The Sensititre® MYCOTB MIC plate for susceptibility testing of *Mycobacterium tuberculosis* to 1st and 2nd line drugs

SUPPLEMENTAL MATERIAL

Contents

Table S1. Results for testing of 99 *M. tuberculosis* isolates from the Republic of Korea using the Sensititre MYCOTB MIC and agar proportions methods

Drug	APM critical concentration (ug/ml)	MYCOTB result	# of isolates Resistant on APM	# of isolates Susceptible on APM	Sensitivity % (95% CI)	Specificity % (95% CI)	% Agreement-Categorical	% Agreement-Conditional
Isoniazid	0.2	Resistant	73	0	100.0	100.0	100.0	99.0
		Susceptible	0	26	(100 , 100)	(100 , 100)		
Isoniazid	1	Resistant	51	1	81.0	97.2	86.9	99.0
		Susceptible	12	35	(71.3 , 90.7)	(91.9 , 102.6)		
Rifampin	1	Resistant	74	0	98.7	100.0	99.0	100.0
		Susceptible	1	24	(96.1 , 101.3)	(100 , 100)		
Rifabutin	0.5	Resistant	52	13	100.0	72.3	86.9	93.9
		Susceptible	0	34	(100 , 100)	(59.6 , 85.1)		
Ethambutol	5	Resistant	49	0	58.3	100.0	64.7	83.8
		Susceptible	35	15	(47.8 , 68.9)	(100 , 100)		
Ethambutol	10	Resistant	12	1	23.1	97.9	58.6	83.8
		Susceptible	40	46	(11.6 , 34.5)	(93.8, 102)		
Ofloxacin	2	Resistant	52	11	100.0	76.6	88.9	95.0
		Susceptible	0	36	(100 , 100)	(64.5 , 88.7)		
Moxifloxacin	0.5	Resistant	45	30	100.0	44.4	69.7	81.8
		Susceptible	0	24	(100 , 100)	(31.19 , 57.7)		

Moxifloxacin	2	Resistant	19	36	100.0	55.0	63.6	76.8
		Susceptible	0	44	(100 , 100)	(44.1 , 65.9)		
Streptomycin	2	Resistant	26	21	96.3	70.8	77.8	98.0
		Susceptible	1	51	(89.2 , 103.4)	(60.3 , 81.3)		
Streptomycin	10	Resistant	20	2	90.9	97.4	96.0	97.0
		Susceptible	2	75	(78.9 , 103.0)	(93.9 , 101.0)		
Amikacin	4	Resistant	45	0	95.7	100.0	98.0	99.0
		Susceptible	2	52	(90.0 , 101.6)	(100 , 100)		
Kanamycin	5	Resistant	51	0	96.2	100.0	98.0	99.0
		Susceptible	2	46	(91.1 , 101.4)	(100 , 100)		
Cycloserine	25	Resistant	2	7	10.0	91.1	74.8	90.9
		Susceptible	18	72	(-3.2 , 23.2)	(84.9 , 97.4)		
Ethionamide	5	Resistant	54	3	87.1	91.9	88.9	98.0
		Susceptible	8	34	(78.8 , 95.4)	(83.1 , 100.7)		
PAS	2	Resistant	57	6	89.1	82.9	86.9	96.0
		Susceptible	7	29	(81.4 , 96.7)	(70.4 , 95.3)		
Abbreviations: APM, agar proportion method; CI, confidence interval; PAS, para-aminosalicylic acid								

