INDSTRIAL TRAINING IN QC MICROBIOLOGY DEPARTMENT OF PHARMACEUTICAL COMPANY

An Industrial Training Report submitted

For the partial fulfillment of the Degree of Master of Science

By

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CERTIFICATE

This is to certify that this training report entitled "**INDSTRIAL TRAINING IN QC MICROBIOLOGY DEPARTMENT OF PHARMACEUTICAL COMPANY**" was successfully carried out by Miss **Shivani L Joshi** towards the partial fulfillment of requirements for the degree of Master of Science in Microbiology of Shri M & N Virani Science College, Rajkot. It is an authentic record of her own work, carried out by her under the guidance of Mr. Himansu Khandhedia for a period of Feb-2021- Apr-2021 during the academic year of 2020-2021 The content of this report, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other University.

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D E C L A R A T I O N

I hereby declare that the work incorporated in the present dissertation report entitled **"INDSTRIAL TRAINING IN QC MICROBIOLOGY DEPARTMENT OF PHARMACEUTICAL COMPANY"** is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of any Degree or a Diploma.

Date: 03-05-2021

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(Name and signature of Student)

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"If I have the belief that I can do it, I shall surely acquire the capacity to do it even if I may not have at the beginning".

- Mahatma Gandhi

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ABBREVIATIONS

Abbreviation	Full form
SCDA	Soyabean casein digest agar
SBDA	Sabouraud dextrose agar
DENA	Dey-engley neutralizing agar
R2AA	Reasoner's 2A agar
TAMC	Total aerobic microbial count
TYMC	Total yeast microbial count
CFU	Colony forming unit
MLT	Microbial limit test
GPT	Growth promoting test
LAF	Laminar air flow
USP	United states pharmacopeia

1. ABSTRACT

A microbiologist in pharmaceutical company is a member of quality control department, the role of the microbiologist is to ensure the quality of raw materials before they are processed in the production area, monitor the microbiological quality of environment (air monitoring) and water, validate the test methods used in testing finished products, and test finished products from microbiological perspective. For the microbiologist to perform an individual duty some devices have to found in lab as already mentioned, also the microbiologist has to prepare media and diluents and calibrate equipment used on the lab based on their SOP (standard operating procedure). Precautions have to be always followed in microbiology department and wearing laboratory uniform and safety clothes. We will discuss here some of the main tests performed in quality control pharmaceutical microbiology department.

2. INTRODUCTION

Farbe Firma was incorporated in the year 2012. It was started as a Dyes' and Pharmaceutical intermediates trading company. We have now ventured to diversify into Injectable Pharmaceutical formulations.

The company has signed an MOU (Memorandum of Understanding) with the Government of Gujarat under the "Vibrant Gujarat 2015". The company aims at providing contract manufacturing services to esteem and ambitious National and Multinational partners. At Farbe Firma "QUALITY" is being imbibed from the roots and considered as a "NECESSITY" and "EDGE" for healthy and long-lasting partnership. The manufacturing site (at GIDC, Ankleshwar) is basically a "brown-field" project.

The site is self-sufficient for all necessary utilities. The machinery installed is one of the best in the segment and promises a smooth and hassle-free production with a high-quality rating. Even if you are looking for high volumes, we assure you to comfortably accommodate and cater your needs in a timely manner.

our vision:

Our ultimate aim is to produce zero defect products and all our systems should act as filters at all levels to ensure that our products are of good quality and fulfil the individual requirements of our customers.

Quality control department:

The quality control (QC) microbiology department plays an essential role in pharmaceutical manufacturing and product release. It is responsible for multiple tasks, including:

- Environmental monitoring (sampling, bioburden counts, microbial identifications, and tracking and trending of data).
- Water analysis, pathogen testing, growth promotion test, sterility test, microbial limit test etc.
- Investigating out of specifications, deviations, and contamination events.
- Implementing and validating QC methods and testing based on release specifications.

These activities must follow the company's quality procedures and federal regulations. Because these establish the state of control of the manufacturing environment and are critical for product release, it is imperative that the QC microbiology laboratory perform tests accurately, reliably, and timely.

