Tuberculosis Survey In Bhaktapur

JAPANESE MEDICAL TEAM

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INTRODUCTION

Tuberculosis Control Programme should be planned and conducted according to the epidemiological situation of tuberculosis in each country. Mortality and morbidity rate of tuberculosis have not been worked out in Nepal so far.

Bhaktapur is one of the old towns where high morbidity of tuberculosis can be presumed by the number of tubercular patients attending the hospital and clinic there.

The first tuberculosis survey at Bhaktapur town was conducted by the Japanese medical team from the 9th of November 1965 to the end of February 1966.

METHOD

- I) The population of Bhaktapur town is approximately 38,000. 6,954 people were examined by tuberculin-test and radiophotography at Bhaktapur Schools and Wards. Their age distribution is shown in (Fig. 1). At each school and ward, all students and inhabitants who came willingly in front of the X-ray van were registered. Their name, age, sex and address were noted down. Previous chemotherapy on tuberculosis and occupation of the people also were recorded on the punch card. (Fig. 2) 0.1cc of Japanese old tuberculin (1:2000) was injected intradermally and they were taken to X-ray van for radiophotography. The students and inhabitants thus examined were asked to report two days after the injection to read the result of tuberculin test. Over 8mm. diameter of induration was taken as a positive reading.

0.05 mg of Japanese Freeze-dried B.C.G. Vaccine was given to the non-reactors by the intradermal technique.

- II) Results of the X-ray examination were deliberately described in order to investigate the relation between X-ray and bacteriological findings. The X-ray findings were classified according to the X-ray classification of the Japanese Society for Tuberculosis.

Fig. 1

Fig 2 INDIVIDUAL PUNCH CARD FOR MASS SURVEY

[illegible]

Fig 2

X-ray Classification of the Japanese Society for Tuberculosis. 1)

I: Far-advanced, cavitary type	
II: Not-far-advanced, cavitary type	
IIIa: Suspected cavity, unstable type	Suspected Inactive Tuberculosis.
IIIb: Non-cavitary, unstable type	
IV: Non-cavitary, stable type	Suspected Inactive Tuberculosis.
V: Healed type	

The following changes are added to the above-mentioned type.

H: Hilar lymph node swelling

Pl: Exudative Pleurisy.

• **X-ray Apparatus.**

Type: CM 100L X-ray High Tension Unit
(Shimadzu Seisakusho LTD, Japan)

Classification: CG-I-100 (JIS)

Rating: Radiographic rating 100KV, 1.0uF

Fluoroscopic rating 100KV, 2. 0mA, 30 second

Power Source: Single phase AC 100 V, 50 or 60 c.p.s.
more than 1.0 KVA capacity

X-ray tube: Circlex 2MGA (triode X-ray tube)
focal spot size; 2×2 mm

• **X-ray camera:**

Type: Cannon Mirror Camera CXM-70

Lens: f 186 mm, 1:063 (G.R.A.)

Fluorescent Screen Size: 400 X 400 mm

Film Size: 70 mm

Frame Size: 63 X 63 mm

Fluorescent Screen: PO

• **X-ray Film:**

Sakura X-ray Film: New Y type 70 mm

• **Developing:**

Developer: Sakura Konidol-X

Fixer: Sakura Konifix-X

• **Exposure conditions:**

Tube Voltage: Adult, 75-85 kv

Children, 65-75 KV

Tube Current: 120-150 uAp

Focus screen distance: 900 mm.

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Tube Current: 120-150 uAp

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III) Bacteriological Examination of Sputum.

The people examined by tuberculin test and Chest X-ray were classified into two groups, suspected pulmonary tuberculosis and non tuberculosis. The former were divided to the suspected active tuberculosis in radiological sense (I,II,IIIa, and IIIb) and the inactive tuberculosis.

Bacteriological examination of sputum was done to this suspected active tuberculosis for the purpose of final diagnosis.

The method described in "A GUIDE TO THE EXAMINATION OF TUBERCLE BACILLI" 2) was followed for staining, culture of sputum and laryngeal swab examination.

Collection of Sputum:— Specimen were obtained mostly at daytime in the sterilized Petri dish and examined within 24 hours.

