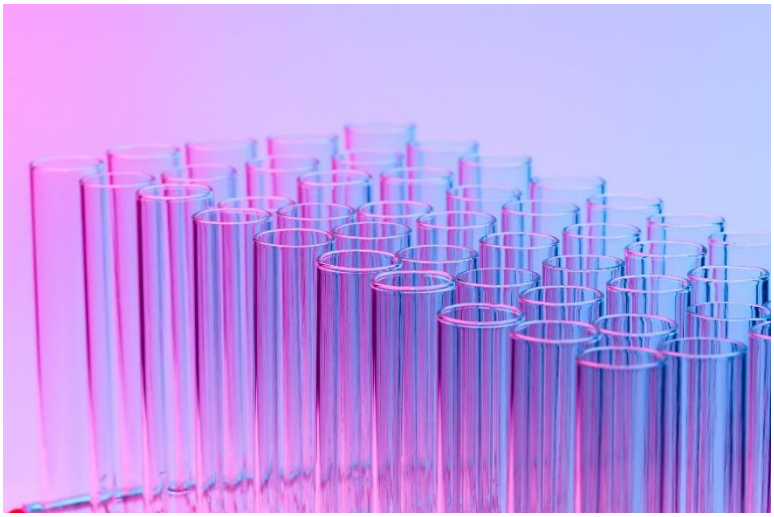
Internship Report on



MIC of Synthetic Ligands

Internship at Atmiya university

Bhavya M. Parmar | 200601033 | Date: 08/2022- 04/2023

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DECLARATION

I hereby declare that the work incorporated in the present training report entitled as **Synthetic Ligand Testing By MIC**, which is being submitted to **Department of Biotechnology**, Faculty of Science as a partial fulfilment of the Degree of Bachelor of Biotechnology, is carried out by me during my academic year 2022-2023 (Final Year Biotechnology). I also hereby declare that the Internship report which I am submitting here is solely my work and has not been submitted by any other student at this university. The information and details written in this report are duly written by me and shall I take sole responsibility for this training report.

Thank You,

Date: _____

Place: _Atmiya University, Rajkot_

X

Bhavya Parmar

BT Sem VI / 200601033

1. ACKNOWLEGEMENTS

I would like to express my sincere gratitude towards previous Dr. **Ravi Ranjan Kumar** who presented me with the opportunity under "**Earn while you Learn**" scheme and saw potential in me and assigned me laboratory sample testing with MIC Broth dilution method.

I am heartly thankful to **Dr. Nutant Prakash Vishwakarma** who continuously encouraged me during my internship period.

Thank you to lab assistants **Mrs. Kairvi Vyas**, **Mrs. Joyati Yadav** and **Mrs. Hetal Patel** who assisted me with my internship program throughout the procedure.

And my peers Anjaliba Jadeja, Aastha Patel, Shreya Kathiriya, Kishan Aghara who assisted me with the wet lab.

It was a great experience to share my skills by mentoring my sub juniors.

2. Abbreviations

µl	Microlitre
CLSI	Clinical and Laboratory Standards
DMSO	Dimethyl Sulfoxide
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
mg	Microgram
MH broth	Muller Hilton broth
MIC	Minimum Inhibitory concentration
ml	Millilitre
NC	Negative Control
nm	Nano Meter
OD	Optical Density
PC	Positive Control
Sample	Drug/Ligand/synthetic compound

3. Introduction

Our esteemed **"Atmiya University"** has many Chemistry and Microbiology scholars that synthesize **Synthetic compounds** which are required to test as to check the Product's efficacy with different set of microbes and check their antimicrobial virtue.

To help with the same, **Biotechnology Department** organised a program called **"Earn While You Learn"** that helps with MIC analysis where the students who are interested in earning as well as improve their wet laboratory skills along with the academics.

Under this program many former batches have already gained experience of exploring their interest in **Microbiology Laboratory testing Environment**.

As well as giving them a start in boosting the level of understanding of laboratory environment and working along with the **Pathogens** for the Ligand testing.

In general, the Protocol is followed along with the Isolated set of Gram Positive as well as Gram Negative and Fungal Strains. P.T.O

The method that is followed is **Minimum Inhibitory Concentration** with **Broth Dilution method** as it Gives clearer results in short period of time with more efficacy.