3. MATERIAL AND METHODS

3.1. Environment Monitoring:

- **Purpose of Environmental Monitoring**
- Where the purpose of these test to find the number of microorganisms found in the air in the production areas or in the microbiology lab during work and whether they are in the limit or not.
- The non-viable particles that do not contain any living organisms are spreading the viable particles throughout your facility, so monitoring for these is critical to maintaining asepsis.
- Sampling procedures to detect the microbial viable count by environmental monitoring

A] By settle plate monitoring or viable passive air sampling:

- Where settled plate method is used where Petri dished containing media is left open in certain places for certain places for certain period of time then closed and transferred in the incubator at 22 ºC - 25 ºC for 3 days and 32 ºC - 35 ºC for 2 days. Examined after the incubation period.

B] By air sampler monitoring or viable active air sampling:

- Where an air sampler is used which, a device is used to collect a predetermined volume of air and pass it on petri dish containing media then the petri dish transferred in the incubator at 22 ºC - 25 ºC for 3 days and 32 ºC - 35 ºC for 2 days. Examined after the incubation period.

C] Surface monitoring by contact plate sampling:

- Where DENA plate used for surface monitoring. Touch surface of agar on selected location for 5 seconds. Transferred the plate into incubator at 22 ºC - 25 ºC for 3 days and 32 ºC - 35 ºC for 2 days. Examined after the incubation period.

3.2 Growth Promotion Tests:

- **Purpose:**
- The growth promotion test is quality control requirement that confirms the ability of a new batch of media to support growth of a predetermined selection of representative microorganisms.
- **Growth Promotion Testing of Solid Media:**
- Use the culture suspension containing not less than 100 cfu for Growth promotion and inhibitory test and Inoculate 10 to 100 CFU of each specified organism in duplicate by either pour plate or spread plate and incubate at specified conditions.

- After completion of incubation, count the number of colonies recovered and Record the result.

- **Acceptance Criteria:**

➢ **Solid Agar Media:** The growth should be obtained within the specified duration and the number of CFU recovered should not differ by a factor greater than 2 from the calculated value of the standardized inoculums (Recovery should be obtained within the range of 70 to 100%).

➢ **Selective and Differential Media:** Characteristic/indicative growth should be obtained within the

- Perform the GPT in duplicate by spread plate/pour plate or direct inoculation technique.
- All the media that are expected to recover the growth on agar surfaces as per below calculation.

Formula Use for % Recovery	**Calculation:** Calculate the Microbial recovery in percentage by equation. $$\% \text{ Recovery} = \frac{\text{Mean CFU observed X 100}}{\text{Inoculated CFU ml}}$$ **Acceptance criteria:** Recovery should not less than 70 to 100 %.

➢ **Pour plate method:**

Add 0.1 mL of test organism culture suspension containing 10 – 100 cfu in to each of the sterile petri plate in duplicate and pour about 15 to 20 mL of sterilized agar media to each plate at a temperature about 45°C to 50°C. Mix well by rotating the plate's clockwise and anti-clockwise direction for homogenous mixing and allow the plates to solidify.

➢ **Spread Plate method:**

Add 0.1 mL of test organism suspension containing 10 -100 cfu on to the surface of media which is already poured in plate. Spread evenly with the help of sterile spreader. In case of inhibitory test, add the suspension containing not less than 100 cfu. Incubate the plates at specified condition. After completion of incubation period count number of cfu and record the observations. Incubate one un-inoculated plate/tube/bottle for each temperature as negative control. Use the sterile media for routine analysis only after the growth promotion test is done and the media is found meeting the growth promotion requirements. Growth obtained shall not differ by a factor greater than 2 from the calculated value for standardized inoculums for growth promotion test. There should not be any growth in the negative control. Perform the growth promotion testing of the ready to use sterile media plates/containers by taking the required number of plates/containers from the particular lot. In case of environmental monitoring media, perform the GPT after pre-incubation using spread plate technique.

- **Growth Promotion Testing of Liquid Media:** Inoculate 10 to 100 CFU of each specified organism in signal test control and incubate at specified conditions. After completion of incubation, observe for growth and record the observation.
- **Acceptance Criteria:** The clearly visible (turbid) growth should be obtained within the specified duration. The selective and differential media should show characteristic/indicative growth. There should not be any growth in the media in case of inhibitory test. There should not be any growth in the negative controls. After completion of GPT it is released for routine.

