

# STUDY OF THE ENUMERATION OF TWELVE CLINICAL IMPORTANT BACTERIAL POPULATIONS AT 0.5 MCFARLAND STANDARD

E. Gayathiri<sup>1\*</sup>, .Bharathi B<sup>2</sup>., Priya K<sup>3</sup>

1. Department of Plant Biology and Plant Biotechnology, Guru Nanak College (Autonomous) Chennai-42.

2. Department of Plant Biology and Plant Biotechnology Loganatha Narayanaamy Govt. college (Autonomous)

3. Department of Biotechnology St. Joseph Arts and Science College, kovur , Chennai

## ABSTRACT:

McFarland standard is a specific scale for the concentration of bacteria /ml. It is used to approximate the number of microbial cells in the liquid suspension. The aim of the present study is to evaluate some clinically important bacterial inoculums with that compared to 0.5 McFarland standard and the bacterial inoculums were serially diluted by 10 fold dilution and spread plated and enumerating the number of colonies by help of colony counter and size of the organisms also been measured by micrometry. The results were read. As per McFarland rule there should be 15 colonies in the 7<sup>th</sup> dilution but all the species vary 15 times to three hundred times more than the expected value because of the size and volume of the organisms the time for the division also yield across amount in the suspension so the colony dilution may differ in each setup. The present study enumerated for 12 bacterial study has brought into light that the number bacterium in given sample could not be calculated by comparing it with McFarland standard because of varying number of population from 20- 324 any for the maintained bacteria

**KEYWORDS:**

McFarland , Serial Dilution, Colony Count, Bacteria

**1. INTRODUCTION:-**

McFarland turbidity standards are used as a reference standard to approximate the number of microbial cells in a liquid suspension. One of the earliest uses of turbidity for the estimation of bacterial populations was in the preparation of vaccines.[1].

BaSO<sub>4</sub> standards [11].

By challenging identification or susceptibility testing procedure with standardized inoculums reproducible and meaningful results can be obtained. It is by use of these standardized inoculums values that the National committee for clinical laboratory standards (NCCLS) has standardized expected results with a give ATTC Microorganisms. The bacterial suspensions are visually compared to the McFarland standards estimating the bacterial density. For *E. coli*, a 0.5 McFarland standard corresponds to  $1.5 \times 10^8$  CFU/ml [2].

McFarland scale is a scale is a scale numbered from 1-10 which represent concentration of bacterial/ml. Though it is originally designed to be used for estimating the concentration of gram negative bacteria like *E. coli* which are much smaller in size and difficult to count with hemocytometer method, now McFarland standard scale is widely adopted for gram positive bacteria for making a inoculums of uniform concentration of bacteria in *In vitro* anti susceptibility protocols.[3]

Currently, McFarland Turbidity Standards consist of uniform polystyrene microparticles, which allow longer shelf life and comparable absorbance values to the original Baso<sub>4</sub> standards .[4] [5]

The discovery of sulfonamide and penicillin are some drug was been susceptible to most of the organisms. So resistance organism is been tested by *In vitro* against anti microbial agents for many types of susceptibility testing a standardized inoculums of bacteria must be used so McFarland standards were devised to correspond to approximate cell densities as required by the method of antimicrobial testing[6]. Hence the aim of the present study is to evaluate some clinically important bacterial inoculums with that compared to 0.5 McFarland standard and the bacterial inoculums were serially diluted by 10 fold dilution and spread plated and enumerating the number of colonies by help of colony counter and size of the organisms also been measured by micrometry.