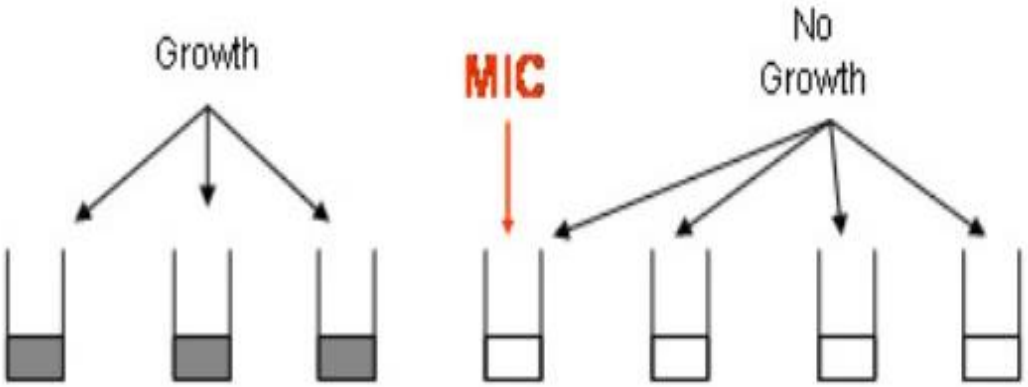
4. DEFINITION

MIC: A method to test the minimum required concentration of any drug that inhabits the growth of a microbe, pathogen, or fungus that is the single Isolate.

Breakpoint: Discriminatory concentrations used in the interpretation of results of susceptibility testing to define isolates as susceptible, intermediate, or resistant (determined by various organizations - **FDA, CLSI, EUCAST**)

Broth dilution method: The broth/tube dilution test is the standard method for determining levels of microbial resistance to an antimicrobial agent. Serial dilutions of the test agent are made in a liquid microbial growth medium inoculated with a standardized number of organisms and incubated for a prescribed time. At the end of the incubation period (generally 18-24 hours), the tubes are visually examined for turbidity. (Figure 1.1)

- For clear and concise interpretation, we use **Microplate Reader** instrument which can read 96 sample with Microtiter Plate which works on colorimeter principle generally ranging from 400-850nm. (Currently at 600nm)



MIC (Figure 1.1)

5. Requirements

5.1. Chemicals

Sample (Synthesized Ligand)

DMSO-Dimethyl sulfoxide (organic solvent) aprotic solvent.

Microbial Cultures (gram positive, negative bacteria and fungus)

MH Media broth

MH Agar Plates 2% (only for isolated colonies storage/isolation of contaminated culture)

70% Alcohol

Agar Agar

5.2. MICROBIAL STRAINS USED

Gram positive Strains:

1. ***Staphylococcus***
2. ***Bacillus subtilis***

Gram Negative Strains

1. ***Salmonella Typhi***
2. ***Pseudomonas aeruginosa***

Fungal Strains:

1. ***Aspergillus flavus***
2. ***Aspergillus niger***

5.3. Instruments

Shaker Incubator

Auto clave

Bunsen burners

Microtiter plate (96 well plate)

Microplate Reader

Micro Pipettes (1000µl, 0.5-10µl)

Micro Tips

Eppendorf's tube (2ml)

Glass plates

Weighing machine

Plastic ware

Fridge/cold storage

Spray Bottle/cotton.

Auto clave

To sterilize lab equipment's and media preparation under high temperature and pressure to eliminate chance of contamination.

MH Media (Muller Hilton Broth)

Media to grow pathogens in as it supports wide spectrum of microbes and is more transparent in nature that aids visual analysis.

SHAKER INCUBATOR

Provides controlled environment for optimal microbial growth condition. By means of temperature and oscillation of media.

Digital Weighing Scale

-Used to measure the unknown amount of sample received.

Eppendorf (2ml)

Used to perform serial dilution as well as ligand testing.

Microtiter plate reader

To perform OD analysis of multiple sample in a single run

Microtiter plate

To load and perform optical density analysis of susceptible.

Micro pipette

Used in serial dilution, ligand preparation, culture inoculation and sample loading on microtiter plate.