3.3 Microbial Limit Test:

Purpose of microbial limit test

Mainly used to check the microbial contamination in different types of water sample used in different purpose in pharmaceutical industries. We performed MLT of purified water, raw water, water for injection by membrane filtration method and pour plate method as per standard procedure according to

Materials:

- Membrane filter assembly
- Filtration flask
- 0.45-micron filter paper
- Sterile forceps
- Water sample
- Peptone
- R_2AA plate
- SBDA plate
- SCDM

Procedure:

- Collect the sample for test.
- Assemble the sterile membrane filtration unit in filtration flask in the LAF. Aseptically remove filtration cup and place the sterile membrane filter (0.45 micron) in filtration unit with the help of sterile forceps and fix the clamp.

- For Negative control incubate media plate as it is. And for Peptone control filter 100 ml of sterile peptone.
- Pre-wet the membrane filter by adding 50 ml sterile 0.1% peptone water and filter it.
- For WFI and PSC add 200 ml of sample to membrane filter and filter it. And for purified water add 1.0 ml of sample to the membrane filter and filter it.
- Rinse the inside wall of filtration cup with 100 ml of sterile peptone water.
- Pick up the membrane filter from the filtration unit with the help of sterile forceps.
- Keep the membrane filter on sterile R2AA plate and SBDA plate and incubate it accordingly at 30 ℃ - 35 ℃ for 5 days and 20 ℃ - 25 ℃ for 7 days. Observe the result.
- For raw water and WPT testing, take 1ml of sample in sterile empty petri plate and pour about 15 to 20 ml R2AA molten agar and take 1ml of sample in sterile empty petri plate and pour about 15 to 20 ml SBDA molten agar.
- incubate the plates at 30 ℃ - 35 ℃ for 5 days and 20 ℃ - 25 ℃ for 7 days. Observe the result.

3.4 Pathogen testing:

➢ **Purpose of pathogen testing**:

After performing MLT of water sample the growth is observed than proceed for further testing is called pathogen testing of selected microorganisms. Pathogen testing performs according to USP guideline.

➢ **Selected organism is:**

1. *Escherichia coil*
2. *Shigella*
3. *Salmonella*
4. *Pseudomonas*
5. *S. aureus*

3.5 Bioburden Test:

- **Bioburden by swab:** The method used for the testing of in-process bioburden sample was by using sterile swab. wet the swab with sterile saline by adding to each of them about 2ml of sterile saline in the tube containing sterile swab. Close the tube immediately after adding sterile saline. Wrap the closed tubes of wetted swab with sterile aluminum foil from outside. select the location to be sampled by swabbing. Sample $25cm^2$ area of the selected location by swab method. Transfer the tube with sample location detail. Carry this tube to Qc microbiology lab for further analysis.

- **By membrane filtration method:** The method used for the bioburden test was membrane filtration method. Assemble the sterile filtration assembly with 0.45µm membrane filter in LAF unit. Pre-wet membrane filter by adding 50ml of sterile 1% peptone water and filter it. Squeeze the swab into the swab sample tube and pre squeeze the tube in the same. Transfer this suspension to the filtration unit. Add sterile 1% peptone water for second time to the swab sample tube and re-squeeze the tube in same. Transfer this suspension to the same filtration unit. Now filter all the contents through the membrane filter. Wash the membrane by filtering 100ml of sterile peptone water. Remove the membrane with sterile forceps and transfer on the surface of soyabean casein digest agar plate which id pre incubated for not less than 48hrs before using the plate ensure that there should not any microbial contamination is there. Incubate plates at 30°c - 35°c for 2 days followed by 20°c - 25°c for 3 days.

- **Pour-Plate Method:** The method used for the testing of internal samples in the process of the bioburden in the study was the pour plate method using Soyabean casein digest agar. Transfer the sample from the tube in sterile empty 90mm plates. Within 20 mins of adding the sample to plates pour approx. 20ml of molten SCDA agar medium maintained at around 45°c into petri dish with sample. Mix the sample with molten agar thoroughly by swirling the contents of plates clockwise for 20 times and for anticlockwise for

  20 times. Allow plates to solidify. Incubate plates at 30°c - 35°c for 2 days followed by 20°c - 25°c for 3 days. After incubation observe for growth and count the number of colonies observed in plate. For negative control transfer 1ml of sterile peptone water into the plate and pour 20ml of molten agar. For media control keep media plate as it is and incubate plate along with test and negative control.