Sputum Culture:— The sputum was mixed with an equal volume of 4% NaOH and after thorough mixing it to a homogeneous substance, 0.1 ml of material was inoculated on 3% Ogawa medium without neutralization, and then kept 37°C incubator for 8 weeks.

The specimens showing no colonies after 8 weeks were described as negative. In the cases where colonies grew, A.F.B. staining was done in doubtful case only.

The guide book was followed to record appearance of the colony.

Laryngeal Swab:

A swab stick tipped with absorbent cotton was used for wiping the epiglottis to obtain the mucus, which was treated by the same way as for sputum.

RESULT

I. Tuberculin Test.

The total number of the people tested was 6,159 of these only 4,725 attended for the reading and the tuberculin positivity was 50.9%. The attendance rate was 76.7%.

Dividing by age group, the tuberculin positivity was 20.8% in the age group under 4, and rose in accordance with the increase of age,

then fell to 57.2% in the age group over 51. (Table 1) classified according to sex the tuberculin positivity was 48.4% in male, 55.6 in female, the latter was higher than the former in all age group except between 31 and 50 (Table 2,3 Fig. 3).

B.C.G. was given to 2,240 tuberculin non-reactors.

TABLE 1 Result of the Tuberculin Test (Total)

Age Years	Total injected	Positive	Negative	Total judged	Attendance rate
— 4	232	40 20.8%	152	192	82.7%
5—10	1396	412 35.3	755	1167	83.6
11—20	1927	735 48.2	790	1525	79.1
21—30	703	340 68.8	154	494	70.2
31—50	1188	580 70.0	248	828	69.7
51—	713	297 57.2	222	519	72.8
Total	6159	2404 50.9%	2321	4725	76.07%

TABLE 2 Result of the Tuberculin Test (Male)

Age years	Total injected	Positive	Negative	Total judged	Attendance rate
— 4	135	21—18.8%	91	112	82.9%
5—10	886	240—32.4	501	741	83.6
11—20	1389	471—43.7	607	1078	77.6
21—30	473	230—68.6	105	335	70.8
31—50	703	353—71.5	141	494	70.2
51—	426	171—54.6	142	313	73.2
Total	4012	1486—48.4%	1587	3073	76.6%

TABLE 3 Result of the Tuberculin Test (Female)

Age years	Total injected	Positive	Negative	Total judged	Attendance rate
— 4	97	19—23.8%	61	80	82.5%
5—10	510	172—40.4	254	426	83.6
11—20	538	264—59.1	183	447	83.1
21—30	230	110—69.2	49	159	69.2
31—50	485	227—68.0	107	334	68.9
51—	287	126—61.2	80	206	71.6
Total	2147	918—55.6%	734	1652	77.0%

Fig. 3 PREVALENCE OF TUBERCULIN (O.T) POSITIVE REACTORS
BY SEX AND AGE AT BHAKTAPUR TOWN

Fig. 3

II) X-Ray Findings.

1) Suspected Tuberculosis

6,954 person were examined by X-Ray and 558 of which were suspected to have pulmonary Tuberculosis on the X-Ray and its rate is 8.0%. The

in male and 2.2% in female, and the female was higher than the male in the age group under 30, and the male was higher than the female in the age group over 31. (Table 5,6 Fig.4).

TABLE 5 Result of the Chest X-Ray Examination (Male)

Suspected pulmonary Tuberculosis												Total of Examination
Age years	Suspected active T.B.					Suspected inactive Healed						
	I	II	IIIa	IIIb	Total	H	PI	IV	V	Total		
— 4				1	1(0.6%)	1			1	3(1.9%)	159	
5—10		2			2(0.2)	3		2	16	23(2.5)	928	
11—20	2	2		4	8(0.5)		1	3	54	66(4.5)	1474	
21—30	1	7	2	7	17(2.9)			11	32	60(10.2)	585	
31—50	6	13	8	18	45(5.3)		2	18	62	127(14.9)	854	
51—		6	4	10	20(4.2)			12	30	62(13.1)	474	
Total	9	30	14	40	93(2.1%)	4	3	46	195	341(7.6%)	4,474	