## 2. MATERIALS AND METHODS:-

1907, McFarland developed a series of barium sulfate solutions to approximate the numbers of bacteria in solutions of equal turbidity, as determined by plate counts. (Table- 1, Figure-1)[7] [8]

**Table 1**

### McFarland standard scale value

MFU	Approximate cell density
0.5 MFU	$1.5 \times 10^8$ Cells/ ml
1 MFU	$3.0 \times 10^8$ Cells/ ml
2 MFU	$6.0 \times 10^8$ Cells/ ml
3 MFU	$9.0 \times 10^8$ Cells/ ml
4 MFU	$12 \times 10^8$ Cells/ ml
5 MFU	$15 \times 10^8$ Cells/ ml
6 MFU	$18 \times 10^8$ Cells/ ml
7 MFU	$21 \times 10^8$ Cells/ ml
8 MFU	$24 \times 10^8$ Cells/ ml
9 MFU	$27 \times 10^8$ Cells/ ml
10 MFU	$30 \times 10^8$ Cells/ ml

\*MFU- McFarland Standard



**Fig:1** - McFarland Standard (0.5)

### PREPERATION OF MCFARLAND STANDARD:-

A. Barium chloride

1.175 grams

Distilled water	100 ml
B. Sulphuric acid(0.36 N)	1.0 ml
Distilled water	100 ml

0.5 ml of solution A was added to 99.5 ml of Solution B and mixed well. It is mixed well with magnetic stirrer then distributed in test tubes with a screw cap of the same size as those containing the bacterial culture, the turbidity of which must be evaluated.

The cap was closed tightly to avoid evaporation of flame sealed. It is agitated vigorously in a vortex before using it.

### **STORAGE OF MCFARLAND STANDARD:**

The prepared McFarland standard solution was stored at 2-30° C in the dark standards. After opening there is a reduced shelf life depending upon storage and contaminations.

### **METHOD OF USING MCFARLAND:-**

Susceptibility testing requires the use of standardized inocula. The 0.5 McFarland standard is recommended for use in the preparation of inocula for performing the antimicrobial disk diffusion susceptibility test.[9]. The inoculum for primary sensitivity testing was usually prepared from a broth that had been incubated for 4-6 hours, when growth was said to be in logarithmic phase then density of the suspension was adjusted by adding the bacterial suspension to a sterile saline test tube to match the density of desired standard.

### **QUANTITATION OF BACTERIA:**

Micro organisms used for screening

The pure clinical bacterial isolates were obtained from the Department of ALMPGIBMS, Taramani Campus

### **GRAM POSITIVE BACTERIA:**

1. *Staphylococcus aureus*
2. *Staphylococcus epidermidis*
3. *Enterococcus faecalis*

### **GRAM NEGATIVE BACTERIA:-**

4. *Escherichia coli*
5. *Salmonella typhi*
6. *Vibrio cholera*

7. *Shigella sonneii*

8. *Proteus vulgaris*

9. *Proteus mirabilis*

10. *Pseudomonas aeruginosa*

### **ATCC Standard:-**

11. *Staphylococcus aureus* ATCC 25923

12. *Escherichia coli* ATCC 25922.

### **MATERIALS NEEDED FOR THE QUANTIFICATION OF MICROORGANISMS:**

Nutrient agar, Nutrient broth, Saline, Petriplates, Micropipettes, Tips, L-rod

Vortex rotator, Ethanol

### **METHOD FOR QUANTIFICATION OF BACTERIA:-**

A standard bacterial were inoculated in nutrient broth. Then it was incubated for 2-4 hrs at 37°C. Later it was compared with McFarland standard. Quantization method was performed by ten-fold dilution method. Estimate the concentration of a broth culture by spread plate technique.

### **COMPARING WITH MCFARLAND STANDARD:-**

Inoculating a known concentration of bacteria into the solution and incubate for 4 hours. Then adjusted the turbidity with that of bacterial suspension. The tubes for the suspension should be the same diameter as the McFarland standard tube. Visually compare this using adequate light and reading the tubes against white card with contrasting black lines. Then adjusted the turbidity with that of bacterial suspension. The tubes for the suspension should be the same diameter as the McFarland standard tube. Visually compare these using adequate lights and reading the tubes against white card with contrasting black lines.