4. RESULT & DISCUSSION

Environment Monitoring:				
Area	**Acceptance criteria according to pharmacopeia (TAMC)**	**Acceptance criteria according to pharmacopeia (TYMC)**	**Observed range of TAMC**	**Observed TYMC**
Grade C	50	Nil	12 to 25	Nil
Grade D	100	Nil	25 to 40	Nil
Environment monitoring by Air sampling				
Grade A	<1	NIL	<1	Nil
Grade B	10	NIL	1 or 2	Nil
Grade C	100	NIL	25 to 35	Nil
Grade D	200	NIL	35 to 45	Nil
Environment monitoring by surface monitoring by contact plate				
Grade A	<1	Nil	<1	Nil
Grade B	5	Nil	1 or 2	Nil
Grade C	25	Nil	10 to 15	Nil
Grade D	50	Nil	15 to 25	Nil

Microbial Limit Test:

Sample	Acceptance criteria according to pharmacopeia (TAMC)	Acceptance criteria according to pharmacopeia (TYMC)	Observed range of TAMC	Observed TYMC
Water for injection	< 10CFU/100ml	Nil CFU/100ml	<10 CFU/100ml	Nil CFU/100ml
Purified Water	100CFU/1ml	Nil CFU/ 1ml	20 to 35 CFU/1ml	Nil CFU/1ml
Raw Water	500CFU/1ml	Nil CFU/1ml	100to200CFU/1ml	NilCFU/1ml
WPT Water	500CFU/1ml	Nil CFU/1ml	100to200CFU/1ml	NilCFU/1ml

Bioburden test:

Sample	Acceptance criteria according to pharmacopeia (TAMC)	Acceptance criteria according to pharmacopeia (TYMC)	Observed range of TAMC	Observed range of TYMC
In-process bulk sample (before filtration)	10CFU/100ml	Nil CFU/100ml	<10 CFU/100ml	Nil CFU/100ml

Growth promotion test:							
Name of Test Strain	**Total Viable Count / 0.1 mL**	**Observed Counts (cfu)**			**Recovery/ Turbidity**	**Complies Yes/No**	**Result**
		Plate I	**Plate II**	**Average**			
P. aeruginoa	70	60	64	62	88%	Yes	Growth promoting
S. aureus	71	62	65	63	88%	Yes	Growth promoting
B. subtilis	65	60	63	61	94%	Yes	Growth promoting
A.brasilienss	40	32	35	33	83%	Yes	Growth promoting
C.albicans	51	40	43	42	82%	Yes	Growth promoting
Negative Control: No growth							

5. CONCLUSION

Industrial training is very much essential for students. It is also a best opportunity to acquire practical knowledge. During my training period, in the company I obtain lots of experience in QC microbiological testing of pharmaceutical products. This will help me to clarify my theory knowledge. I hope that it will help me in my future profession.

During our training period, we had seen the various instruments and apparatus in the company. The highly sophisticated instruments that work precisely must be operated with intense care for optimum use. We could acquire a lot of information regarding the latest instruments and working principles.

It was taught to us that; the cGMP guidelines are to be strictly followed in the industries in each and every section. We also learn about various microbiological test like microbial limit test, bioburden test, pathogen testing, growth promoting test, sterility test etc.

Apart from all that, the training was very interesting with lots of things to be learned. It helped us to acquire knowledge on punctuality, regularity and working environments in companies. The friendly working environment in Farbe Firma pharmaceuticals will remain in our mind in near future. Hence, we can say that our goal of industrial training is fulfilled. We acknowledge the great help of Farbe Firma pharmaceuticals.

6. REFERENCE

- United states pharmacopeia (USP 43), general chapter <1115>, MONITORING OF BIOBURDEN
- FDA. Updated 510(k) sterility review guidance K90-1; nil guidance for industry and FDA. 2002
- The United States Pharmacopeia (**USP**) Chapter <**1116**> MICROBIAL EVALUATION OF CLEAN ROOMS AND OTHER CONTROLLED ENVIRONMENT
- United states pharmacopeia, general chapter ⟨1231⟩ WATER FOR PHARMACEUTICAL PURPOSES
- SOP-042-003, Procedure for Handling of Microbiologiacal media., Farbe Firma
- SOP-042-021, Microbial Limit Test., Farbe Firma
- SOP-042-021, Microbial Environmental Monitoring by Settle plate (Passive Air sampling), by Air sampler (Active Air sampling) and Surface sampling by Contact plate., Farbe Firma
- SOP-042-018, Bio burden of bulk or in-process sample, Farbe Firma

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