6. PROCEDURE

6.1 Sanitization

working plate-form needs to be sterilized with 70% alcohol and all the work is needed to be done inside four flame to avoid contamination.

To make 70% alcohol first add 30 parts of water then add remaining parts of Alcohol till it reaches the 100 parts mark.

6.2 Sterilization

Every lab equipment's used in performance of testing are required to be sterilized in auto clave to avoid contamination in broth incubation in further steps ahead.

6.3 Media Preparation

MH Media broth (Muller Hilton Broth) 1L for microbial culture as well as serial dilution of drug in known concentration.

MH media is Used as standard media for MIC as it can support the growth of larger microbe pool then other medias and is also better for visual screening due to its clarity after dissolution.

6.4 Ligand Preparation

The ligands received for testing are in variety of proportions hence first they are weighed on scale.

Standard Required proportion of Ligand needs to be in ratio of 1000µl:100mg of DMSO – ligand Respectively.

Hence after weighing the amount of drug the Amount of DMSO is found out by simple cross Multiplication.

As DMSO is **aprotic Organic solvent** it is suitable for a large range of synthetic and as well as natural ligands along with test and it has low toxicity towards Microbes in small amount of Exposure.

6.5 Broth Serial Dilution

1. In first Eppendorf tube add **1000µl** broth and next **4** tubes **500µl.**

2. next add **10µl** drug dissolved in **DMSO** to tube 1 and mix it well with pipette

3. then **collect 500µl** of broth from tube 1 and **add** it to **next tube** and repeat the step till last of broth tube. which will serially dilute the drug concentration.

4. from the last tube remove the excess 500µl broth and discard it.

NOTE : PRELABEL THE TUBES WHICH REPRESENT ITS DILUTION FACTOR (LATER NECESSARY)

6.6 Microbial Inoculation

In current step the Inoculation of desired species of microbe (G+; G-, Fungus) in ligand diluted broth is done to check growth inhibition rate of the compound at different known concentrations (5µl).

6.7 Microbial Control (Positive Control)

Alongside test performance another set of normally prepared MH broth is Inoculated with similar amount of microbial culture as it was introduced in the broth with Synthetic compound.

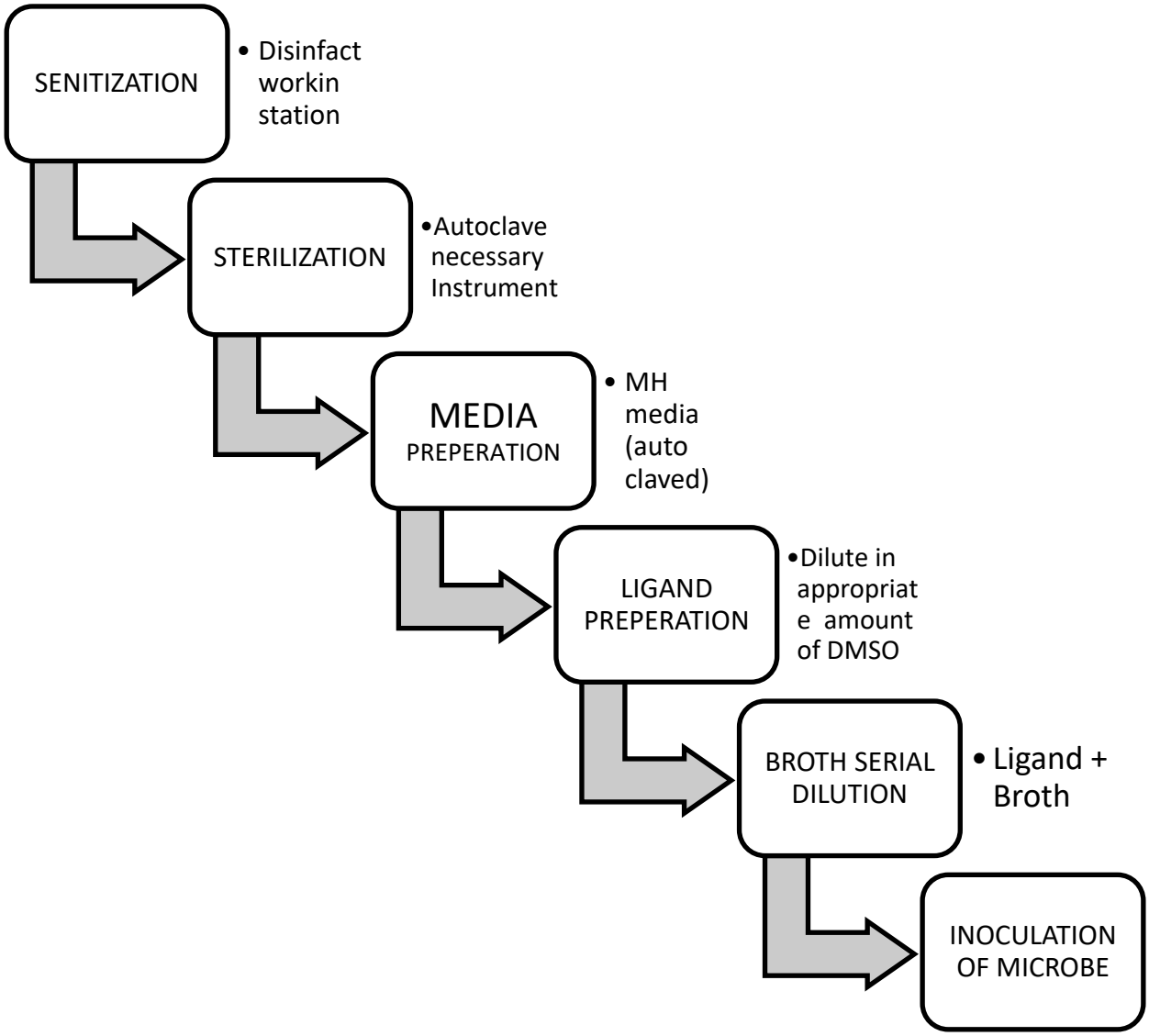
This is intended to check **Normal growth** of the microbial cultures capability to grow under same time and condition that is being given to Ligand Infused broth. And is termed as Positive Control.