Table S2. Results for testing of 98 *M. tuberculosis* isolates from Uganda using the Sensititre MYCOTB MIC and agar proportions methods								
Drug	APM critical concentration (ug/ml)	MYCOTB result	# of isolates Resistant on APM	# of isolates Susceptible on APM	Sensitivity % (95% CI)	Specificity % (95% CI)	% Agreement-Categorical	% Agreement-Conditional
Isoniazid	0.2	Resistant	52	3	100.00	93.5	96.9	99.0
		Susceptible	0	43	(100 , 100)	(86.3 , 100.6)		
Isoniazid	1	Resistant	43	1	97.7	98.2	98.0	99.0
		Susceptible	1	53	(93.3 , 102.1)	(94.6 , 101.7)		
Rifampin	1	Resistant	31	0	96.9	100.0	99.0	100.0
		Susceptible	1	66	(90.9 , 102.9)	(100 , 100)		
Rifabutin	0.5	Resistant	26	1	96.3	98.6	98.0	98.0
		Susceptible	1	70	(89.2 , 103.4)	(95.9 , 101.3)		
Ethambutol	5	Resistant	20	3	83.3	96.0	92.9	100.0
		Susceptible	4	71	(68.4 , 98.2)	(91.5 , 100.4)		
Ethambutol	10	Resistant	5	1	100.0	98.9	99.0	100.0
		Susceptible	0	92	(100 , 100)	(96.8 , 101.0)		
Ofloxacin	2	Resistant	1	1	100.0	99.0	99.0	100.0
		Susceptible	0	96	(100 , 100)	(97.0 , 101.0)		
Moxifloxacin	0.5	Resistant	1	1	100.0	99.0	99.0	100.0
		Susceptible	0	96	(100 , 100)	(97.0 , 101.0)		

Moxifloxacin	2	Resistant	0	0	0.0	100.0	100.0	100.0
		Susceptible	0	98		(100 , 100)		
Streptomycin	2	Resistant	10	5	100.0	94.3	94.9	98.0
		Susceptible	0	83	(100 , 100)	(89.5 , 99.2)		
Streptomycin	10	Resistant	5	3	83.3	96.7	95.9	99.0
		Susceptible	1	89	(53.5 , 113.2)	(93.1 , 100.4)		
Amikacin	4	Resistant	0	0	0.0	100.0	100.0	100.0
		Susceptible	0	98		(100 , 100)		
Kanamycin	5	Resistant	0	0	0.0	100.0	100.0	100.0
		Susceptible	0	98		(100 , 100)		
Cycloserine	25	Resistant	1	8	100.0	91.8	91.8	100.0
		Susceptible	0	89	(100 , 100)	(86.3, 97.2)		
Ethionamide	5	Resistant	11	2	55.0	97.4	88.8	99.0
		Susceptible	9	76	(33.2 , 76.8)	(93.9 , 100.9)		
PAS	2	Resistant	1	1	100.0	98.97	99.0	100.0
		Susceptible	0	96	(100 , 100)	(97.0 , 101.0)		
Abbreviations: APM, agar proportion method; CI, confidence interval; PAS, para-aminosalicylic acid								

Table S3. Results for testing of 25 *M. tuberculosis* isolates from the Supranational Laboratory using the Sensititre MYCOTB MIC and agar proportions methods

Drug	APM critical concentration (ug/ml)	MYCOTB result	# of isolates Resistant on APM	# of isolates Susceptible on APM	Sensitivity % (95% CI)	Specificity % (95% CI)	% Agreement-Categorical	% Agreement-Conditional
Isoniazid	0.2	Resistant	16	0	100.0	100.0	100.0	100.0
		Susceptible	0	9	(100 , 100)	(100 , 100)		
Isoniazid	1	Resistant	14	0	93.3	100.0	96.0	100.0
		Susceptible	1	10	(80.7 , 106.0)	(100 , 100)		
Rifampin	1	Resistant	16	0	100.0	100.0	100.0	100.0
		Susceptible	0	9	(100 , 100)	(100 , 100)		
Rifabutin	0.5	Resistant	12	1	100.0	92.3	96.0	96.0
		Susceptible	0	12	(100 , 100)	(77.8, 106.8)		
Ethambutol	5	Resistant	3	2	75.0	90.5	88.0	100.0
		Susceptible	1	19	(32.6 , 117.4)	(77.9 , 103.0)		
Ethambutol	10	Resistant	1	1	100.0	95.8	96.0	100.0
		Susceptible	0	23	(100 , 100)	(87.8 , 103.8)		
Ofloxacin	2	Resistant	10	1	100.0	93.3	96.0	100.0
		Susceptible	0	14	(100 , 100)	(80.7 , 106.0)		
Moxifloxacin	0.5	Resistant	10	3	100.0	80.0	88.0	100.0
		Susceptible	0	12	(100 , 100)	(59.8 , 100.2)		