### **ENUMERATION TECHNIQUE:-**

Set Ten tubes 12X100 mm in the rack



Pipette 0.9 μl of sterile saline into each tube using sterile disposable tip



Using sterile tip transfer 0.1 μl of culture suspension into the 1<sup>st</sup> tube and mix well transfer 0.1 μl to the 2<sup>nd</sup> tube



Discard the tip into the discarding jar and new tip were changed for every dilution, the dilution is carried out till the 9<sup>th</sup> tube and transfer 0.1  $\mu$ l from the 9<sup>th</sup> tube to the discarding charge.

↓  
10<sup>th</sup> tubes were made as control

### SPREAD PLATE TECHNIQUE:-

Sterile nutrient agar plates were dried and to that 0.1  $\mu$ l of suspension from the 6<sup>th</sup> tubes is transferred to the plate

↓  
Gently rotate plate with L-Rod  
↓  
Similarly transfer 0.1  $\mu$ l suspension to the plate labeled upto 9<sup>th</sup> dilution  
↓  
Incubate at 37° c for 24 hrs  
↓  
Count the number of colonies present in all the plated by using colony counter

**Formula:- Number of Colonies X Dilution**

### 3.RESULT & DISCUSSION:

McFarland standard is adopted as an easy method to know approximate number of bacteria from a given broth samples. This was initially adopted to enumerate the gram positive. However this method has been widely adopted to use an inoculum of approximate uniform number of bacteria in inoculums meant for In Vitro susceptibility test. The NCCLS methods present 0.2  $\mu$ l of 0.5 McFarland standard inoculum for antimicrobial susceptibility test for bacteria.

The present study was aimed to know the number of bacteria in 1 ml of 0.5 McFarland inoculums of twelve clinically important species of bacteria and the enumeration study has taken into consideration that no. of bacteria in 1 ml of 0.5 McFarland standard broth standard inoculum would have 150 million bacteria. As the present study had used 0.1ml for the enumeration purpose, so 15 million bacteria would present in it. As this number is uncountable by colony counter the McFarland standard compared broth inoculum was diluted tenfold in a serial manner and was found that single digit number of bacteria is expected at eight dilution but however

the results obtained here at the 8th dilution for each species are varying for 15 times to 300 times more than the value expected for McFarland standards.

The term approximate the number differ to a small variability in the number of population namely 5 times higher or lower value. Hence however none of the test bacterium had population number around 10 at 8th dilution. The nearest number was for *Proteus mirabilis* and the number was 22 following *Enterococci faecalis* which has 37. All the other bacteria had a population between 75 to 325. (Table :2).

The result show highly variable number of bacterial population among the twelve bacterial chosen for study. Hence a given volume inoculum compound with the McFarland standard may not be true for bacteria of different species. As far as the *In Vitro* anti microbial susceptibility is concerned this may not affect the result because species transferred to dilution of serial tubes will be same.

However it should be make clear to the colony forming unit for any number of McFarland standard inoculums will be highly varying for different species of bacterium as we have inferred for our results.

Correlation was attempted to know whether the volume of the cell has anything to do with variation in the number of microbial in given volume of inoculum. It is so larger bacterium will have fewer number and smaller bacterium will have more number of population. Ironically the results obtained may depends upon the growth of the organisms.

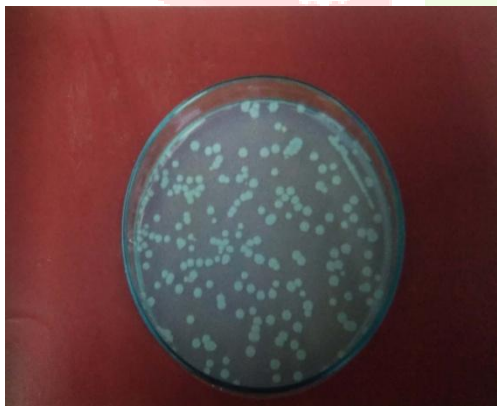
Although used universally, McFarland turbidity Standards become uncertain with other organisms as different species of bacteria differ in size and shape as do yeast and mould [10] .We hypothesize that the difference found is due to subjectivity of the visual determination of bacterial densities when working with McFarland turbidity Standards.

