

REVISED DRAFT AMENDMENTS

Date: 15th July 2020

The following proposed revised draft amendments are placed for stakeholders comments, if any. The comments may be sent to the Indian Pharmacopoeia Commission through email id: lab.ipc@gov.in

The proposed changes in (Highlighted part) Microbiological Assay of Antibiotics in General Chapters of Microbiology for IP addendum/New Edition is given below:

2.2.10. Microbiological Assay of Antibiotics. Page 50

Page 52, Table 3

Change to: Table 3 - Stock solutions and test dilutions of Standard Preparation

Antibiotic	Assay Method	Prior Drying	Standard Stock Solution			Final diluent	Test Dilution Median dose µg or Units per ml	Incubation temp (°)
			Initial solvent (further diluent, if different)	Final Stock Concentration per ml	Use before (number of days)			
Amikacin	B	No	Water	1 mg	14	Water	10 µg	32 - 35
Amphotericin B	A	Yes	DMSO ¹¹	1 mg	Same day	B5	1.0 µg	29 - 31
Bacitracin	A	Yes	0.01M HCl	100 units	Same day	B1	1.0 Unit	32 - 35
Bleomycin	A	Yes	B6 ⁸	2 units	14	B6	0.04 Unit	32 - 35
Capreomycin	B	Yes	Water	1 mg	7	Water	100 µg	36-37.5
Carbenicillin	A	No	B1	1 mg	14	B6	20 µg	36 - 37.5
Chlortetracycline	A ¹	No	0.1M HCl	1 mg	4	Water	2.5 µg	37 - 39
	B ¹⁰	No	0.1M HCl	1 mg	4	Water	0.24 µg	35 - 37
Colistimethate sodium	A	Yes	Water	12700 Units	Same day	B4	1.0 Unit	35 - 39
	B	Yes	Water	12700 Units	Same day	B6	1.0 Unit	35 - 37
Colistin sulphate	A	No	Water	1 mg	14	B6	1.0 µg	35 - 37
Erythromycin	A	Yes	Methanol	1 mg	14	B2	1.0 µg	32 - 35
			(10 mg / ml) ⁸ (B2)					
Framycetin	A	Yes	B2	1 mg	14	B2	1.0 µg	30 - 37
	B ¹³	Yes	B2	1 mg	14	B2	10 µg	35-37
Gentamicin	A	Yes	B2	1 mg	30	B2	0.1 µg	36 - 37.5
	B ¹³	Yes	Water	1 mg	30	B6	0.5 µg	35-37
Gramicidin	B	Yes	Methanol	1000 Units	30	B6 ¹²	1.0 Unit	35 - 37
Kanamycin sulphate	A ¹	No	B2	800 units	30	B2	0.8 Unit	37 - 39
	B ²	No	Water	1000 units	30	Water	10 Units	32 - 35
Neomycin	A	Yes	B2	1 mg	14	B2	1.0 µg	36 - 37.5
	B ¹³	Yes	Water	1 mg	14	B2	1.0 µg	35-37
Netilmicin sulphate	A	Yes	Water	1 mg	7	B2	0.2 µg	32 - 35
Novobiocin	A	Yes	Ethanol	1 mg	5	B4	0.5 µg	34 - 36
			(10 mg/ml) ⁹ , (B2)					
Nystatin	A	Yes	DMF ⁷	1000 units	Same day	B4	20 Units	29 - 31
Oxytetracycline	A ³	No	0.1M HCl	1 mg	4	B3	2.5 µg	32 - 35
	B ²	No	0.1M HCl	1 mg	4	Water	0.24 µg	35 - 37
Polymyxin B	A	Yes	Water, (B4)	10,000 Units	14	B4	10 Units	35 - 39
Sisomicin	A	Yes	B2	1 mg	14	B2	0.1 µg	32 - 35
Spiramycin	A ⁴	No	Methanol	3200 Units	1	B2	20 Units	30 - 32
	B ¹³	No	Methanol	3200 Units	1	B6	50 Units	35-37
Streptomycin	A ⁴	Yes	Water	1 mg	30	Water	1.0 µg	32 - 35
	B ⁵	Yes	Water	1 mg	30	Water	30 µg	35 - 37
Teicoplanin	A	Yes	B4	1000 Units	Same day	B4	1.0 Unit	35 - 37
Tetracycline	A ³	No	0.1M HCl	1 mg	1	Water	2.5 µg	32 - 35
	B ⁶	No	0.1M HCl	1 mg	1	Water	0.24 µg	35 - 37
Tobramycin	B	Yes	Water	1 mg	14	Water	2.5 µg	32 - 35
Tylosin	A	No	Methanol (2.5% v/v) ⁹	1000 Units	30	40 Volumes of Methanol & 60 in B6	0.25 Unit	32-35