Moxifloxacin	2	Resistant	4	1	80.0	95.0	92.0	100.0
		Susceptible	1	19	(44.9 , 115.1)	(85.5 , 104.6)		
Streptomycin	2	Resistant	9	2	100.0	87.5	92.0	100.0
		Susceptible	0	14	(100 , 100)	(71.3 , 103.7)		
Streptomycin	10	Resistant	9	0	100.0	100.0	100.0	100.0
		Susceptible	0	16	(100 , 100)	(100 , 100)		
Amikacin	4	Resistant	7	0	100.0	100.0	100.0	100.0
		Susceptible	0	18	(100 , 100)	(100 , 100)		
Kanamycin	5	Resistant	9	2	100.0	87.5	92.0	100.00
		Susceptible	0	14	(100 , 100)	(71.3 , 103.7)		
Cycloserine	25	Resistant	1	7	100.0	70.8	72.0	100.0
		Susceptible	0	17	(100 , 100)	(52.7 , 89.0)		
Ethionamide	5	Resistant	3	1	100.0	95.4	96.0	100.0
		Susceptible	0	21	(100 , 100)	(86.8 , 104.2)		
PAS	2	Resistant	0	0	0.0	100.0	100.0	100.0
		Susceptible	0	25		(100 , 100)		
Abbreviations: APM, agar proportion method; CI, confidence interval; PAS, para-aminosalicylic acid								

Table S4. Sensititre® MYCOTB MIC plate drug concentrations that optimize sensitivity and specificity using APM as the reference comparator. Recommended critical concentrations for use in the liquid MGIT system are also shown.

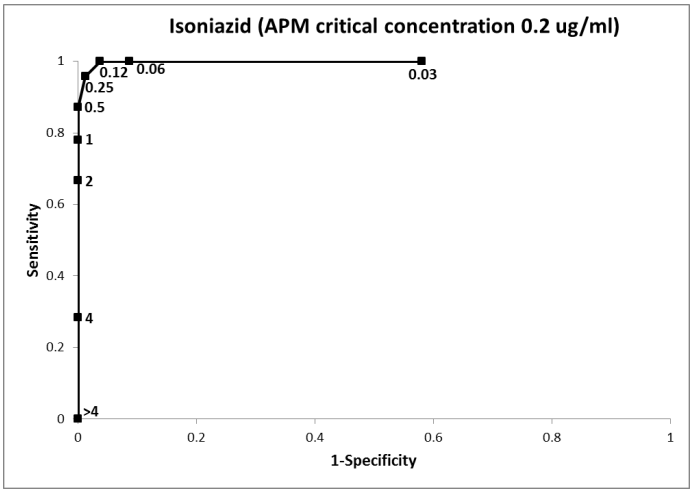
Drug	APM critical concentration (ug/ml)	Optimal MYCOTB plate concentration(s) (ug/ml)	Area under the Receiver Operator Characteristic curve	Recommended MGIT critical concentration (ug/ml)*
Isoniazid	0.2	0.12-0.25	1.00	0.1
Isoniazid	1	0.5	0.98	
Rifampin	1	0.5-1	1.00	1
Rifabutin	0.5	0.5-1	0.96	0.5
Ethambutol	5	2	0.88	5
Ethambutol	10	2-4	0.85	
Ofloxacin	2	4	0.99	2
Moxifloxacin	0.5	1	0.95	0.25
Moxifloxacin	2	2	0.94	
Streptomycin	2	2-4	0.98	1
Streptomycin	10	4	0.99	
Amikacin	4	1-2	1.00	1
Kanamycin	5	5	0.98	2.5
Cycloserine	25	Unable to assess	0.56	Not recommended
Ethionamide	5	1.2-2.5	0.96	5
PAS	2	2	0.96	4

