Vaidya et al., 2015

Volume 1 Issue 1, pp. 55-71

Year of Publication: 2015

DOI- https://dx.doi.org/10.20319/lijshls.2015.s11.5571

This paper can be cited as: Vaidya, S., Huliyalkar, V., Gohil, A., Chowdhary, A., Shah, D., & Ranjan Sengupta, S. (2015). Evaluation of Radiorespirometric Technique for Detection of Mycobacterium Tuberculosis Strains from Pulmonary Tuberculosis Patients. LIFE: International Journal of Health and Life-Sciences, 1(1), 55-71.

EVALUATION OF RADIORESPIROMETRIC TECHNIQUE FOR DETECTION OF MYCOBACTERIUM TUBERCULOSIS STRAINS FROM PULMONARY TUBERCULOSIS PATIENTS

Shashikant Vaidya
Department of Clinical Pathology, Haffkine Institute for Training, Research and Testing, Mumbai, India
shashikantvaidya@hotmail.com

Vibhavari Huliyalkar
Grant Government Medical College and Sir J.J.Hospital, Mumbai, India
vibhahuliyalkar@gmail.com

Auna Gohil
Grant GovernmentMedical College and Sir J.J. Hospital, Mumbai, India
arunagohiljj@gmail.com

Abhay Chowdhary
Grant Government Medical College and Sir J.J. Hospital, Mumbai, India
abhaychowdhary@yahoo.com

Damayanti Shah
Radiation Medicine Centre, Bhabha Atomic Research Centre, Mumbai, India

Satya Ranjan Sengupta
Haffkine Institute for Training, Research and Testing, Mumbai, India

Abstract

A simple and rapid radiorespirometric method, for early detection of tubercle bacilli from sputum samples has been developed. A biphasic liquid scintillation vial system is used for detection of $^{14}CO_2$ produced by metabolism of acetate 1-2-^{l4}C on glycerol free Lowenstein-Jensen Media (LJM). Total 207 sputum samples were tested by smear, visual growth method (VGM) and radiorespirometric method (RRM) for detection of Mycobacterium tuberculosis (M.tuberculosis) strains. Total 27.54 % sputum samples were scored positive by smear, 21.7% samples by VGM and 33.3 % samples by RRM. There is significant difference between three methods related to sensitivity, specificity, positive and negative predictive value for detection of M. tuberculosis strains. RRM was found to be most sensitive and specific method amongst three. The average detection time by RRM was 9.48 days, compared to 34.47 days by VGM. There is a correlation of number of bacilli in smear and rate of growth in RRM and VGM.

Keywords

Radiorespirometric Method, Visual Growth Method, Mycobacterium Tuberculosis

---

1. Introduction

Tuberculosis (TB) persists as a leading global cause of death. It is a major public health problem, particularly in the developing world where the prevalence of infection is reported to be as high as 40 % and the annual risk of infection is 2-4 % (Baily, 1983).

After major and sustained progress in reducing the burden of TB, marked by declines for over a century in some industrialized countries, (Stýblo et al., 1969; Vynnycky & Fine, 1997; Wilson, 1990) global TB control efforts have faced new challenges due to the HIV epidemic and the rise of drug resistance in several settings (Corbett et al., 2003; WHO, 2004).

These all mentioned factors contribute to increase the early detection of TB using easy and suitable method. The following methods aids in TB detection in simplified manner. The primary isolation of tubercle bacilli from clinical specimens by classical visual growth method (VGM) currently used for diagnosis are not so sensitive and are time consuming. VGM is still considered as the gold standard because of being 100 % specific. It is essential in situations

like drug sensitivity testing, change of drug regimen in resistant cases, epidemiological surveys, mass case-finding programs, and identification of *Mycobacterial* species and assessment of tuberculosis programs (Bhargava et al., 2001).

The replication time of tubercle bacilli cannot be reduced significantly by further improvement in culture media or culture conditions since it is genetically controlled.

Therefore the best way of achieving early detection of tubercle bacilli is by sensitive techniques that will permit their recognition after completion of only a few cycles of replication. During metabolic activity of the bacteria instead of visual growth, reduces detection time considerably. There is a need for evolving rapid, less cumbersome and more sensitive culture methods for detection of mycobacteria.

Radiorespirometry (RRM) is one such technique which has received such attention over the past decade for detection of microorganisms. A radiometric method utilizing ^{14}C labeled carbon source in biphasic vial system has been described as an efficient system for early detection of bacterial growth. Hence radioactivity detected from accumulated $^{14}CO_2$ respired from metabolically active bacteria and absorbed on alkaline absorbent paper is taken as index of growth (Buddemeyer, 1974).The biphasic vial system originally described (Ganatra et al., 1980) and further modified (Deodhar et al., 1983) was used for measurement of the respired $^{14}CO_2$. The optimization of experimental conditions were followed using Lowenstein Jensen media (LJM) and ^{14}C-labelled acetate as a substrate as indicated (Shah et al., 1984).