TABLE 6 Result of the Chest X-Ray Examination (Female)

Age years	Suspected Pulmonary Tuberculosis										Total of Examination
	Suspected active T.B.					Suspected inactive Healed					
	I	II	IIIa	IIIb	Total	H	PI	IV	V	Total	
— 4									1	1(0.9%)	112
5--10			1	1	2(0.4)			2	14	16(3.3)	542
11--20	3			6	9(1.5)			6	38	53(8.8)	596
21--30	3	1	5	8	17(5.5)		1	4	12	34(11.0)	310
31--50	4	8	1	8	21(3.5)	1	1	9	37	69(11.5)	601
51--		4		3	7(2.2)			6	29	42(13.2)	319
Total	10	13	7	26	56(2.2)	1	2	27	131	217(8.8%)	2489

FIG 4 PULMONARY TUBERCULOSIS ON RADIOLOGICAL EXAMINATION
BY SEX AND AGE AT BHAKTAPUR TOWN

2) Suspected Non Tuberculosis

The findings of suspected non tuberculosis was discovered on the chest X-ray of 180 persons (2.6%), the most was bronchiectasis 87 (1.3%), the second was bronchitis 36 (0.5%), pneumonia 33 (0.5%) and so on.

Dividing by age group, bronchiectasis and cardiac cases predominated in higher age groups, pneumonia was dominant in younger ages. (Table 7, 8 and 9).

TABLE 7 Result of the Chest X-Ray Examination (Total)

Age years	Non Tuberculosis						Total of the Examined
	Bronchiectasis	Bronchitis	Pneumonia	Cardiac	Others	Total	
— 4			7			7(2.6%)	271
5—10	9	3	15	1	1	30(2.0)	1,470
11—20	11	7		1	1	25(1.2)	2,070
21—30	4	5	3	2		14(1.6)	895
31—50	25	10		5		40(2.7)	1,455
51—	38	11	2	7	6	64(8.1)	793
Total	87	36	33	16	8	180(2.6%)	6,954

TABLE 8 Result of the Chest X-Ray Examination (Male)

Age years	Non Tuberculosis						Total of the Examined
	Bronchiectasis	Bronchitis	Pneumonia	Cardiac	Others	Total	
— 4			6			6(3.8%)	159
5—10	5	1	10		1	17(1.8)	928
11—20	10	4	3		1	18(1.2)	1474
21—30	2	3	2	1		8(1.4)	585
31—50	14	2		1		17(2.0)	854
51—	21	8	1	1	3	34(7.2)	474
Total	52	18	22	3	5	100(2.2%)	4474

TABLE 9 Result of the Chest X-Ray Examination (Female)

Age years	Non Tuberculosis						Total of the Examined
	Bronchiectasis	Bronchitis	Pneumonia	Cardiac	Others	Total	
— 4			1			1(0.9%)	112
5—10	4	2	6	1		13(2.4)	542
11—20	1	3	2	1		7(1.2)	596
21—30	2	2	1	1		6(1.9)	310
31—50	11	8		4		23(2.8)	601
51—	17	3	1	6	3	30(9.4)	319
Total	35	18	11	13	3	80(3.2%)	2480

III. Bacteriological Examination.

1) A.F.B. findings is shown in Table 10.

TABLE 10 X-Ray Classification and A.F.B Findings

X-Ray Classification		Total	Positive on Smear	Positive on Culture (Smear Negative)	Total of Positive	Positive Ratio	Negative
Suspected Active TB	I	18	16(88.9%)	0	16	88.9(%)	2
	II	36	27(75.0)	5	32	88.9	4
	IIIa	16	11(68.8)	1	12	75.0	4
	IIIb	54	4(7.4)	8	12	22.2	42
Total		124	58(46.8%)	14(11.3%)	72	58.1	52
Other	H	2	0	0	0		2
	PI	4	0	0	0		4
	IV&V	41	0	0	0		41
	Total	47	0	0	0		47

Among 149 suspected active T.B. cases, sputa of 124 cases were subjected to bacteriological examination. The rest could not be examined because of obtaining no sputa.