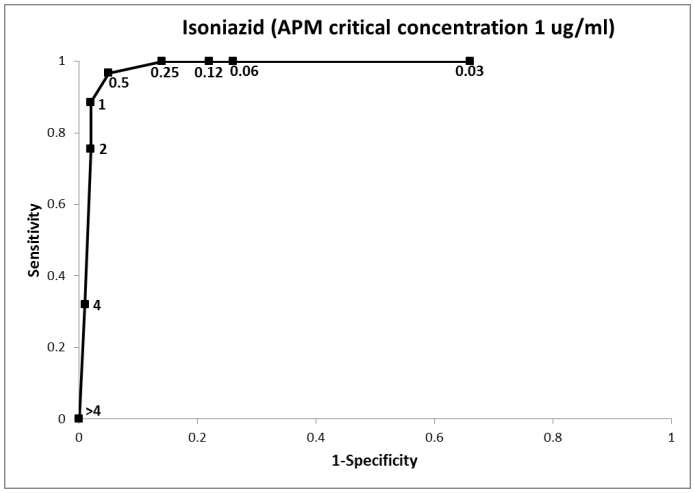
***Clinical Laboratory Standards Institute**. Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes: approved standard. CLSI document M24-A (ISBN 1-56238-550-3). Clinical Laboratory Standards Institute, Wayne, PA, 2003.

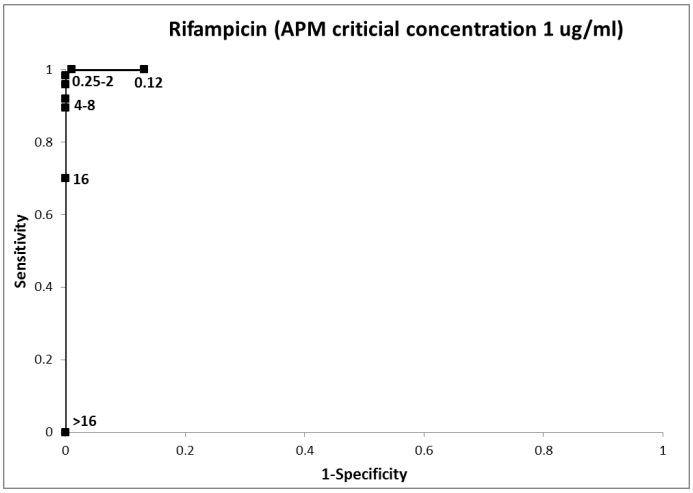
Abbreviations: APM, agar proportion method; MGIT, Mycobacterial Growth Indicator Tube; PAS, para-aminosalicylic acid

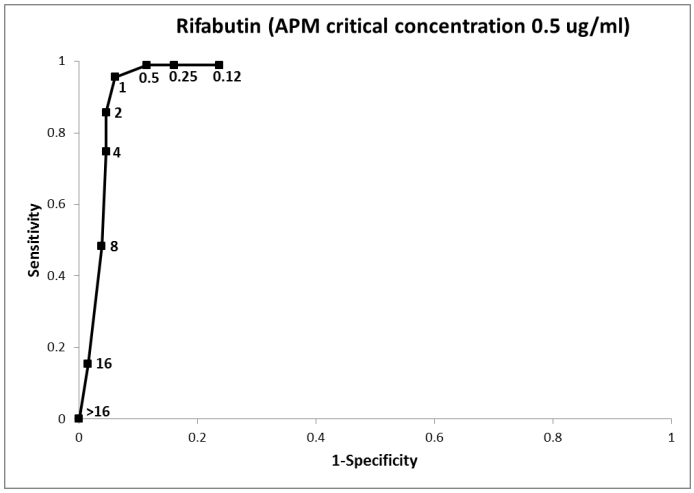
Figure S1. Receiver operator characteristic (ROC) curves for each tested drug.