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		Volumes of B2						
B ¹⁰	No	Methanol	1000 Units	30	Methanol & B2 (1:1)	4 Units	36 -38	
Tyrothricin	B	Yes	Ethanol	1 mg	Same Day	Ethanol	1.0 Unit	36 - 38
Vancomycin	A	No	Water	1 mg	7	B3	10 µg	32 - 35
	B ¹³	No	Water	1 mg	7	B2	10 µg	37 - 39

1. With *Bacillus pumilus* ATCC 14884 as test organism;
2. With *Staphylococcus aureus* ATCC 29737 as test organism;
3. With *Bacillus cereus var. mycoides* ATCC 11778 as test organism;
4. With *Bacillus subtilis* ATCC 6633 as test organism;
5. With *Klebsiella pneumoniae* ATCC 10031 as test organism;
6. With *Staphylococcus aureus* ATCC 29737 as test organism;
7. DMF = Dimethylformamide;
8. In column 4 & 7, B denotes buffer solution and the number following refers to the buffer number in table 2;
9. Initial concentration of stock solution;
10. With *Staphylococcus aureus* ATCC 9144 as test organism;
11. **DMSO**: Dimethyl sulphoxide;
12. Addition of a detergent may be necessary to avoid absorption on the material during the dilutions for example 0.1 mg per ml of polysorbate 80;
- 13 With ***Staphylococcus aureus ATCC 6538P*** as Test organism

Page 54, Table 4

Change to: Table 4 - Test Organisms for Microbiological Assay of Antibiotics

Antibiotic	Test Organism	ATCC ¹ No.
Amikacin	<i>Staphylococcus aureus</i>	29737
Amphotericin B	<i>Saccharomyces cerevisiae</i>	9763
Bacitracin	<i>Micrococcus luteus</i>	10240
Bleomycin	<i>Mycobacterium smegmatis</i>	607
Capreomycin	<i>Klebsiella pneumoniae</i>	10031
Carbenicillin	<i>Pseudomonas aeruginosa</i>	25619
Chlortetracycline	<i>Bacillus pumilus</i>	14884
	<i>Bordetella bronchiseptica</i>	4617
	<i>Escherichia coli</i>	10536
Colistimethate - sodium	<i>Escherichia coli</i>	9637
	<i>Bordetella bronchiseptica</i>	4617
Colistin sulphate	<i>Escherichia coli</i>	10536
	<i>Kocuria rhizophila</i>	9341
Erythromycin	<i>Bacillus pumilus</i>	14884
Framycetin	<i>Bacillus subtilis</i>	6633
	<i>Staphylococcus aureus</i>	6538P
Gentamicin	<i>Staphylococcus aureus</i>	6538P
	<i>Staphylococcus epidermidis</i>	12228
	<i>Bacillus pumilus</i>	14884
Gramicidin	<i>Entrococcus hirae</i>	10541
	<i>Staphylococcus aureus</i>	6538P
	<i>Bacillus pumilus</i>	14884
Kanamycin sulphate	<i>Staphylococcus aureus</i>	29737
	<i>Bacillus pumilus</i>	14884
Neomycin	<i>Bacillus subtilis</i>	6633
	<i>Staphylococcus epidermidis</i>	12228
	<i>Staphylococcus aureus</i>	6538P
Netilmicin sulphate	<i>Staphylococcus aureus</i>	6538P
Novobiocin	<i>Staphylococcus epidermidis</i>	12228
Nystatin	<i>Saccharomyces cerevisiae</i>	2601
Oxytetracycline	<i>Bacillus cereus var, mycoides</i>	11778
	<i>Staphylococcus aureus</i>	29737
Polymyxin B	<i>Bordetella bronchiseptica</i>	4617
Sisomicin	<i>Staphylococcus epidermidis</i>	12228
Spiramycin	<i>Bacillus subtilis</i>	6633
	<i>Staphylococcus aureus</i>	6538P
Streptomycin	<i>Bacillus subtilis</i>	6633
	<i>Klebsiella pneumoniae</i>	10031
Teicoplanin	<i>Bacillus subtilis</i>	6633
Tetracycline	<i>Bacillus cereus</i>	11778
Tobramycin	<i>Staphylococcus aureus</i>	29737
	<i>Staphylococcus aureus</i>	29737
Tylosin	<i>Staphylococcus aureus</i>	9144
	<i>Kocuria rhizophila</i>	9341
Tyrothricin	<i>Entrococcus hirae</i>	10541
Vancomycin	<i>Bacillus subtilis</i>	6633
	<i>Staphylococcus aureus</i>	6538P