Among 124 cases examined, 58 cases (46.8%) were A.F.B. positive on smear and 14 cases (11.3%) which were A.F.B. negative on smear revealed positive on culture.

The positive ratio of A.F.B was 58.1% in average. The 52 cases remained negative not only on smear but on culture.

2) 47 cases among H, PI, IV, and V types were examined, and A.F.B. in the sputa of these cases was negative.

3) In comparison between positive ratio of sputum culture and smear of IIIb type cases, the former (22.2%) is significantly higher than the latter (7.4%).

*a) Total number of sputa examined was 472 (270 of smear and 202 of culture). The 20 specimen of laryngeal swab were examined on culture (the positive ratio of A.F.B. was 22.5% in average by the observation up to date of Feb. 20).

b) All 22 cases which were A.F.B. positive on smear revealed positive on culture also.

c) The colonies grown on the 3% Ogawa media were observed between 4 to 6 weeks after inoculation, there are no colonies grown within 2 weeks.

d) The 8 cases were undeterminable because of the growth of saprophytes especially of moulds.

e) Using 3% Ogawa medium direct method, the sensitivity tests were demonstrated in the 15 cases in which the development of resistance were surmised as a result of previous chemotherapies.

The drug concentration of SM, PAS, and INH was 10ug/ml, 100ug/ml of SM, 1ug/ml and 10ug/ml of PAS, and 5ug/ml of INH respectively.

The results on the sensitivity tests in these cases will be reported after final judgement because the cultivation is still going on.

f) Bacteriological examination of each non-tuberculosis pulmonary diseases were not enforced.

IV Final Diagnosis.

149 suspected active tuberculosis cases were discovered by the X-ray examination, and the 72 cases revealed A.F.B. positive on sputum examination among them, so that it can be determined that the 72 cases were active pulmonary tuberculosis which were 58.1% of 124 sputum examined

Unfortunately the sputum of 25 cases was not available. But estimation can be carried out by means of $25 \times 58.1/100 = 14.5$ Total of active pulmonary tuberculosis cases are $72 + 14.5 = 87$ which is 1.3% of 6,954 total cases examined. (Table 11)

TABLE 11 X-Ray Classification and Sputum Examination

X-Ray Classification	X-Ray Classification	Sputum Examination			
		done			
		Total	A F B Positive	Positive Ratio	not done
I	19	18	16	88.9%	1
II	43	36	32	88.9	7
IIIa	21	16	12	75.0	5
IIIb	66	54	12	22.2	12
Total	149	124	72	58.1%	25

A F B Positive estimated in the not done group $= 25 \times \frac{58.1}{100} = 14.5$

TREATMENT

The purpose of our project at Bhaktapur was researching the epidemiological situation of pulmonary tuberculosis over there. However it was preferable to treat the cases found not only for personal advantage of patient but also to fulfill the public health demand.

The treatment was applied to the cases those who revealed I, II, IIIa, IIIb and H or PI on the X-ray findings. To the cases of IV on the X-ray finding which had been previously treated the treatment were given after the consideration about the history of A.F.B. findings and treatment.

These cases were called on at the Bhaktapur T.B. Clinic, and their X-ray findings, previous chemotherapy and A.F.B. findings on sputum smear or culture were recorded on the charts.

The programme of chemotherapy for each case was decided according to these description on the chart and the treatment started immediately.

The different diagnosis from non-tuberculosis pulmonary disease had been done by means of consideration about age, X-ray findings, clinical appearance and histories. The definite diagnosis of each non-tuberculosis pulmonary disease was not enforced.

TABLE 12 Active Tuberculosis of Sex and Age

Age years	Total	Male	Female
— 4	1(0.4%) 271	1(0.6%) 159	0
5—10	2(0.1) 1,470	1(0.1) 928	1(0.2%) 542
11—20	4(0.2) 2,070	3(0.2) 1,474	1(0.2) 596
21—30	18(2.0) 895	8(1.4) 585	10(3.2) 310
31—50	35(2.5) 1,454	24(2.8) 854	11(1.8) 601
51—	12(1.5) 793	8(1.7) 474	4(1.3) 319
Total	72(1.6%) 6,953	45(1.0%) 4,474	27(1.1%) 24,083

The number of active tuberculosis was 72 which was 58.1% of the 124 sputum examined. Among 72 active cases, 61 are keeping on the treatment (84.7%). In addition to these 61 active tuberculosis 86 suspected tuberculosis cases were treated.