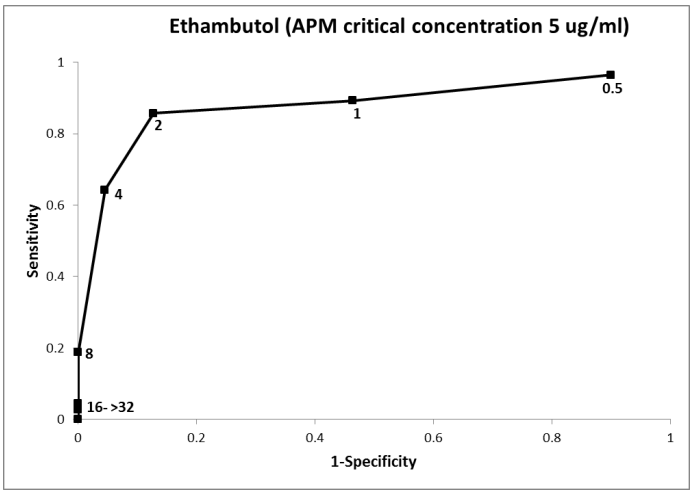
Numbers in bold are MYCO TB plate minimum inhibitory concentrations.

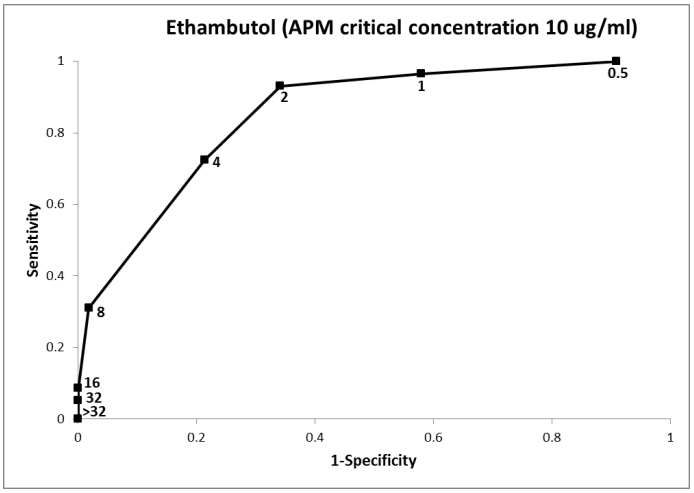


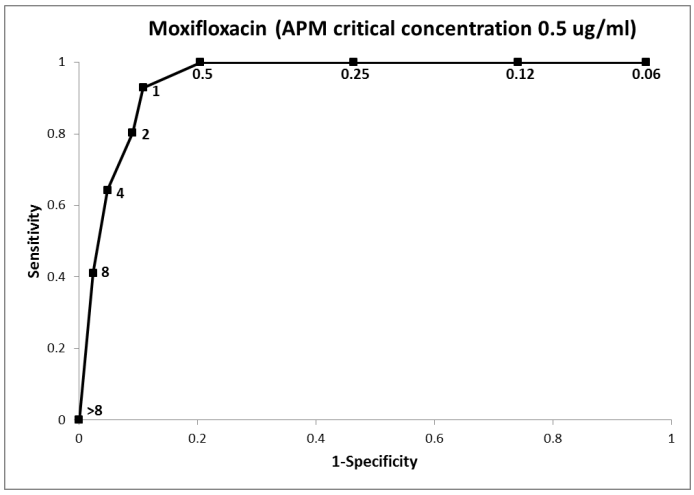


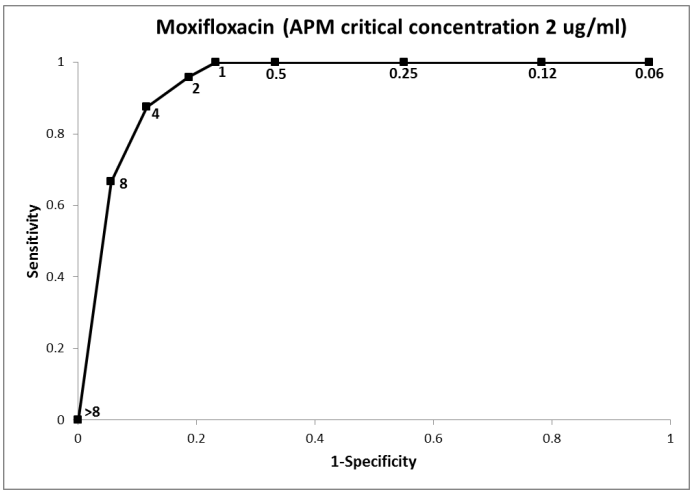


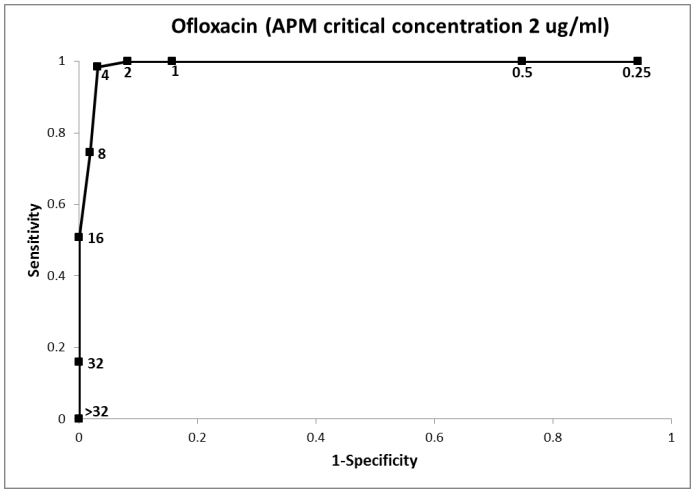