6.8 Blank (Negative Control)

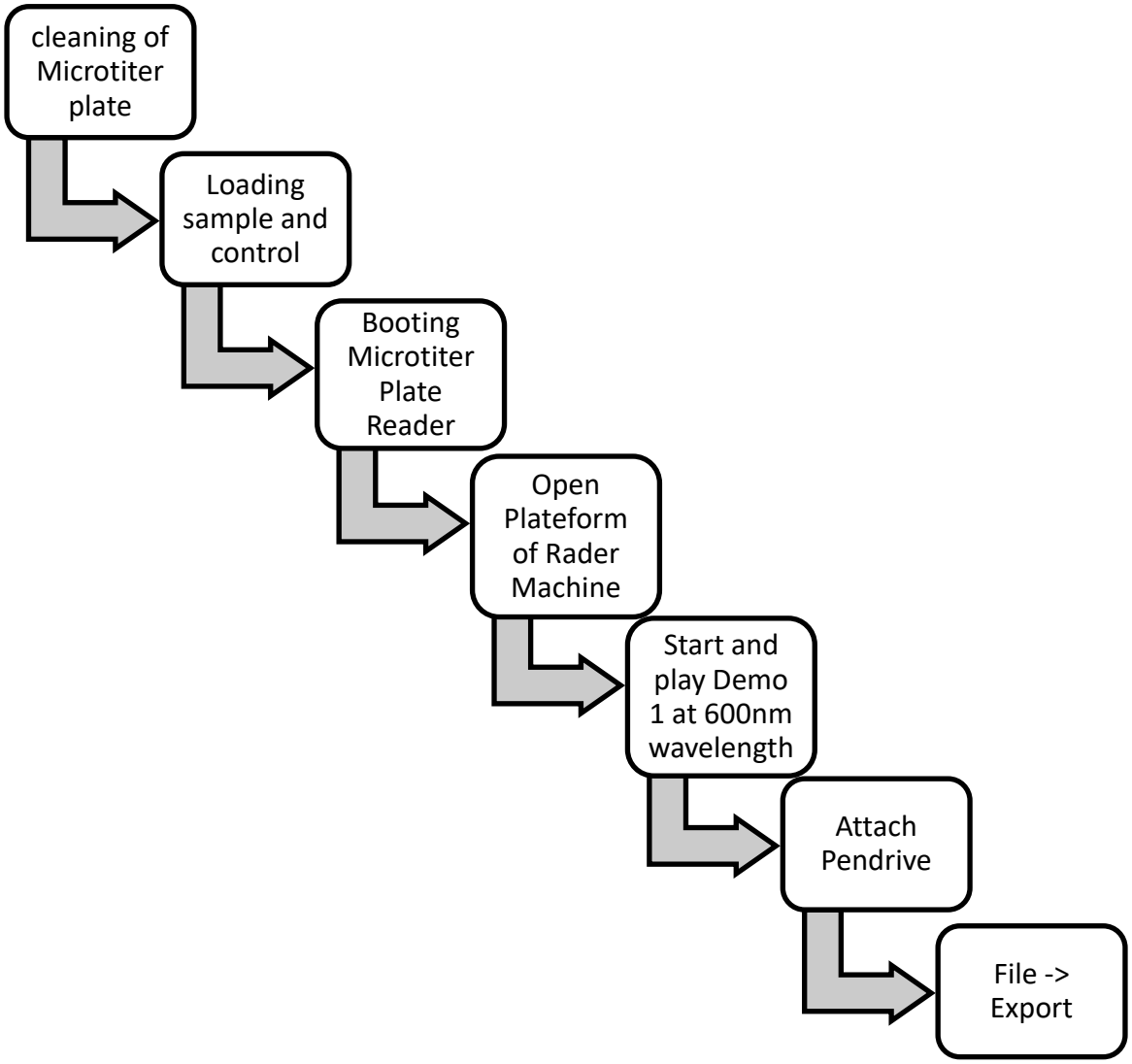
Blank is another set of control that is made by just Diluting the Drug in the same ratio as the test Eppendorf's to **exclude the OD** generated by Drug during analysis.

