding Company for Biological Products and Vaccines.

**VSVRI is Veterinary Serum and Vaccine Research Institute.

N.B. The samples should be transported bat 2 – 8 °C.

Table 1. Types of Biological products used in the study

The Product	No.of examined samples
1. Antisera	
1.1 Tetanus antitoxin	40
1.2 Diphteria antitoxin	20
1.3 antiscorpion serum	20
1.4 Snake venom antiserum	20
2. Killed Bacterial Vaccine	
2.1 DTP Vaccine	20
2.2 DT Vaccine	20
2.3 Typhoid Vaccine	20
3. Inactivated Viral Veterinary Vaccine	
3.1 Canine Distemper	20
3.2 Paramyxo Vaccine	20
3.3 Parvo Vaccine.	20

3.1.2. Media used:

3.1.2.1. Media for bacteriological examination:

3.1.2.1.1. Solid media for primary isolation:

• Nutrient agar medium *(Oxoid, 1992)*. It was used for detection of chromogenic bacteria.

• MacConkey's agar medium *(Oxoid, 1992)*. It was used for isolation of various types of Enterobacteriaceae.

• Blood agar base medium *(Oxoid, 1992)*. It was used for detecting haemolytic activities.

3.1.2.1.2. Fluid media for primary isolation:

• Fluid thioglycollate medium. It was used for detection of ***Staphylococcus aureus***, ***Pseudomonas aeruginosa*** and ***Clostridium sporogenes.***

3.1.2.2. Media for mycological examination:

Fluid media for fungal isolation:

• Soybean casein digest medium. It was used for detection of mould and yeast.

3.2. METHODS:

3.2.1. Collection of samples:

Test the number of samples specified in Table 1. If the contents of each article are of sufficient quantity (see Table 3), they may be divided so that equal appropriate portions are added to each of the specified media.

The samples should be transported in 2-8°C.

Table 2. Minimum Number of samples to Be Tested in Relation to the Number of Articles in the Batch

Number of Articles in the Batch	Number of Articles to be tested
Not more than 100 articles	10% or 4 articles, whichever is greater
More than 100 but not more than 500 articles	10 articles
More than 500 articles	2% or 10 containers, whichever is less

3.2.2. Procedure of samples collection from an article:

Great care must be taken on our consideration when opening an article, so that the sample to be tested for sterility is not contaminated by microorganisms present on the exterior of the container. The exterior surfaces of ampoules and

closures of vials and bottles were cleansed with a suitable decontaminating agent, and the containers must be placed in an environment that prevents recontamination of the exterior surfaces. If the vial contents are packaged under vacuum, admit sterile air by means of a suitable sterile device, such as a needle attached to a membrane filter holder containing a sterilizing grade filter.

3.2.3. Quantity of samples:

When using the direct transfer method, use the quantities in Table 3.

3.2.4. Volume of Medium:

The volume of medium used in the test is not less than the volume indicated in Table 3.

Table 3. Quantities of samples to be tested

Container content (mL)	Minimum volume taken from each product container for each medium	Minimum volume, in mL, of Each Medium
less than 10	1 mL, or entire contents if less than 1 mL	15
10 to less than 50	5 mL	40
50 to less than 100	10 mL	80

3.2.5. Incubation Condition:

The inoculated fluid media were incubated aerobically for not less than 14 days at 32.5±2.5°C for bacteriological examination or at 22.5±2.5° C for mycological examination. Observe the tubes of media on a periodic basis over than 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary. The inoculated solid media were incubated aerobically for 24-48hours at 37°C. The inoculated media were examined for bacterial growth.

3.2.6. Testing Facilities:

The following two types of facilities are used for sterility testing.

3.2.6.1. Clean Rooms and Clean Zones:

A clean room of a sterility testing facility is maintained under microbiological control criteria appropriate for the critical zones in an

aseptic processing facility. When a clean zone is used for sterility testing, it must also meet the same microbiological control criteria.

3.2.6.2. Isolators

Isolators are free-standing environments that allow aseptic manipulations to be made from outside the controlled environment. Isolator systems protect the test articles and sterility test supplies from contamination during aseptic handling. Transfer ports from a dedicated autoclave or decontamination ports are used. The interior of the isolator must also meet the same microbiological control criteria.

3.2.7. Bacteriological examination:

3.2.7.1. Cultivation on Fluid thioglycollate medium:

1. Ten test vessels shall be used for media (according to table 2). Each test vessels contain sufficient medium to negate the bacteriostatic or fungistatic activity in the inoculums as determined (according to table 3).

2. Inoculum:

Ten final container samples from each serial and each subserial shall be tested. One ml from each sample was inoculated into a corresponding individual test vessel of culture medium (according to table 3) and were Incubated for an observation period of 14 days at 30-35°C.

If the inoculum renders the medium turbid so that the absence of growth cannot be determined by visual examination, subculture shall be made on the third to seventh day. Portions of the turbid media in amount of not less than 1.0 ml shall be transferred to 20 to 25 ml of fresh medium and incubated the balance of 14-day period.