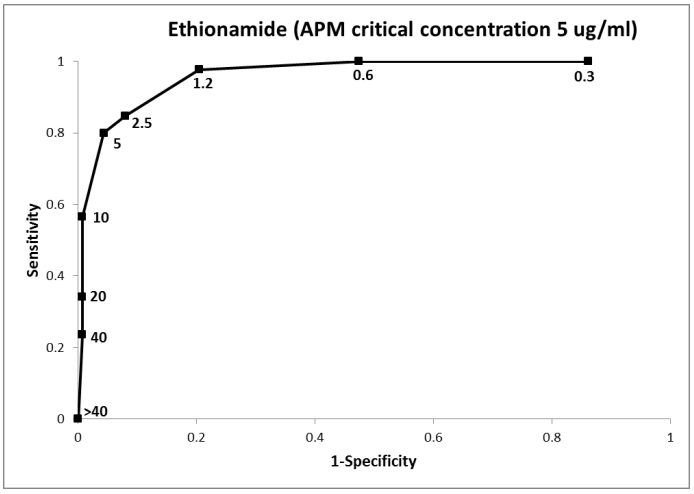


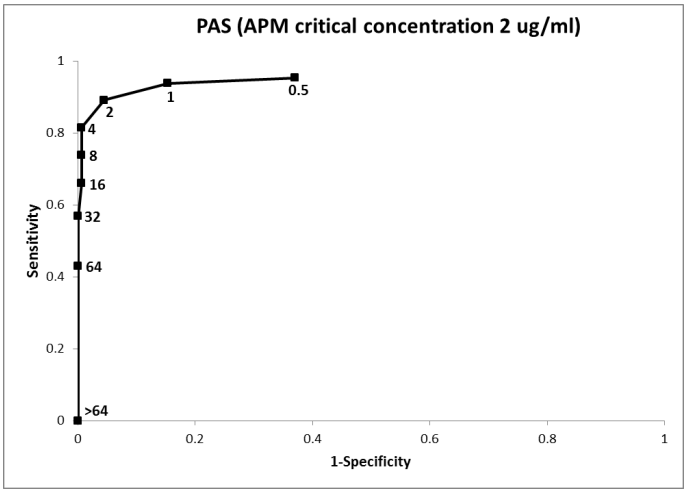


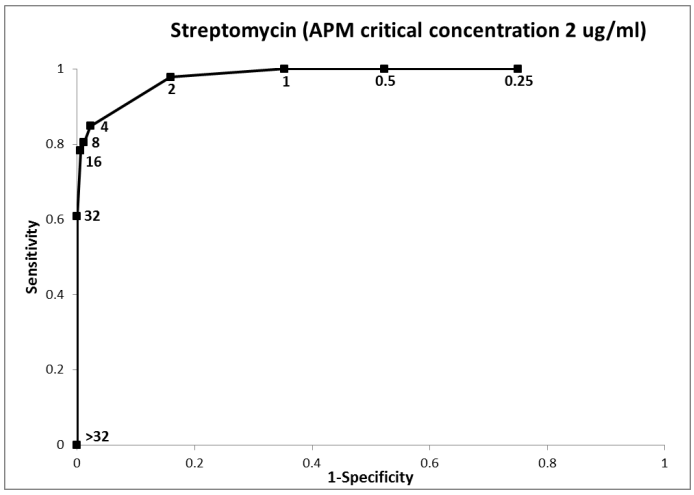

Moxifloxacin (APM critical concentration 0.5 ug/ml)
Sensitivity
1-Specificity
0.5
0.25
0.12
0.06
1
2
4
8
>8

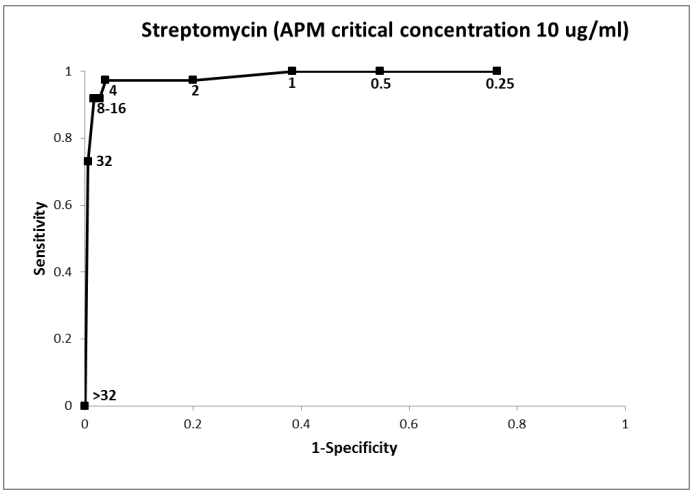