**Table : 2**

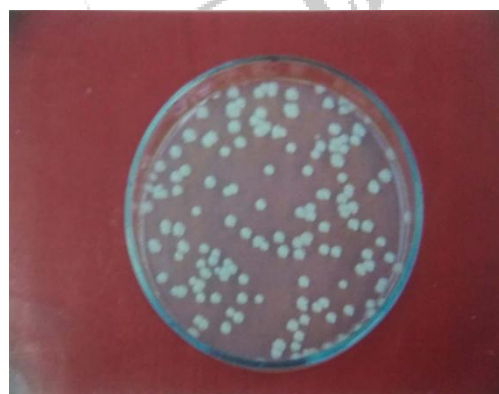
**ENUMERATION OF GRAM POSITIVE BACTERIA IN 0.1 ML OF BROTH AT 0.5 MCFARLAND STANDARD**

S.NO	GRAM POSITIVE ORGANISMS	DILUTION		
		$10^7$	$10^8$	$10^9$
1.	<i>Staphylococcus aureus</i>	461	234	120
2.	<i>Staphylococcus aureus atcc</i>	470	234	120

3	<i>Enterococcus faecalis</i>	82	37	17
4	<i>Staphylococcus epidermis</i>	242	117	48
S.NO	GRAM NEGATIVE ORGANISMS	DILUTION		
		$10^7$	$10^8$	$10^9$
1	<i>Escherichia coli</i>	640	325	142
2.	<i>Salmonella typhi</i>	232	148	84
3	<i>Proteus vulgaris</i>	42	22	10
4.	<i>Shigella sonnei</i>	147	75	40
5	<i>Pseudomonas aeruginosa</i>	162	77	44
6	<i>Vibrio cholerae</i>	640	39	207
7	<i>Escherichia coli ATCC</i>	410	214	9



**Fig 2:** Comparison of *Staphylococcus aureus* with 234 colonies in 8<sup>th</sup> dilution



**Fig 3:** Comparison of *Staphylococcus aureus* with 0.5 McFarland shows 120 colonies in 9<sup>th</sup> dilution





Fig 4:

Comparison of *Staphylococcus aureus* ATCC with 0.5 McFarland shows 240 colonies in 8<sup>th</sup> dilution

Comparison of

*Staphylococcus aureus* ATCC with 0.5 McFarland shows 120 colonies in 7<sup>th</sup> dilution

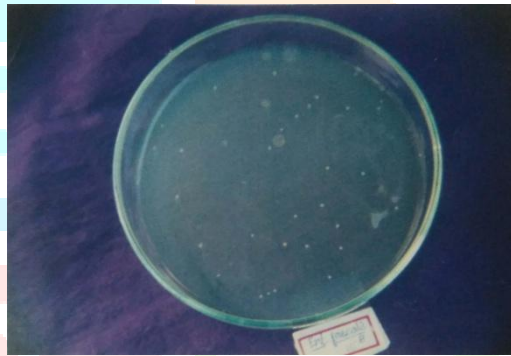
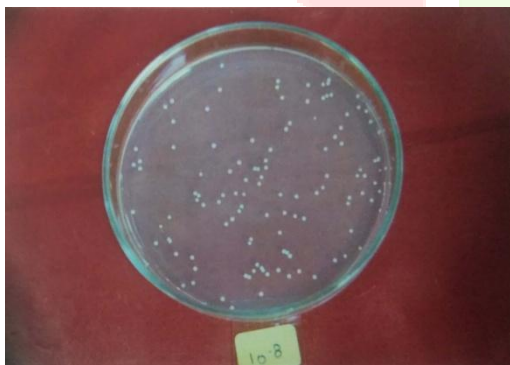