Regarding chemotherapy, Streptomycin (SM), Isoniazid (INH) and Thiosemicarbazone (TBI) were combined by means of SM+ INH+TBI, INH+ TBI and INH alone. Their doses, SM was given 0.5g or 1.0g, INH 0.3g and SBI 0.15g daily for adult. It was remarkable that Kanamycin, Cycloserin and Ethionamide were given as secondary anti-tuberculous medicines to 3 cases who had received the injection of SM over 300 g previously and from 1 case of them resistant A.F.B. against over 100 ug per ml of SM on the sensitivity test was obtained.

The treatment of T.B. patients who were found by our survey was carried on in cooperation with Japanese and Nepali doctors.

The final evaluation of the treatment must be done at least one year later, because pulmonary tuberculosis is one of chronic diseases. So serious side-effects except dermatitis, transient nausea and slight dizziness did not call for withdrawal of drug.

The relation between X-ray classification and previous chemotherapy of the active tuberculosis cases. Among 72 active tuberculosis cases 38 patients were previously treated and 34 were not treated by chemotherapy.

Dividing by X-ray classification, I (cavitary-far advanced) was more in the treated group than in the non-treated and vice-versa, II (cavitary not far advanced) (Table 13). Regarding the 146 cases with the suspicious findings of active tuberculosis, 82 patients were previously treated and 67 were not treated by chemotherapy.

TABLE 13 X-Ray Classification and Previous Chemotherapy of the Active Tuberculosis

X-Ray Classification	Total	Previously Chemotherapy	
		not done	done
I	16	3(8.8%)	13(34.2%)
II	32	20(58.8)	12(31.6)
IIIa	12	5(14.7)	7(18.04)
IIIb	12	6(17.6)	6(15.8)
Total	72	34(100.0)	38(100.0)

Dividing by X-ray classification, I and II (with cavity) or IIIa (suspected cavity) were more in the treated group than in the non treated, and vice-versa-

DISCUSSION

Regarding tuberculin positivity and active tuberculosis rate, both were well co-related in dividing by age and sex. It is suggested that tuberculin positivity can be used as an indicator of the prevalence of tuberculosis in this area.

The tuberculin positivity and active tuberculosis rate of age group between 5 and 30 was higher in female than in male as with the results shown by other countries.

It is remarkable that 68.3% of non-tuberculosis cases were chronic bronchial disease.

Regarding sputum examination, positive ratio of A.F.B. of IIIb type was 22.2% on culture and 7.4% on smear, the former was significantly higher than the latter. Therefore, it is very important for not only case finding but also for treatment that sputum culture would be added to sputum examination.

All cases of A.F.B. positive on sputum smear and culture belong to the group of suspected active tuberculosis on X-ray (Table 10). It could be said that suspected active tuberculosis (I, II, IIIa, and IIIb) on "the X-ray Classification of the Japanese Society for Tuberculosis" enclose all of the A.F.B. positive cases which are finally diagnosed as active tuberculosis.

Therefore, it is useful to read and describe X-ray findings according to the Japanese Classification for not only case findings of active tuberculosis but also for treatment.

It is our belief that even A.F.B. negative cases which are radiologically active might be going to be bacteriologically positive if they are not treated 3). Accordingly, mass X-ray examination is also useful for early case finding as well as sputum examination. Radiophotography should be utilized for that purpose.

TABLE 14 X-Ray Classification and Previous Chemotherapy of the Radiologically Suspected Active T.B. Cases

Classification of T.B.	Total	Previously Chemotherapy	
		not done	done
I	19	4 (49%)	15 (22.4%)
II	43	28 (31.6)	15 (22.4%)
IIIa	21	9 (11.1)	12 (17.9)
IIIb	66	41 (52.4)	25 (37.3)
Total	149	82 (100.0)	67 (100.0)

Previously Chemotherapy

not done.....