Moxifloxacin (APM critical concentration 2 ug/ml)
Sensitivity
1-Specificity
1
0.5
0.25
0.12
0.06
2
4
8
>8

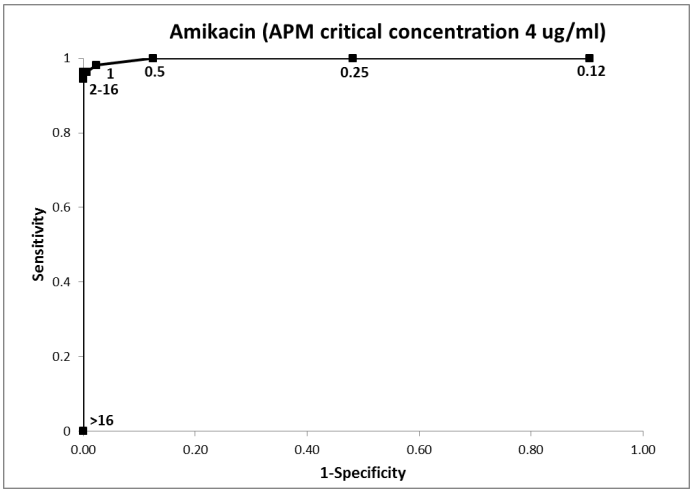

Ofloxacin (APM critical concentration 2 ug/ml)
Sensitivity
1-Specificity
4
2
1
0.5
0.25
8
16
32
>32