1. American Type Culture Collection, 21301 Park Lawn Drive, Rockville, MD20852, USA.

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Page 55, Table 5

Change to: Table 5 – Preparation of inoculum

Test organism	Incubation conditions				Suggested inoculum composition		
	Medium/ Method of Preparation	Temp. (°)	Time	dilution factor	Medium	Amount (ml per 100 ml)	Antibiotics assayed
<i>Bacillus cereus</i> <i>var. mycoides</i> (11778)	A ¹ /2	32-35	5 days	-	F	As required	Oxytetracycline Tetracycline
Bacillus pumilus (14884)	A ¹ /2	32-35	5 days	-	D	As required	Chlortetracycline Framycetin Kanamycin sulphate Neomycin Gentamicin
Bacillus subtilis (6633)	A ¹ /2	32-35	5 days	-	E D B A E A	As required As required As required As required As required	Framycetin Neomycin Spiramycin Streptomycin Teicoplanin Vancomycin
<i>Bordetella bronchiseptica</i> (4617)	A/1	32-35	24 hr	1:20	H H H	0.1 0.1 0.1	Colistimethate sodium Colistin sulphate Polymyxin B
<i>Escherichia coli</i> (10536)	A/1	35-39	24 hr	1:20	H	0.1	Colistimethate sodium
<i>Escherichia coli</i> (9637)	A/1	35-37	24 hr	-	H	0.1	Colistin sulphate
<i>Enterococcus hirae</i> (10541)	A/1	36-38	16-18 hr	-	C C	0.1 1.0	Colistimethate sodium Gramicidin
<i>Klebsiella pneumoniae</i> (10031)	A/1	36-37	24 hr	1:25	C	1.0	Tyrothricin
Kocuria rhizophila (9341)	A/1	32-35	24 hr	1:40	C	0.1	Capreomycin Streptomycin
<i>Micrococcus luteus</i> (10240)	A/1	32-35	24 hr	1:35	D	1.5	Erythromycin
<i>Mycobacterium smegmatis</i> (607)	A/1	32-35	24 hr	1:40	D	1.2	Tylosin
<i>Pseudomonas aeruginosa</i> (25619) ²	A/1	36-37.5	48 hr	As determined	A	0.3	Bacitracin
<i>Saccharomyces cerevisiae</i> (9763)	J/4	36-37.5	48 hr	As determined	I	1.0	Bleomycin
<i>Saccharomyces cerevisiae</i> (2601)	A/1	36-37.5	24 hr	1:25	H	0.5	Carbenicillin
Staphylococcus aureus (6538P)	G/3	29-31	48 hr	As determined	G	1.0	Amphotericin B
	G/3	29-31	48 hr	As determined	G	1.0	Nystatin
	A/1	32-35	16-18 hr	-	C	3.0	Framycetin
	A/1	32-35	24 hr	-	C	1.0	Gentamicin
	A/1	32-35	16-18 hr	-	C	1.0	Gramicidin
	A/1	32-35	16-18 hr	-	C	0.5	Neomycin
	A/1	32-35	24 hr	-	A	1.0	Netilmicin sulphate
	A/1	32-35	16-18 hr	-	C	0.5	Spiramycin
<i>Staphylococcus aureus</i> (29737)	A/1	37-39	24 hr	-	C	As required	Vancomycin
	A/1	32-35	24 hr	1:20	C	0.1	Amikacin Doxycycline Oxytetracycline Tetracycline Tobramycin
					C	0.2	Kanamycin sulphate
Staphylococcus aureus (9144)	A/1	35-37	16-18 hr	-	C	0.25	Tylosin
<i>Staphylococcus epidermidis</i> (12228)	A/1	32-35	24 hr	1:40	D	0.03	Gentamicin
					D	0.4	Neomycin
					A	4.0	Novobiocin
					D	0.03	Sisomicin

1. Use Medium A containing 300 mg of manganese sulphate per litre.
2. For *Pseudomonas aeruginosa* in the assay of carbenicillin, use the dilution yielding 25 percent light transmission, rather than the stock suspension, for preparing the inoculum suspension.

Amphotericin B, IP 2018, Volume II, Pg No.1236

Change From:

Amphotericin B has a potency of not less than 750 Units per mg, calculated on the dried basis.

Change To:

Amphotericin B has a potency of not less than 750 µg of C₄₇H₇₃NO₁₇ per mg, calculated on the dried basis.