Examine the contents of all test vessels for macroscpic microbial growth during the incubation period. When demonstrated by adequate controls to be invalid, the test may be repeated. For each set of test vessels representing a serial or subserial in a valid test, the following rules shall apply:

(i) If no growth is found in any test vessels, the serial or subserial meets the requirements of the test.

(ii) If growth is found in any test vessel, one retest to rule out faulty technique may be conducted using 20 unopened final container samples.

(iii) If growth is found in any test vessels of the final test, the serial, subserial, or ingredients to be used in the preparation of a biological product, as the case may be, is unsatisfactory.

3.2.7.2. Cultivation on solid medium:

1. Three plates of each nutrient agar medium, macConkey's agar medium and blood agar medium were inoculated as subculture from the inoculated fluid thioglycolate medium.

2. Incubate for 24-48 hours at 37°C.

3.2.8. Mycological examination:

Cultivation on Soybean casein digest medium:

Ten test vessels shall be used for media (according to table 2). Each test vessels shall contain sufficient medium to negate the bacteriostatic or fungistatic activity in the inoculums as determined (according to table 3). 10 final container samples from each serial and each subserial shall be tested. One ml from each sample shall be inoculated into a corresponding

individual test vessel of culture medium (according to table 3) and incubation shall be for an observation period of 14 days at 20-25°C.

If the inoculum renders the medium turbid so that the absence of growth cannot be determined by visual examination, subculture shall be made on the third to seventh day. Portions of the turbid mediums in amount of not less than 1.0 ml shall be transferred to 20 to 25 ml of fresh medium and incubated the balance of 14-day period. Examine the contents of all test vessels for macroscopic microbial growth during the incubation period. When demonstrated by adequate controls to be invalid, the test may be repeated. For each set of test vessels representing a serial or subserial in a valid test, the following rules shall apply:

(i) If no growth is found in any test vessels, the serial or subserial meets the requirements of the test.

(ii) If growth is found in any test vessel, one retest to rule out faulty technique may be conducted using 20 unopened final container samples.

(iii) If growth is found in any test vessels of the final test, the serial, subserial, or ingredients to be used in the preparation of a biological product, as the case may be, is unsatisfactory.

4. RESULTS

4.1. Results of bacteriological examination:

4.1.1. Results of bacteriological examination using fluid media:

The examination of 10 samples from each biological product which are Tetanus antitoxin serum, Diphteria antitoxin serum, polyvalent antiscorpion serum, antisnake serum, DTP Vaccine, DT Vaccine, Typhoid Vaccine, Canine Distemper vaccine, Paramyxo Vaccine, Parvo Vaccine (totally 100 samples) cultivated into two tubes of fluid thioglycollate media and incubated for 14 days at 30-35°C. The examination of the 100 samples revealed that the percentage of bacterial contamination was 0 %.

4.1.1.1 Examination of human biologics

4.1.1.1.1 Examination of human antisera:

Table (4) shows that the examined samples from each of Tetanus antitoxin serum, Diphteria antitoxin serum, polyvalent antiscorpion serum and antisnake serum are free from bacterial contamination.

Table (4): Results of bacteriological examination of human antisera cultivated into fluid media.

Tetanus Antitoxin		Diphtheria Antitoxin		Anti-Scorpion serum		Anti-Snack serum	
No. of examined samples	**Results**	**No. of examined samples**	**Results**	**No. of examined samples**	**Results**	**No. of examined samples**	**Results**
10	Negative	10	Negative	10	Negative	10	Negative

4.1.1.1.2 Examination of human killed bacterial vaccine:

Table (5) shows that the examined samples from each of DTP vaccine, DT vaccine and Typhoid vaccine are free from bacterial contamination.

Table (5): Results of bacteriological examination of human killed bacterial vaccine cultivated into fluid media.

DTP Vaccine		DT Vaccine		Typhoid Vaccine	
No. of samples	**Results**	**No. of samples**	**Results**	**No. of samples**	**Results**
10	Negative	10	Negative	10	Negative

4.1.1.2 Examination of veterinary killed bacterial vaccine:

Table (6) shows that the examined samples from Canine Distemper vaccine, paramyxo vaccine and Parvo vaccine are free from bacterial contamination.

4.1.1.1.2 Examination of human killed bacterial vaccine:

Table (5) shows that the examined samples from each of DTP vaccine, DT vaccine and Typhoid vaccine are free from bacterial contamination.

Table (5): Results of bacteriological examination of human killed bacterial vaccine cultivated into fluid media.

DTP Vaccine		DT Vaccine		Typhoid Vaccine	
No. of samples	**Results**	**No. of samples**	**Results**	**No. of samples**	**Results**
10	Negative	10	Negative	10	Negative

4.1.1.2 Examination of veterinary killed bacterial vaccine:

Table (6) shows that the examined samples from Canine Distemper vaccine, paramyxo vaccine and Parvo vaccine are free from bacterial contamination.

