

Microbiological Testing in a Nigerian Pharmaceutical Company Microbiology Laboratory

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Abstract

This is a case study of microbiology testing performed in a pharmaceutical company microbiology laboratory in Lagos, Nigeria which consists of the following ; routine laboratory safety and cleanliness, operation and care of laboratory instruments, preparation of glassware- ready for use, sterilization and sterilization techniques, proper methods of collection of samples for microbiological testing, inoculation and incubation of samples, detection of contaminants, pollution monitoring of the air and environment of the factory and many more.

This laboratory experience provides a broad insight and knowledge of microbiological control techniques as applied to the Nigerian Pharmaceutical industry which will facilitate the ability of microbiologists to be able to control the air, environment and samples in pharmaceutical companies in the locality.

Keywords: Microbiological; Testing; Nigerian; Pharmaceutical; Laboratory

Introduction

One of the oldest and biggest pharmaceutical companies in Nigeria with branches in other parts of Nigeria, that is into the manufacturing of drugs and other pharmaceutical products was visited for training. Every pharmacy shop in Nigeria has their products in its shelf because of the confidence and trust consumers repose on their products.

The company is a big organization made up of many departments which in turn are split into many units. Some of these departments are the finance services department, the quality control department, production department, material control department and the site and engineering department.

Each department is split into units, for example: the quality control department is split into units like the chemical analysis laboratory, finished products unit, raw materials unit, microbiology laboratory and the instrument room.

The staff of the company were warm and courteous. On our arrival to the company, we were taken on an exciting tour of the departments with general explanation of their operations, organization structure, function of the department and its relationship with other departments, line of command structure of the department or organigram.

We were introduced to the employees of the departments and shown our place of work and to whom we will be responsible and our payment system.

We were supplied with protective clothing and information about the location of toilets, washing facilities, canteen, medical room, notice boards, main products of the company, house-keeping, accident reports, laboratory equipment and materials etc.

Routine Laboratory Safety and Cleanliness

On entry into any of the laboratories, there is a slogan which is popular in each of them- "SAFETY NOW. SAFETY ALWAYS". Another slogan reads- "PROUD TO BE SAFE". Safety in the laboratory was fully emphasized.

We were told to:

1. Allow the autoclave to cool down before opening it because of its explosive nature.
2. Use woolen hand gloves to bring down boiled agar medium from hot plates or to remove dry heat sterilized glass wares from the oven.
3. Destroy grown cultures on plates by autoclaving before disposal.
4. Clean table tops, bench tops and laminar flow work stations thoroughly before and after work on them.
5. Handle glass wares and other fragile items gently and carefully.
6. Prevent water from dripping on any instrument.
7. Wash glass wares with detergent and water before air drying and sterilization.
8. Wear clean caps and laboratory coats always.
9. Ensure that all materials that are to be used for laboratory work is clean and many more.

Operation and Care of Laboratory Equipment

All laboratory equipment apart from the incubators must be switched off and unplugged from the power source at the close of work. The incubator is not switched off because it is used to incubate samples for about 3- 5 days.

We were taught how to operate and work competently with all the instruments in the microbiology laboratory. The instruments in the microbiology laboratory includes the autoclaves, microscope, hot plate, colony counter, refrigerator for storing chemicals and reagents, laminar flow hood or work station, water bath and electronic weighing balance.

Preparation of Glass Ware- Ready for Use

The glass wares are washed with detergent and water, then turned upside down on a clean table and allowed to dry. When they dry, they are put into the hot air oven for dry heat sterilization at 170 °C for a minimum of 1 ½ hours. The glassware used always are petri dishes and short bijou bottles for collection of water and drug samples.

The used old cultures in petri dishes are melted in the B&T Autoclave model with purge 13 minutes, sterilization 15 minutes and cooling 40 minutes. They are poured into the sink before they solidify. Water is also run on it so that the used agar medium from the used petri dish will not block the sink.

Sterilization and Sterilization Techniques

The items we sterilize in the laboratory include already boiled agar medium, prepared Ringer's solution and plastic wares. The above-mentioned items are sterilized with moist heat in the autoclave with purge of 13 minutes, sterilization 15 minutes and cooling 40 minutes before use.

Grown or old cultures are destroyed in the autoclave before they are discarded. The plastic petri-dishes are wrapped in a "Sterilin paper bag", autoclaved and discarded. The glass wares are sterilized in the dry heat oven at 170 °C for a minimum of 1 ½ hours. The laminar flow work station is scrubbed with ethanol before use.

The bench tops are also cleaned with ethanol. The floor of the laboratory is scrubbed with household bleach or fumigated as soon as there is need to do so in order to keep the laboratory under sterile conditions. The same applied to the production and packaging areas of the company.

Proper Methods of Collection of Water and Drug Samples

The samples collected for microbiological testing are water samples from the production areas ie; potable water and deionized water from liquid room where syrup and other "liquid-like" drugs are prepared, deionized water from the granulation room where granules "solid tablets and capsules" are prepared. Other samples collected are drug samples which are tested microbiologically for the presence of contaminants. Some of these samples tested include raw materials for the preparation of the drugs.