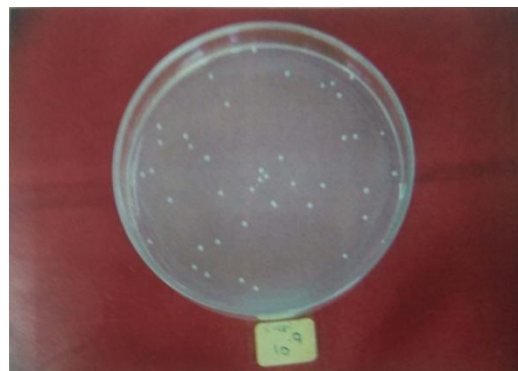
Fig 6: Comparison of *Enterococcus faecalis*

with

0.5 McFarland shows 37 colonies in 8<sup>th</sup> dilution



Fig



7: Comparison of *Staphylococcus epidermidis* with Fig 8: Comparison of *Staphylococcus*

*epidermidis* with 0.5 McFarland shows 117 colonies in 8<sup>th</sup> dilution 0.5 McFarland shows 48 colonies in 9<sup>th</sup> dilution

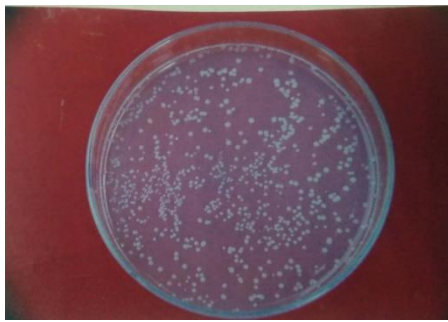


Fig 9: Comparison of *Escherichia coli* with colonies in 8<sup>th</sup> dilution

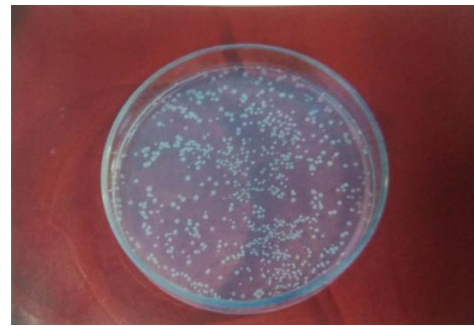


Fig 10: Comparison of *Escherichia coli* with 0.5 McFarland shows 325 colonies in 9<sup>th</sup> dilution

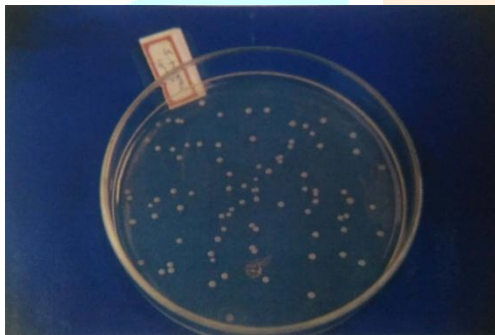


Fig 11: Comparison of *Salmonella typhi* with 148 colonies in 8<sup>th</sup> dilution

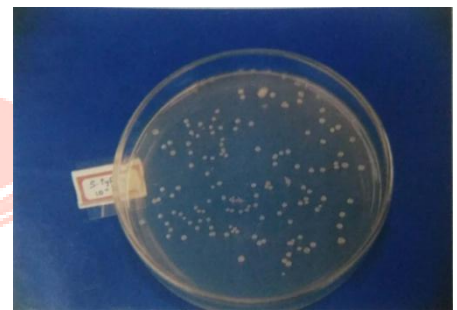


Fig 12: Comparison of *Salmonella typhi* with 0.5 McFarland shows 84 colonies in 9<sup>th</sup> dilution

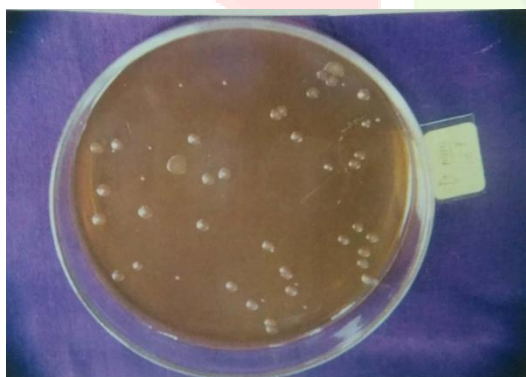


Fig 13: Comparison of *Proteus mirabilis* with 0.5 McFarland shows 42 colonies in 8<sup>th</sup> dilution in 9<sup>th</sup> dilution

