MICROBIOLOGICAL ASSAY

Method of Analysis No.: Micro/MoA 002

Adapted from the USP 34 NF 29 2011 Vol. 1 Page 70 (Antibiotics – Microbial Assay)

MICROBIOLOGY LAB NO.	DATE RECEIVED	DATE TEST SET	DATE OF RESULTS

SAMPLE AND STANDARD PREPARATION

Preparation of Standard solution:
Taking into consideration its potency, weigh accurately a weight of the standard equivalent to approx. 16mg of Erythromycin into a 25mL volumetric flask. Add a little methanol and sonicate for 10 minutes to dissolve. Allow to cool and then make up to volume with methanol. Dilute 5mL of the resultant solution to 25mL using Buffer Solution pH 8.0. This gives solution **S_3** i.e., Standard Stock Solution (~0.128 mg/mL).
The standard solution should be prepared in duplicate (Std A & B).

NB: The esteric form of Erythromycin in the Standard used should be the same as that in the sample, i.e. **Stearate**.

Preparation of the Sample Solution:
Weigh accurately a weight of the tablet powder equivalent to approx. 40mg Erythromycin to a 50mL volumetric flask. Add a little methanol and sonicate for 10 minutes to dissolve. Allow to cool and then make up to volume with methanol. Take 10mL of this solution into a 25mL volumetric flask and top up with methanol. Transfer 10mL of the resultant solution to a 25mL volumetric flask and make up to volume with Buffer Solution pH 8.0. This gives solution **T_3** i.e., Sample Stock Solution (~0.128 mg/mL).
The sample solution should be prepared in triplicate (Test A, B, & C).

Preparation of the test solutions:
Dilute both Solutions **S_3** and **T_3** as follows:
Dilute 5mL to 10mL using Buffer Solution pH 8.0; this gives solutions **S_2** and **T_2** respectively.
From the S_2 and T_2 solution take 5mL and dilute to 10mL with Buffer Solution pH 8.0; this yields solutions **S_1** and **T_1** respectively.

Preparation of Innoculum:
From a recently grown slant of *Bacillus pumilus,* subculture onto a plate of Nutrient Agar and incubate at 35 ^{o}C for 5 days or until sufficient growth is attained. Harvest the growth using sterile water or normal saline into a test-tube or sterile bottle.

Preparation of the Media:
Weigh Antibiotic Assay Medium No. 1 and reconstitute with water as prescribed by the manufacturer to give a volume sufficient for analysis. Autoclave at 121^{o}C for 15minutes. Allow cooling to about 50 ^{o}C before adding the innoculum (approx. 4mL of the suspension of *Bacillus pumilus* harvested). Swirl the bottle to mix the innoculum while avoiding introduction of air bubbles.

Preparation of the plates:
Measure out 25mL of the inoculated media using a measuring cylinder into each of the plates to be used for the assay. Let the plate settle for about 1hour. When the media has hardened enough, make 6 cylindrical

wells using the borer and the template guide in each plate. Label the wells with the solutions to be put into each well, in the following order: T_2-T_3-T_1- S_3-S_1- S_2.
Each assay uses a total of 18 plates, thus:

- 3 plates having Std A and Test A test solutions,
- 3 plates having Std A and Test B test solutions,
- 3 plates having Std A and Test C test solutions,
- 3 plates having Std B and Test A test solutions,
- 3 plates having Std B and Test B test solutions,
- 3 plates having Std B and Test C test solutions.

Performing the Test:
Using the100-µL micropipette transfer 100 µL of each of the Solutions into the appropriately labeled wells. After completion, allow the petri dishes to stand for 2 hours before incubating them at 35 ◦C for about 18-24 hours.
Read the diameters of the zones of inhibition using a caliper and record them in the table.

Calculations:
Calculate the amount of Erythromycin in each of the samples using the formulae below:

$E = \frac{1}{4}[(S_3+T_3)-(S_1+T_1)]$
$F = \frac{1}{3}[(T_3+T_2+T_1)-(S_3+S_2+S_1)]$
b = E/log Dose Ratio
m = F/b
Antilog m = Factor
% Label Claim = Factor X $\frac{[S\ t\ d]}{[Smp]}$ X 100

Where S_3, S_2, S_1, T_3, T_2, T_1 is the average diameters per each sample
[S t d] is the Final Concentration of Erythromycin Standard, and
[Smp] is the Expected Concentration of Erythromycin Sample

Zone Diameters (mm)						
Std Weight A (mg)		**Std Weight B (mg)**		**Smp Vol/Mass A (mL/mg)**		
Std Potency						
Sample A / Standard A						
Petri Dish	**S_1**	**S_2**	**S_3**	**T_1**	**T_2**	**T_3**
1						
2						
3						

Sample A / Standard B						
Petri Dish	**S_1**	**S_2**	**S_3**	**T_1**	**T_2**	**T_3**
1						
2						
3						
Smp Vol/Mass B (mL/mg)						
Sample B / Standard A						
Petri Dish	**S_1**	**S_2**	**S_3**	**T_1**	**T_2**	**T_3**
1						
2						
3						
Sample B / Standard B						
Petri Dish	**S_1**	**S_2**	**S_3**	**T_1**	**T_2**	**T_3**
1						
2						
3						
Smp Vol/Mass C (mL/mg)						
Sample C / Standard A						
Petri Dish	**S_1**	**S_2**	**S_3**	**T_1**	**T_2**	**T_3**
1						
2						
3						
Sample C / Standard B						
Petri Dish	**S_1**	**S_2**	**S_3**	**T_1**	**T_2**	**T_3**
1						
2						
3						

Sample and Standard Preparation			
Analyst:		**Head, Biological Analysis Unit:**	
Date:		Date:	
Analyst:			
Date:			