Table (6): Results of bacteriological examination of veterinary killed bacterial vaccine cultivated into fluid media.

Canine Distemper Vaccine		Paramyxo Vaccine		Parvo vaccine	
No. of samples	**Results**	**No. of samples**	**Results**	**No. of samples**	**Results**
10	Negative	10	Negative	10	Negative

4.1.2. Results of bacteriological examination using solid media:

The examination of 10 samples from each biological product which are Tetanus antitoxin serum, Diphteria antitoxin serum, polyvalent antiscorpion serum, antisnake serum, DTP Vaccine, DT Vaccine, Typhoid Vaccine, Canine Distemper vaccine, Paramyxo Vaccine, Parvo Vaccine (totally 100 samples) cultivated onto three plates of each of nutrient agar, macConkey's agar and blood agar media and incubated for 24 - 48 hours at 37°C. The examination of the 100 samples revealed that the percentage of bacterial contamination was 0 %.

4.1.2.1 Examination of human biologics

4.1.2.1.1 Examination of human antisera :

Table (7) shows that the examined samples from each of Tetanus antitoxin serum, Diphteria antitoxin serum, polyvalent antiscorpion serum, antisnake serum are free from bacterial contamination.

Table (7): Results of bacteriological examination of human antisera cultivated onto solid media.

Tetanus Antitoxin		Diphtheria Antitoxin		Anti-Scorpion serum		Anti-Snack serum	
No. of examined samples	Results	No. of examined samples	Results	No. of examined samples	Results	No. of examined samples	Results
10	Negative	10	Negative	10	Negative	10	Negative

4.1.2.1.2 Examination of human killed bacterial vaccines:

Table (8) shows that the examined samples from each of DTP vaccine, DT vaccine and Typhoid vaccine are free from bacterial contamination.

Table (8): Results of bacteriological examination of human killed bacterial vaccine cultivated onto solid media.

DTP Vaccine		DT Vaccine		Typhoid Vaccine	
No. of samples	**Results**	**No. of samples**	**Results**	**No. of samples**	**Results**
10	Negative	10	Negative	10	Negative

4.1.2.2 Examination of veterinary killed bacterial vaccine:

Table (9) shows that the examined samples from each of Canine Distemper vaccine, Paramyxo vaccine and Parvo vaccine are free from bacterial contamination.

Table (9): Results of bacteriological examination of veterinary killed bacterial vaccine cultivated onto solid media.

Canine Distemper Vaccine		Paramyxo Vaccine		Parvo Vaccine	
No. of samples	**Results**	**No. of samples**	**Results**	**No. of samples**	**Results**
10	Negative	10	Negative	10	Negative

4.2. Results of mycological examination:

4.2.1. Results of mycological examination using fluid media:

The examination of 10 samples from each biological product which are Tetanus antitoxin serum, Diphteria antitoxin serum, polyvalent antiscorpion serum, antisnake serum, DTP Vaccine, DT Vaccine, Typhoid Vaccine, Canine Distemper vaccine, Paramyxo Vaccine, Parvo Vaccine (totally 100 samples) cultivated to two tubes of Soybean casein digest media and incubation for 14 days at 20-25°C.

The examination of the samples except the Tetanus antitoxin serum revealed that the percentage of fungal contamination was 0 %.

The examination of the Tetanus antitoxin serum revealed that there is growth is found in one test vessel at the 7^{th} day, one retest to rule out faulty technique may be conducted using 20 unopened final container samples.

4.2.1.1 Examination of human biologics

4.2.1.1.1 Examination of human antisera:

Table (10) shows that the examined samples from Diphteria antitoxin serum, polyvalent antiscorpion serum, antisnake serum are free from mycological contamination. The results revealed one of the examined samples from Tetanus antitoxin serum evidence of growth is found.

4.2.1.1.2 Examination of human killed bacterial vaccine:

Table (11) shows that the examined samples from DTP vaccine, DT vaccine and Typhoid vaccine are free from mycological contamination.

Table (11): Results of mycological examination of human killed bacterial vaccine cultivated into fluid media.

DTP Vaccine		DT Vaccine		Typhoid Vaccine	
No. of samples	**Results**	**No. of samples**	**Results**	**No. of samples**	**Results**
10	Negative	10	Negative	10	Negative

Table (10): Results of mycological examination of human antisera cultivated into fluid media.

Tetanus Antitoxin			Diphtheria Antitoxin		Anti-Scorpion serum		Anti-Snack serum	
No. of examined samples	Results		No. of examined samples	Results	No. of examined samples	Results	No. of examined samples	Results
10	1 Positive	9 Negative	10	Negative	10	Negative	10	Negative

4.2.1.2 Examination of veterinary killed bacterial vaccine:

Table (12) shows that the examined samples from Canine Distemper vaccine, Paramyxo vaccine and Parvo vaccine are free from mycological contamination.