I	II	IIIa	IIIb	H	pl
---	----	------	------	---	----

done.....

I	II	IIIa	IIIb	pl
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0 10 20 30 40 50 60 70 80 90 100

Among radiological active tuberculosis cases, far-advanced cavitary forms which were low sensitive to chemotherapy were included much higher (22.0%) in the previously treated group than (4.4%) in the non treated group, and non cavitary forms which were highly sensitive to chemotherapy were included higher (54.9%) in the previously non treated group than (38.3%) in the treated group. In these facts, it is suggested that early stage of the disease might be easily found by mass X-ray examination and they could be prevented from becoming far advanced by application of adequate treatment.

Regarding treatment, in some cases under treatment, conversion from positive to negative on A.F.B. in sputa and slight improvement of X-ray findings were observed at serial examinations. The final evaluation of the treatment must be done at least one year later, because pulmonary tuberculosis is one of chronic diseases. It is most important for the treatment of tuberculosis to be continued regularly by adequate chemotherapy till negative X-ray finding. But most of the patients fell back to collect the drug with the improvement of their symptoms and the drug is not taken for the full course of treatment.

This matter should be emphasized in the case of the first treatment, because it is usual that chemotherapy at the first start is more effective than in subsequent phase, and irregular treatment will lead to development of resistant strain of tubercle bacilli against anti-tuberculosis drugs.

Fig 5 PREVALENCE OF ACTIVE TUBERCULOSIS
BY SEX AND AGE

FIGURE 5

CONCLUSION

The Japanese Medical Team conducted the pulmonary Tuberculosis Survey at Bhaktapur, Kathmandu valley, Nepal from 6th Nov. 1965 to the end of Feb. 1966.

6,954 inhabitants (male 4,474 and female 2,480) were examined by tuberculin test and radiophotography.

Tuberculin positivity 50.9% (male 48.4%, female 55.6%) radiological active tuberculosis rate 2.1% (male 2.1%, female 2.2%) and non tuberculosis pulmonary disease 2.6% (male 2.2%, female 3.2%). Bacteriological examination of sputum were done by sputum smear and culture. Active tuberculosis rate 1.0% (male 1.0%, female 1.17%). B.C.G. were given to 2,240 tuberculin non reactors.

References

- 1) X-ray Classification of the Japanese Society for Tuberculosis, 48, Photofluorography in Japan, April, 1960.
- 2) A Guide to the Examination of Tubercle Bacilli, a part of a Guide to the Laboratory Examination, supervised by the Japanese Ministry of Health and Welfare, Published by the Japanese Association for Public Health.
- 3) Japan anti-Tuberculosis in Japan:
Treatment of Pulmonary Tuberculosis in Japan

X-RAY CLASSIFICATION OF JAPANESE SOCIETY FOR TUBERCULOSIS

A. Character of Lesions.

O: No pathological finding

I: Far-advanced, cavitory type

The sum of the extent of disease exceeds the total area of one lung and the sum of cavities exceeds the extent of category I (see extent of disease).

II: Not—far—advanced, cavitory type

Cavitory tuberculosis other than I

III: Non—cavitory, unstable type

All or some of the lesions are unstable. No cavity exists.

IV: Non-cavitory, stable type

Stable lesions only.

V: Healed type

Healed lesions only.

The following change are added to the above-mentioned type:

H: Hilar lymph node swelling

PL: Pleurisy with effusion.

OP: Postoperative residue

B. Extent of Disease

1. Extent not reaching the horizontal line of the upper margin of the frontal end of the second rib.
2. Extent between the categories I and 3.
3. Extent wider than that of one lung.

C. Site of Disease

r: Only in right lung

l: Only in the left lung

b: Bilateral disease.

THE METHOD OF EXAMINATION FOR DRUG RESISTANCES OF TUBERCLE BACILLI CURRENTLY EMPLOYED IN JAPAN

In almost all laboratories in Japan, the drug resistance test of tubercle bacilli is carried out according to the Guidance of the Examination of Tubercle Bacilli determined by the Committee of the Laboratory Technique of the Ministry of Health and Welfare.