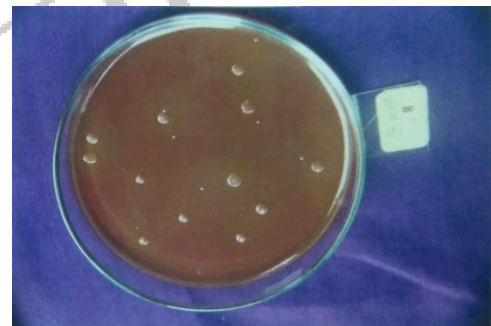
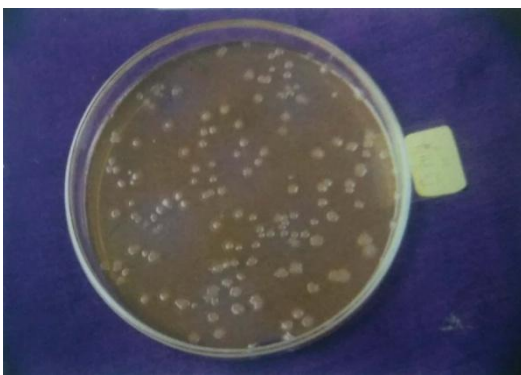
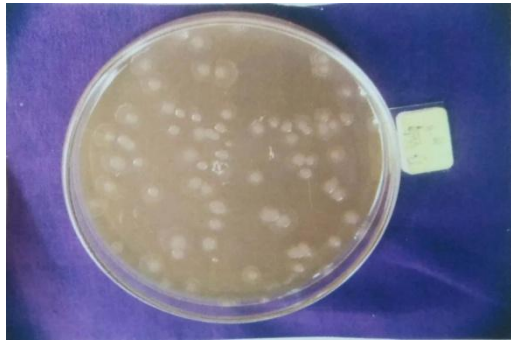