Table (12): Results of mycological examination of veterinary killed bacterial vaccine cultivated into fluid media.

Canine Distemper Vaccine		Paramyxo Vaccine		Parvo Vaccine	
No. of samples	**Results**	**No. of samples**	**Results**	**No. of samples**	**Results**
10	Negative	10	Negative	10	Negative

4.2.2. Results of retest of the tetanus antitoxin serum:

Table (13) shows that the examined 20 samples from Tetanus antitoxin serum are free from mycological contamination. So, the growth that is found in the primary examination was due to faulty technique.

Table (13): Results of mycological re-examination of Tetanus antitoxin serum cultivated into fluid media.

Tetanus Antitoxin serum	
No. of samples	**Results**
20	Negative

5. DISCUSSION

Infectious disease continues to be one of the most important constraints on the efficient production of farm livestock in both developing and developed countries. Vaccination is increasingly being viewed as the more sustainable option which plays an important role in human and animal disease control.

Sterility management is the system that combines the continuous efforts of research and development (R&D), production, quality control (QC) and quality assurance (QA) in reducing and controlling the level of microbiological contamination are personnel, air and equipment.

Tests of sterility are necessary for assessing samples from a product that should be microbiologically sterile, since they give informations on the presence of viable aerobic or anaerobic bacteria and viable fungi.
During the incubation period and at its conclusion, the contents of all of the vessels examined for macroscopic evidence of microbial growth, such as the development of turbidity.

Sterility tests are designed to determine the presence of bacteria and fungi in or on test devices or solutions. The present work was planned to throw some light on the role of sterility tests to determine the presence of various bacteria and fungi contamination in the human and veterinary biological products which produced locally.

Bacteriological examination of human biologiacls which are Tetanus antitoxin serum, Diphteria antitoxin serum, polyvalent antiscorpion serum, antisnake serum, DTP Vaccine, DT Vaccine, Typhoid Vaccine revealed that the bacterial contamination represents 0% as indicated in Tables 3, 4, 6, 7.

The results obtained during this study revealed the 0% of bacteriological contamination of veterinary biologicals which are Canine Distemper, Paramyxo Vaccine and Parvo Vaccine (Table 5, 8).

The mycological examination of the tested human biologicals except the Tetanus antitoxin serum revealed that the mycological contamination represents 0% as indicated in Tables 9, 10. One of the examined samples from Tetanus antitoxin serum is mycologically contaminated

The results obtained during this study revealed the 0% of mycological contamination of veterinary biologicals which are Canine Distemper, Paramyxo Vaccine and Parvo Vaccine (Table 11).

According to ***the Pharmacopeia of the Untied States of America, (1970 and 1975)*** if no evidence of growth is found, the product meets the requirements for test for the sterility. If evidence of growth is found, the lot of product fails to meet the requirements for the test for the sterility, unless it can be demonstrated by retest or by other means that the test was invalid for causes unrelated to the product.

For sterility tests of products in which the sterilization process has been monitored by use of biological indicators, if no evidence of growth is found in test vessels containing either the indicator only, or the indicator and product under test, the product meets the requirements of the test for sterility. If the test vessels containing either indicator only, or the indicator and product under test, show evidence of growth, the product fails to meet the requirements of the test for sterility, unless it can be demonstrated by retest or by other means that the test was invalid for causes unrelated to the product.

If the nature of the material or other factors make the reading of the tubes uncertain, we should follow the procedures as in ***the Europian pharmacopoeia, (1971)*** the suspect tubes may be subcultured and readings taken after a further period of incubation. If growth of micro-organisms occurs, repeat the test. If growth of the same micro-organism occurs in the second test, the preparation being examined is deemed to have failed the test. If in the second test, growth of different micro-organisms occurs, repeat the test a third time. The preparation being examined passes the test if no growth of micro-organisms then occurs.

The World Health Organization **(1973)** assured in its technical report series that if no evidence of growth is found in any of the vessels inoculated for the test for sterility, the final product meets the requirements for this test. If evidence of growth is found, the preparation tested fails to meet the requirements for the test for sterility, unless it can be demonstrated to the

satisfaction of the national control authority either by retests or by other means, that the test was invalid.

The United States Pharmacopeia, (1990) and Pharmaceutical Inspection Convention (2002) divided the interpretation of the sterility tests into two stages:

FIRST STAGE - At the prescribed intervals during and at the conclusion of the incubation period, examine the contents of all of the vessels for evidence of microbial growth, such as the development of turbidity and / or surface growth. If no growth is observed, the article tested meets the requirements of the test for the sterility. If microbial growth is found, but a review in the sterility testing facility of the monitoring, materials used, testing procedure and negative controls indicates that inadequate or faulty aseptic technique was used in the test itself if the first stage is declared invalid and may be repeated.

If microbial growth is observed but there is no evidence invalidating the first stage of the test, proceed to the second stage.