Actual blank in technical term for just the Incubation of broth alongside test Eppendorf's to check the accuracy of whole procedure by scanning for growth of contamination.

7. **Steps of MIC Broth method**



8. **Data Extraction steps by Microtiter plate and Reader**



9. **Data Analysis and Interpretation**

Data extraction

Trimming down unnecessary data

Arrangement of data

Formula application

Observations

9.2 Formula for interpretation

1. **OD of Negative Control – OD of culture with ligand**
2. **Compare the Result with Positive Control to Observe significant Growth in Test Cultures**

	PC	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
Drug		0.682	0.651	0.586	0.476	0.355
Bacillus megaterium	0.744	0.766	0.602	0.652	0.589	0.804
Staphylococcus aureus	0.856	0.601	0.49	0.52	0.65	0.655
Bacillus subtilis	0.756	0.643	0.608	0.692	0.65	0.655
Escherichia coli	0.795	0.512	0.89	0.887	1.239	1.186
Enterobacter aerogenes	0.671	0.676	0.737	0.636	0.831	0.726
Pseudomonas aeruginosa	0.801	0.576	0.568	0.549	0.708	0.642
Aspergillus flavus	0.513	0.519	0.442	0.477	0.546	0.498
Aspergillus niger	0.513	0.479	0.456	0.47	0.522	0.533

The Highlighted OD is the represented dilution factor at which the Ligand is efficiently Inhibiting the microbe's growth.

10. **CONCLUSION**

Internship at Atmiya University provided me a great opportunity to get real world exposure of the Biotechnology field and how it help's human's welfare.

Here, by exploring new Ligands Susceptibility towards a pathogen and its Importance in real life application and necessity in clinical aspects.

This Internship improved my time management skills along with academics and stipend lab work.

Opportunity to mentor my junior fellows was Presented to me by my **HOD of Biotechnology Dr. Nutant Prakash Vishwakarma** which improved my mentoring capabilities.

Along with all the learnings I gained from this internship, developed my **analytical observations.**

Through this internship I was obliged to work with the following techniques such as **Handling Micropipette**, **Maintaining Sterilized Conditions** and **Pathogen handling.**

As well as **Evolve** Different Methods for work **Optimization Method** to Complete more task under time crunch under stressful condition. Which was a decisive factor for many of the students that could not continue with the internship after a jiffy entry.

Completing this task was one of the most Rewarding Project that I got chance to participate in.