All are subjected to bacteriological analysis.

The apparatus used for collection of samples apart from the normal laboratory coat and cap include hand gloves, ethanol, spatula, sterilized bijou bottles, cotton wool, nose mask and basket.

The sterile bottles remain sterilized as long as they are not opened. They are only opened when the sample is to be collected. In the case of solid granule samples, the spatula is scrubbed with cotton wool soaked in ethanol with hand gloves and mask on.

After scrubbing the spatula with ethanol, the spatula is used to take the granular solid samples. The bottle is opened and the granule samples are put into the bottle and tightened immediately.

As for the liquid samples like water and syrup, the pipe carrying the liquid is allowed to run for about two minutes before the sample is collected. The cap of the sterile bijou bottle is opened and the liquid sample is run into the bottle and the bottle will then be closed and tightened immediately.

Samples like raw materials in jerry cans are drawn into sterilized bottles with pipette and tested in the laboratory.

Inoculation and Incubation of Samples

The surface of the laminar flow hood work station is scrubbed with ethanol before inoculation begins. Before then, the agar medium to be used have been prepared already as well as the Ringer's solution. The agar used in the laboratory include McConkey Broth for coliform bacteriology test. This agar and the various other agar media are prepared according to manufacturer's instructions on the label on the agar bottle given by the manufacturers of the Agar powder.

For example, for MacConkey broth by Himedia International laboratories India, their instruction on the label of the agar bottle read this way;" Suspend 40 gms in 1000ml distilled water. Boil to dissolve completely. Dispense into tubes with inverted Durham tubes. Sterilize by autoclaving at 15lbs pressure (121 °C) for 15 minutes.

Other agar media used in the laboratory include Tryptone Soya Agar and Peptone Dextrose Agar (TSA and PDA).

TSA is used to test water samples. TSA supports the growth of bacteria and is incubated for 3 days in the 37 °C Gallenkamp incubator while the PDA supports the growth of molds and yeast. The PDA is incubated for 5 days in the Gallenkamp cooled incubator at 23 °C to 25 °C.

In the inoculation process, glass petri dishes are arranged in such a way that the PDA and TSA will test each sample. The controls were also made.

Before the inoculation of the granules, 5 grams of the granule sample is taken and put into 45 ml of Ringer's solution that have already been sterilized in the autoclave.

The granules are mixed thoroughly by shaking and turning them round gently. 1 ml of the sample solution is then dropped in a sterile dish carefully, and not to expose the sterile petri dishes to contamination during the opening. The petri dish is slightly opened and the sample solution is put in gently and the cover of the petri dish is closed immediately and flamed to avoid contamination.

Before putting the sample solution into the sterile petri-dish, the mouth of the bottle is flamed. For liquid samples, 1ml of the

liquid sample is put like that into the sterile petri dishes. The already prepared and autoclaved agar medium is then poured by pour plate method into the petri dish containing the 1ml liquid sample.

The petri- dish is then shaken gently and rocked carefully so as to mix the contents. Splashing of the agar medium on the body or cover of the petri dish should be avoided. After mixing and rocking gently, the agar medium is allowed to solidify.

After its solidification, the TSA medium that promotes bacterial growth is incubated for 3 days at 37 o C while the PDA medium that promotes the growth of moulds and yeast is incubated for 5 days in a 23 oC – 25 oC cooled incubator.

After incubation, the colonies are counted with a colony counter and recorded. Contaminated samples are destroyed with moist heat by autoclaving before disposal. Those that passed the test are not contaminated. They were then recorded for further use in the production of the drugs ie; the samples that passed the test are used to produce the drugs while contaminated ones are not used. Contaminated ones are discarded after autoclaving by moist heat decontamination.

The environment of the factory is also tested microbiologically by randomly setting petri dishes containing agar medium around the area to be tested. The air and environment of the packaging and production areas are often tested this way. Areas that are contaminated by microorganisms are fumigated.

Contaminated areas are indicated by the presence of growth (after incubation) on the agar medium in the petri dish placed in such places.

The presence of microbial growth on the agar medium in the petri-dish is an indication of microbial contamination of such areas. Those contaminated areas will then be fumigated.

Conclusion

This laboratory experience gave us a broad insight and knowledge of microbiological control techniques as applied to the pharmaceutical industry in Nigeria, West Africa. With this experience, we can now control the air, environment and samples microbiologically in any pharmaceutical company microbiology laboratory in the locality.

Authors Contributions:

PIA: Concept and design of the study, reviewed the literature, manuscript preparation, critical revision of the manuscript, collected data and review of study.

ACO: Concept and design of the study, reviewed the literature, manuscript preparation, critical revision of the manuscript, collected data and review of study.

ECO: Conceptualized study, literature search, prepared first draft of manuscript and critical revision of the manuscript.

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