SECOND STAGE - The minimum number of specimens selected is double the number tested in the first stage. The minimum volumes tested from each specimen and the media and incubation periods are the same as those indicated for the first stage. If no microbial growth is found, the article tested meets the requirements of the test for sterility. If growth is found, the result so obtained is conclusive that the article tested fails to meet the requirements of the test for sterility. If it can be demonstrated that the second stage was

invalid because of faulty or inadequate aseptic technique in the performance of the test the second stage may be repeated.

The media should be examined for macroscopic evidence of microbial growth during the incubation period. When the material being tested renders the medium turbid, so that the presence or absence of microbial growth cannot be determined readily by visual examination 14 days after the incubation started, transfer suitable portions of the medium to fresh vessels of the same medium. Continue incubation of the original and of the transfer vessels for a total period of not less than 14+7 days from the original inoculation.

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test may be considered invalid only when one or more of the following conditions are fulfilled:

a) the data of the microbiological monitoring of the sterility testing facility show a fault.
b) a review of the procedure used during the test in questions reveals a fault.
c) microbial growth is found in the negative controls.
d) after determination of the identity of the micro-organisms isolated from the test the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and / or the technique used in conducting the sterility test procedure.

If the test is declared to be invalid it is repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test the product examined complies with the test for sterility. If microbial growth is found in the repeat test the product examined does not comply with the test for
sterility. The previous procedures are mentioned at ***The European Pharmacopoeia, (1998 and 2000)***

To approve that the product which examined does not comply with the test for sterility, microscope examination for each tube which appears to have macroscopic growth should be applied. Microscopic examination is described in the testing protocol of ***the Center for Veterinary Biologics and National Veterinary Services Laboratories (1999)*** which concluded that

1. On day 14 of the incubation period, examine all test vessels for cloudiness due either to the product or a contaminant. If it is not possible to determine if the cloudiness is due to a contaminant, then subculture the serial. To subculture, place 1 ml from the test vessel into 40 ml of fresh SCDM, using a sterile individually packaged 1-ml pipette. If less than 10 test vessels of a serial are cloudy, then subculture those that are or at least 3 vessels. If all 10 test vessels are cloudy, then subculture 3 randomly picked test vessels at each incubation temperature. Incubate these subculture tubes for an additional 3 days.
2. Prepare 1 microscope slide from each tube which appears to have macroscopic growth.

After these slides have dried, Gram stain and observe them with a microscope. Enter the number of tubes with growth and no-growth as well as the Gram stain results, in the log book for this test code. Initial and date in the log book as the person taking the serials off test.

3. If extraneous growth is observed in 2 or 3 test vessels and confirmed by Gram stain, then conduct 1 retest using 20 unopened final container samples.
4. If no extraneous growth is found in 9 or 10 test vessels of the initial test or 19 or 20 vessels of the retest, the serial is satisfactory (SAT).
5. If extraneous growth is found in 4 or more test vessels of the initial test or 2 or more of the retest, the serial of biologic is unsatisfactory (UNS).
6. If a serial is found unsatisfactory, freeze 3-4 ml of contaminated media. Label the tube containing the 3-4 ml with the test code, the serial's test number, and the date. Save the Gram-stained microscope slides.

To apply the sterility test and to be assured from the results, this test performed in a clean room or isolators as mentioned in ***the untied states pharmacoeia,(2000)***

Clean Rooms and Clean zones - When performing the sterility test in a clean room or clean zone, the article meets the requirements of the test of sterility when no microbial growth is observed and confirmed microscopically, the article does not meet the requirements of the test for sterility. However if the microbial growth can be without a doubt ascribed to failed aseptic techniques or materials used in conducting the sterility testing procedure, the test is invalid and must be repeated. If microbial growth is not

observed the article tested meets the requirements of the sterility test. If microbial growth is observed and confirmed microscopically, the article tested does not meet the requirements of the sterility test.

Isolators - When performing the sterility test in an isolator, the article meets the requirements of the test for sterility when no microbial growth is observed. When microbial growth is observed and confirmed microscopically, the article does not meet the requirements of the test. If the microbial growth can be without a doubt ascribed to a loss of physical integrity of the isolator or to gross contamination due to faulty aseptic techniques or unsterile materials within the isolator enclosure, the test is invalid. Repeat the test as indicated above.

Pharmaceutical Inspection Convention, PIC/S. (2002)

1. A test may be repeated only when it can be demonstrated that the test was invalid for causes unrelated to the product being examined. The European Pharmacopoeial restricts criteria to one or more of the following conditions only:

   the test the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

NOTE 1: When conditions (a), (b) or (c) apply then the test should be aborted prior to the completion of the incubation period.

NOTE 2: If a stasis test is performed at the end of the test incubation period, failure of challenge micro-organisms to grow in this test also invalidates the test.