Order of Examination.

When staining of a smeared sputum preparation indicates microscopically the presence of tubercle bacilli, the direct method is adopted as a rule. In the case of negatives by this microscopic examination, attempts are made to isolate tubercle bacilli by culturing and test the bacilli according to the indirect method.

Techniques of Examination

a) The table below indicates the media to be used and concentrations of drugs to be tested which vary depending on the test drug.

Test drug	Medium for		Concentration ug/ml
	Direct method	Indirect method	
Streptomycin	3% Ogawa medium	1% Ogawa Medium	0, 10, 100
PAS	" "	"	0, 1, 10
INH	"	"	0, 1, 5
TB-1	1% Ogawa medium	1% Ogawa medium	0, 1, 10
Viomycin	Kirchner agar	Kirchner agar	0, 10, 100
Kanamycin	"	"	0, 10, 100

b) Preparation of media

3% Ogawa medium

Monopotassium Phosphate (KH_2PO_4) ————— 3g

Sodium glutamate ————— 1g

Distilled water ————— 100ml.

The above constituents are mixed and dissolved. To 100ml of this solution are added 6ml of 2% malachite green, 6ml of glycerine and 200ml

of fresh egg. After well mixed, the medium is distributed into sterile tubes in the amount of 5 to 7ml, slanted then coagulated and sterilized by heating at 85-90°C for one hour.

1% Ogawa medium

Monopotassium Phosphate (KH_2PO_4)	1g
Sodium glutamate	1g
Distilled water	100ml

The above are mixed to prepare the mother solution, and the subsequent procedures are the same as those for the proceeding 3% Ogawa medium.

Kirchner agar

Monopotassium Phosphate (KH_2PO_4)	4g
Bisodium Phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$)	3g
Magnesium Sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	0.6g
Sodium citrate	2.5g
Asparagine	5g
Glycerine	20g
Distilled water	1,000ml
Malachite green (0.2%)	1ml
Agar agar	15g

Procedure for the Direct Method:-

a) On 3% Ogawa medium: Sputum is added with an equal amount 6%-8% NaOH, well homogenized by stirring, and without neutralization poured into medium tubes in the amount of 0.1ml each.

b) On 1% Ogawa or Kirchner agar medium: in this case, sputum is added with a 5 to 10 times larger amount of 4% NaOH, homogenized and left at room temperature for 30 minutes. Then it is centrifuged at 2,000 rpm for 10 minutes. The sediment is given one drop of phenol red indicator and neutralized by the addition of 4% HCl. After that, seeding of each medium tube is done with 0.1ml of this material.

Procedure for the indirect method:-

Tubercle bacilli are isolated by culturing on Ogawa medium. Colonies on cultures are harvested after an incubation period of approximately 3

by pressing between a pair of filter paper, weighed aseptically into a round-bottomed flask containing crystal beads. The flask is shaken by hand for 2 to 3 minutes, then after the addition of sterile distilled water further shaken for another few minutes, and then with a calculated amount of sterile distilled water to make a suspension. For inoculation in a 1:100 or 1:1,000 dilution is made in the medium in 0.1 ml amounts, therefore the inoculum includes bacilli.

The timing of the result is done after 4 weeks' cultivation at 37°C, on the following criteria.

Complete resistance: When the control tube shows a countable colony of more than 25 (up to about 200), the highest drug concentration allows the formation of colonies whose number exceeds 75% those of the control are too numerous to count, the highest drug concentration which allows the growth of similarly confluent colonies is called complete resistance. In such a case, of course, a retest is needed to decide whether the resistance is really complete or not.

Incomplete resistance: The test medium tubes showing an obvious reduction in the colony number of less than 75% as compared with the control are regarded as being incompletely resistant.

Example:

1. Streptomycin, 10mg-complete resistance and 100mg-incomplete.				
Colony No.	Control	10ug	100ug	
	128	110	21	
2. Streptomycin, 10ug-complete resistance (equivocal) and 100ug-incomplete resistance.				
Colony No.	Control	10ug	100ug	
	++++	++++	++	

++++: Presumably more than 2,000
 +++: Presumably more than 200 and less than 500.