Fig 14: 0.5 McFarland shows 22 colonies



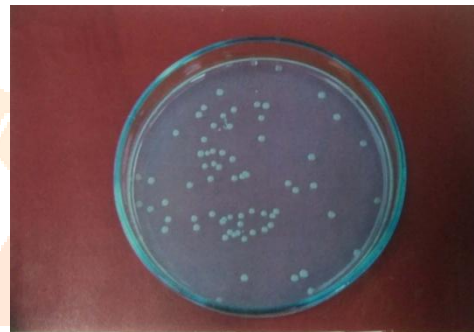


**Fig 15:** Comparison of *Proteus vulgaris* with 0.5 McFarland shows 140 colonies in 8<sup>th</sup> dilution

**Fig 16:** Comparison of *Proteus vulgaris* with 0.5 McFarland shows 82 colonies in 9<sup>th</sup> dilution



**Fig 17:** Comparison of *Shigella sonnei* with 0.5 McFarland shows 75 colonies in 8<sup>th</sup> dilution



**Fig 18:** Comparison of *Shigella sonnei* with 0.5 McFarland shows 40 colonies in 9<sup>th</sup> dilution



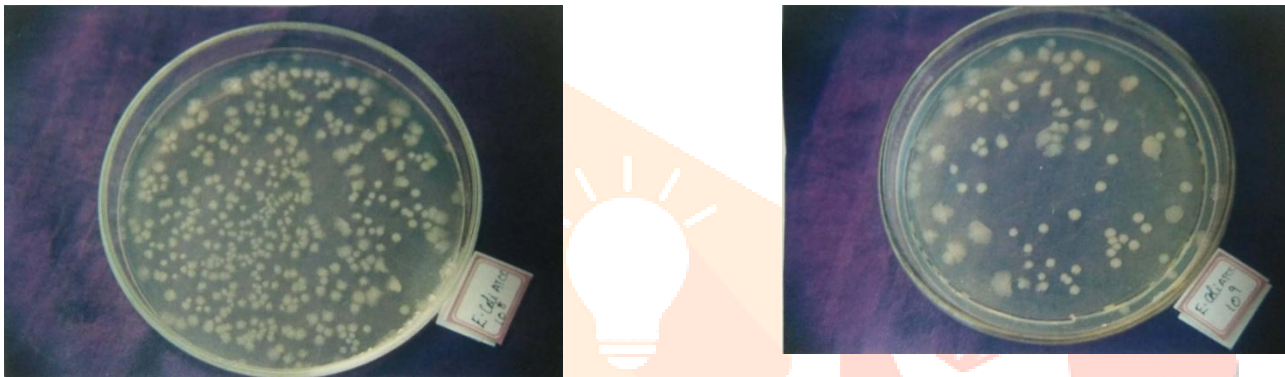
**Fig 19:** Comparison of *Pseudomonas aeruginosa* with 0.5 McFarland shows 77 colonies in 9<sup>th</sup> dilution



**Fig 20:** Comparison of *Pseudomonas aeruginosa* with 0.5 McFarland shows 44 colonies in 8<sup>th</sup> dilution



**Fig 21:** Comparison of *Vibrio cholerae* with 0.5 McFarland shows 2077 colonies in 9<sup>th</sup> dilution



**Fig 22:** Comparison of *Escherichia coli* with 0.5 McFarland shows 214 colonies in 8<sup>th</sup> dilution

**Fig 23:** Comparison of *Escherichia coli* 0.5 McFarland shows 96 colonies in 9<sup>th</sup> dilution

**Flow chart:-**

As per McFarland rule the 0.5 ml of McFarland standard when compared to 1 ml of bacterial suspension should contain  $1.5 \times 10^7$  CFU/ml

↓  
So 1<sup>st</sup> tube 0.1ml of dilution must contain 15,00,0000 CFU/ml

↓  
So 2<sup>nd</sup> tube 0.1ml of dilution must contain 15,00,0000 CFU/ml

↓  
3<sup>rd</sup> tube of 0.1 ml of dilution must contain 1,50,000 CFU/ml

↓  
4<sup>th</sup> tube of 0.1 ml of dilution must contain 1,50,00CFU/ml

↓

5<sup>nd</sup> tube of 0.1 ml of dilution must contain 1,50,0 CFU/ml

↓

6<sup>nd</sup> tube of 0.1 ml of dilution must contain 1,50 CFU/ml

↓

7<sup>nd</sup> tube of 0.1 ml of dilution must contain 15 CFU/ml

↓

8<sup>nd</sup> tube of 0.1 ml of dilution must contain 1.5 CFU/ml

↓

9<sup>nd</sup> tube of 0.1 ml of dilution must contain 0.15 CFU/ml

#### 4. SUMMARY:

McFarland standard is a specific scale For the concentration of bacteria /ml.It is used to approximate the number of microbial cells in the liquid suspension.

It is devised to counts an individual cells and are designed to correspond to approximate cell densities as required by the method of antimicrobial testing.Bacterial suspension in 1 ml of Nutrient broth in incubated for 4 hrs and adjust with the turbidity with McFarland standard and spread plated and counted using colony counter and the size of the microorganisms as measured by micrometry.The results were readed

#### 5. CONCLUSION:-

As per McFarland rule there should 15 colonies in the 7<sup>th</sup> dilution but all the species vary 15 times to three hundred times more than the expected value because of the size and volume of the organisms the time for the division also yield across amount in the suspension so the colony dilution may differ in each setup.The present study enumerated for 12 bacterial study has brought into light that the number bacterium in given sample could not be calculated by comparing it with McFarland standard because of varying number of population From 20- 324 any for the maintained bacteria

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