NOTE 3: For condition (d) to apply as the sole criterion used to invalidate a test, it is necessary to demonstrate that a micro-organism isolated from the product is identical to an isolate from the materials and/or the environment. This determination entails the use of a sensitive typing technique such as a molecular typing technique or other techniques similar to those used for epidemiological studies.

However, if tests are performed competently in a clean room environment the chance of simultaneous adventitious contamination occurring in the environment, test sample and negative controls is negligible. Provisions that allow repeat testing based on morphological or biochemical characterisation of environmental and/or product contaminants should not be permitted. It is possible for the environment to become contaminated by the samples under test, which may contain multiple micro-organisms that are difficult to differentiate without employing sensitive typing techniques.

2. If contamination, which is established to be unrelated to the product, occurs in the original test, the test may be repeated with the same number of test samples as used in the original test, with negative product controls tested concurrently.
3. If contamination is detected in the repeat test performed on the same number of test samples, the product does not comply with the test for sterility and the entire batch should be rejected. The European Pharmacopoeia1 does not permit further testing of the sample under any circumstances.

The same guideline for sterility testing of biological products were proposed by ***TGA Therapeutic Goods Administration, 2002***

1. If microbial growth is not evident in any of the vessels inoculated with the product, the sample tested complies with the test for sterility, provided that growth of challenge organisms has been demonstrated in the stasis test (if performed), in growth promotion tests on the batches of media used and in test method validation. This interpretation applies even if growth occurs in negative product control vessels.
2. If microbial growth is evident the product does not comply with the test for sterility unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product being examined.
3. If microbial growth is evident, the criteria for invalidating the test are:

(a) the data of the microbiological monitoring of the sterility testing facility show a fault;

(b) a review of the testing procedure used during the test in question reveals a fault;

(c) microbial growth is found in the negative product controls;

(d) after determination of the identity of the micro-organisms isolated from the test, the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or technique used in conducting the sterility test procedure.

4. When conditions (a), (b) or (c) apply, the test should be aborted prior to the completion of the incubation period.
5. If condition (d) is to be used as the sole criterion for invalidating a sterility test, it is necessary to employ sensitive typing techniques to demonstrate

that a micro-organism isolated from the product test is identical to a micro-organism isolated from the materials and/or the environment. While routine biochemical/phenotypical identification techniques can demonstrate that two isolates are not identical, these methods are not sufficiently sensitive or reliable enough to provide unequivocal evidence that two isolates are from the same source. Suitably sensitive tests (for example, molecular typing with RNA/DNA homology) are those accepted by microbiologists conducting epidemiological studies to determine that micro organisms are clonally related and have a common origin. Repeat testing based on the biochemical or phenotypical characterisation of environmental and/or product isolates should not be permitted. The test environment can be contaminated by actual product samples, which may contain multiple micro- organisms that are difficult to speciate without employing sensitive typing techniques.

6. If the test is declared to be invalid it may be repeated with the same number of units as in the original test.
7. If there is no evidence of growth in any vessels inoculated with the product during the repeat test the product passes the test for sterility. This interpretation applies even if growth occurs in negative product control vessels.
8. If there is evidence of growth in the test vessels the product fails the test for sterility. Further testing is not permitted under any circumstances.
9. If two consecutive tests on the same product give evidence of growth in control vessels, or consecutive working sessions give evidence of growth

in controls, or there is any other evidence of breakdown in testing methods, then there should be a complete review of all facilities and testing procedures to determine the cause of the contamination. Further tests on samples should be suspended until the review is completed.

The result recorded in this study indicated that the production of the tested products had to be in accordance with current good manufacturing practice (GMP) and quality control (QC) had to be in accordance with both GMP and good laboratory practice (GLP).

So, the importance of this study is to ensure that the production conditions fully meet the requirements of GMP/GLP.

6. SUMMARY

Sterility tests are designed to determine the presence of bacteria and fungi in or on test devices or solutions.

The standard USP sterility test and EP specifies using both soybean casein digest media [SCDM] for mycological examination and fluid thioglycollate media [FTM] for bacterial examination. SCDM is incubated at 20-25 °C; FTM is incubated at 30-35 °C. Both media are normally incubated for 14 days .

The tested materials are:

20 samples from each of the following samples:

1. Human Biologics:
1.1 Antisera:

1.1.1 Tetanus antitoxin of horse origin.

1.1.2 Diphteria antitoxin of horse origin.

1.1.3 Purified polyvalent antiscorpion serum of horse origin.

1.1.4 Snake venom antiserum of horse origin.

1.2 Killed Bacterial Vaccine:

2.1 Adsorbed DTP Vaccine

2.2 Adsorbed DT Vaccine

2.3 Typhoid Vaccine

2. Veterinary Vaccine:

2.1 Canine Distemper Vaccine.

2.2 Paramyxo Vaccine.

2.3 Parvo Vaccine.

The result of this study reveals that the tested materials are free from any bacterial or fungal contamination

Sterility tests should never be used as the primary means of verification of sterility because the method is subject to many variables including technique, environment, media, etc.