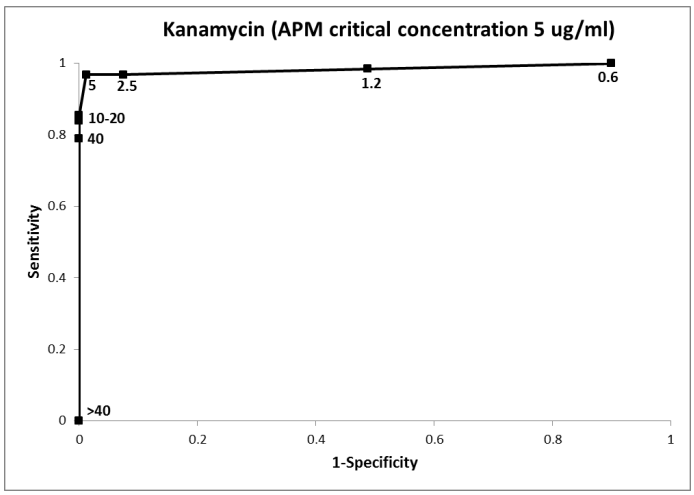

Ethionamide (APM critical concentration 5 ug/ml)
Sensitivity
1-Specificity
1.2
0.6
0.3
2.5
5
10
20
40
>40

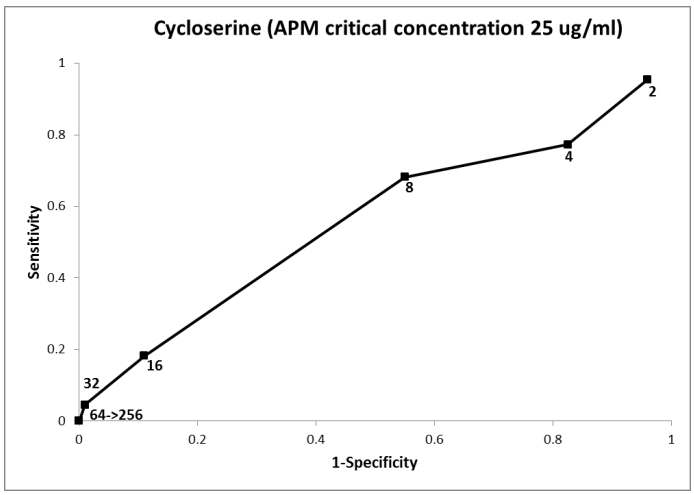

PAS (APM critical concentration 2 ug/ml)
Sensitivity
1-Specificity
1
0.5
2
4
8
16
32
64
>64


Streptomycin (APM critical concentration 2 ug/ml)
Sensitivity
1-Specificity
2
1
0.5
0.25
4
8
16
32
>32


Streptomycin (APM critical concentration 10 ug/ml)
Sensitivity
1-Specificity
2
1
0.5
0.25
4
8-16
32
>32


Amikacin (APM critical concentration 4 ug/ml)
Sensitivity
1-Specificity
0.5
0.25
0.12
1
2-16
>16


Kanamycin (APM critical concentration 5 ug/ml)
Sensitivity
1-Specificity
5
2.5
1.2
0.6
10-20
40
>40


Cycloserine (APM critical concentration 25 ug/ml)
Sensitivity
1-Specificity
2
4
8
16
32
64->256