Sterility test methodologies vary for radiation sterilized products. Resistance tables only incorporate aerobes, and thus SCDB media is normally used alone for radiation verification tests.

So, the importance of our study is to ensure that the production conditions fully meet the requirements of GMP/GLP.

7. REFERANCES

1- The Pharmacopeia of the Untied States of America, Sixteenth Revision. U.S.P. XVI. October 1, (1960):855-858.

2- The Pharmacopeia of the Untied States of America, Seventeenth Revision. September 1, (1965):829-832.

3- The Pharmacopeia of the Untied States of America, Eighteenth Revision. September 1, (1970):851-857.

4- European Pharmacopoeia, volume II, (1971):53-57.

5- World Health Organization, Technical report series No.530. Twenty-fifth Report (1973):41-57.
General Requirements for the sterility of biological substances

6- The Unıied States Pharmacopeia, Nineteenth Revision. July 1, (1975):592-595

7- Seyfarth H., (1975) Jul.;
A comparison of the assay regulations for sterility testing between the USP XIX and the European Pharmacopoeia. Zentralbl Bakteriol; 160(4-5):432-42.

8- Christianson GG and Koski TA. (1983) Apr.;
A comparison of a disposable membrane filtration system with a direct inoculation system for the sterility testing of veterinary biologics. J Biol Stand;11(2):83-9.

9- Pappalardo G, Hirschi B, Pannatier A and Francioli P. (1988) Dec;
Comparative study between liquid and solid media for the detection of bacterial contamination in intravenous solutions.
Clin Pharm Ther.13(6):411-6.

10-Winkler D, Lukaszewicz V. (1989);
Long-term analysis of sterility testing of immunobiological preparations.
State Control Institute for Immunobiological Preparations in Central Institute of Hygiene, Microbiology and Epidemiology of GDR, Berlin.
J Hyg Epidemiol Microbiol Immunol. 33(3):317-22.

11- The United States Pharmacopeia, (1990) January 1; 1483-1488.

12- Akers MJ, Wright GE, Carlson KA. (1991) Nov;
Sterility testing of antimicrobial-containing injectable solutions prepared in the pharmacy. Am J Hosp Pharm.; 48(11):2414-8.

13- Bathgate H, Lazzari D, Cameron H, McKay D. (1993) Sept.– Oct.
The incubation period in sterility testing
J Parenter Sci Technol.;47(5):254-7.

14- Van Doorne H, Van Kampen BJ, Van der Lee RW, Rummenie L, Van der Veen AJ and De Vries WJ. (1998) Jul-Aug;
Industrial manufacture of parenteral products in The Netherlands. A survey of eight years of media fills and sterility testing.
PDA J Pharm Sci Technol. 52(4):159-64

15- European Pharmacopoeia, Third Edition, (1998):21-24.

16- Center for Veterinary Biologics and National Veterinary Services Laboratories Testing Protocol, Supplemental Assay Method for Sterility Testing of Live Viral Vaccines and Master Seed Virus Samples.
Date: April 21, (1999), Number: STSAM0908.01

17- The United States of America Pharmacopeia, (2000):1483-1488.

18- European Pharmacopoeia, Asian Edition. (2000) January 1:45-48.

19- Diachenko IuV, Levitskii AP. (2001) Mar;
Soybean bacteriological culture media and prospects for their use in clinical bacteriology.
Klin Lab Diagn:49-51.

20- Boll Chim Farm. (2002) Nov-Dec;141(6):453-6.
Incubation time in sterility tests for pharmaceutical products.
Bugno A, Pinto Tde J. Instituto Adolfo Lutz, Sao Paulo, Brasil.

21- Bugno A, Pinto Tde J. (2002) Sept.-Oct.
Comparative study between culture media employed in sterility test.
Instituto Adolfo Lutz, SP, Brasil. Boll Chim Farm.;141(5):367-71.

22- Pharmaceutical Inspection Convention; Pharmaceutical Inspection Co-operation Scheme,PIC/S
Recommendation on sterility testing, (2002) 1 November:1-12.

23- Therapeutic Goods Administration, (2002).
Guidelines for Sterility, Testing of Therapeutic Goods: 3-32.

24- Bugno A, Pinto Tde J. (2003);
The influence of incubation conditions in sterility tests.
PDA J Pharm Sci Technol. 57(6):399- 403.

25- Pharmaceutical Inspection Convention; Pharmaceutical Inspection Co-operation Scheme,PIC/S
Recommendation on sterility testing, (2004) 4 May,:3-14.

26- FDA Guide to inspection of microbiological pharmaceutical quality control laborarories.

27- Code of Federal Regulations. (2002) Title 9, Volume 1.
Revised as of January 1:10-14.

List of Abbreviations

1- **USP** : United States Pharmacopeia.
2- **EP** : European Pharmacopeia.
3- **CFR** : Code of federal regulations.
4- **SCDM** : Soya been casein digest media.
5- **CFU** : Colony forming unit.
6- **FTM** : Fluid thioglycollate media.
7- **VACSERA**: Holding Company for Biological Products and Vaccines.
8- **VSVRI** : Veterinary Serum and Vaccine Research Institute.
9- **DTP** :Diphteria, Tetanus Toxoid & Pertussis.
10- **DT** : Diphteria & Tetanus Toxoid.
11- **GMP** : Good manufacturing process.
12- **GLP** : Good laboratory practice.
13- **QA** : Quality assurance.
14- **QC** : Quality control.
15- **R&D** : Research and development.
16- **SAL** : Sterility Assurance Level.

الملخص العربى

التلوث البكتيرى لبعض المستحضرات البيولوجية المنتجة محلياً

تعتبر اللقاحات و الامصال هى خط الدفاع الأول ضد الأمراض سواء فى المجال البيطرى أو البشرى لذا كان من الضرورى ضمان فاعلية هذه المنتجات وأيضا ضمان خلوها من الملوثات سواء البكتيرية أو الفطرية حت لا تتحول بدورها إلى مصدر للإصابة بالأمراض.

تم تعريف التعقيم بمنع وجود الكائنات الحيَّةالدقيقة وهى تتم بالتسخين، الترشيح، المعالجة بأكسيد الإثيلين أو بالأشعة المؤينة مع توافر الظروف العقيمة للإنتاج.

إنّ الهدفَ من هذه الدراسة هو الكشف عن الملوثات البكتيرية و الفطرية التى من الممكن أن نجدها في المُنتَجاتِ الحيويةِ البشرية والبيطرية. عندها يمكن تحديد هذه الملوثات و معالجتها حتي يمكن حل المشاكل الناتجة عنها.

تم فى هذه الدراسة استخدام الميديا الصلبة مثل النيترنت اجار (Nutrient agar) و الماكونكى اجار(MacConkey's agar) و الـ (Blood agar) على أن يتم التحضين عند درجة حرارة ٣٧ درجة مئوية لمدة ٤٨ ساعة.

تنص إختبارات العقامة القياسية فى الفارماكوبيا الأمريكية و الأوربية على استخدام ميديا الصويا بين كازين [SCDM] للفحص الفطرى على أن يتم التحضين عند درجة حرارة ٢٠- ٢٥ درجة مئوية، وأيضاً على استخدام ميديا الثيوجليكولات السائلة [FTM] للفحص البكتيرى على أن يتم التحضين عند درجة حرارة ٣٠- ٣٥ درجة مئوية لمدة ١٤ يوم فى كلتا الحالتين.

فى هذه الدراسة تم فحص ٢٠ عينة من كل من :-

١- منتجات بيولوجية للإستخدام الآدمى وهى:-

- مصل ضد داء الكزاز.

- مصل ضد الدفتيريا.

- مصل مُنَقَّى ضد سم العقرب.

- مصل ضد سم الثعبان.

- اللقاح الثلاثى.

- اللقاح الثنائى.

- لقاح ضد التيفويد.

٢- منتجات بيولوجية للاستخدام البيطرى وهى:-

- لقاح الكانين ديستمبر.

- لقاح الباراميكسو.

- لقاح البارفو.

تكشف نتائج هذه الدراسة أن العينات المُختبرة مطابقة للمواصفات القياسية لخلوها من التلوث الجرثومى والفطرى.

لابد من الأخذ فى الاعتبار أن إختبارات العقامة لا يجب أنْ تُستعمل كوسائل أساسية لتحقق التعقيم لأن هذه الإختبارات خاضعة للعديد من المتغيرات تتضمن التقنية، البيئةالمحيطة، الميديا، وخلافه.

لذا، تؤكد هذه الدراسة على ضرورة أن يتبع الإنتاج شروط ممارسة التصنيع الجيدة وممارسة المُختبر الجيدة لضمان جودة وسلامة المنتجات الحيوية سواء كانت للاستخدام الآدمى أو البيطرى.

تحـــت إشـــــراف

الأستاذ الدكتور / محمد على عقيلة

أستاذ ورئيس قسم الميكروبيولوجيا

كلية الطب البيطرى

جامعة الأسكندرية

الدكتور / سامى عبدالسلام خليل

أستاذ مساعد الميكروبيولوجيا

كلية الطب البيطرى

جامعة الأسكندرية

التلوث البكتيرى لبعض المستحضرات البيولوجية المنتجة محلياً

رسالة مقدمة من

ط.ب./ مروة لطفى غانم

بكالوريوس العلوم الطبية البيطرية- كلية الطب البيطرى – جامعة القاهرة- ١٩٩٣

مقدمة إلى

قسم الميكروبيولوجيا

كلية الطب البيطرى

جامعة الأسكندرية

للحصول على

درجة الماجستير فى العلوم الطبية البيطرية

{ تخصص ميكروبيولوجيا وفطريات ومناعة}

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