2. Material and Methods

2.1 Samples

Total 207 sputumsamples were collected from patients attending the Out Patient Department of Tuberculosis and Chest Diseases Department of Grant Medical College and Sir J.J. Groups of Hospitals, Mumbai. These patients were diagnosed as cases of pulmonary tuberculosis on clinical and radiological evidence.

Smears were made from these samples and stained by Ziehl- Neelsen's method. Further samples were subjected to concentration procedure by modified Petroff's method. In this method 2% Sodium hydroxide and 1% Sodium lauryl sulfate in equal quantities are used for decontamination. The sample is kept at 37°C for 30 minutes (Petroff, 1915). Digested and

neutralized samples were tested by VGM as well as RRM.

2.2 Substrate

Sodum-acetate-1-2-^{14}C (sp. act. 49.3 mCis/mM) was obtained from Isotope Division of Bhabha Atomic Research Centre in India.

2.3 Media

1.8 ml glycerol free LJM was solidified in inner metabolic vial for radiometry, by inspissation at 80°C for 1 hour in a upright position.

2.4 Ram

The biphasic vial system was prepared in the same way as described (Shah et al., 1984)**.**The inner metabolic vial containing 1.8 ml of LJM was layered with 100 µl of 1.0 µCi of *Sodium*-acetate-1-2-^{14}*C* followed by 100 µl of digested neutralized sputum sample. For radiometric test, the pH of the sediment before the inoculation on LJM slant was adjusted in the range of 6.5 to 7.5 by adding PBS (0.05 M, pH 7.0). The sputum sediments, which remained viscous after first digestion were re-digested prior to their inoculation in radiometric vials.

For positive control, a suspension of the standard strain of *M. tuberculosis* H37Rv was inoculated, while for negative control, vials were inoculated with heat killed *M. tuberculosis*H37Rv strain. These vials were incubated at 37°C for 72 hours prior to its transfer in the outer detection vial containing scintillation fluors and alkali as described above. The vial assembly was then incubated at 37°C and the $^{14}CO_2$ was monitored every alternate day, in Packard liquid scintillation counter for two weeks.

A five times rise in $^{14}CO_2$count rate above chemical blank (vials without sputum sample) was considered as criteria for the presence of the organism. The time period at which it attained this value was noted. The vials were opened aseptically and smears were made to confirm the presence of acid fast bacilli. The $^{14}CO_2$ was monitored daily for two weeks. Visual method results were scored after 6 weeks. The follow up was done every week.

3. Results

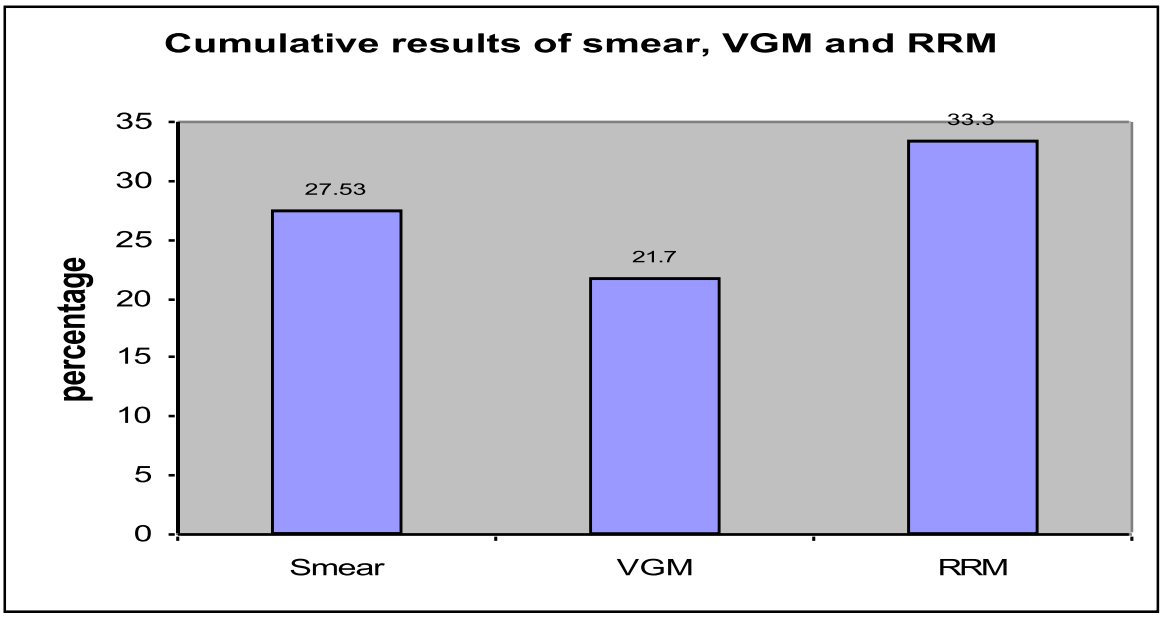

Figure 1: *Sputum positivity by smear, VGM and RRM method for M.tuberculosis*

Amongst three methods studied, RRM detected highest number of M tuberculosis strains in sputum samples (33.3%) followed by smear (27.53 %) and VGM technique (21.7 %). VGM detected least number of *M. tuberculosis* strains amongst three methods.[Figure 1]

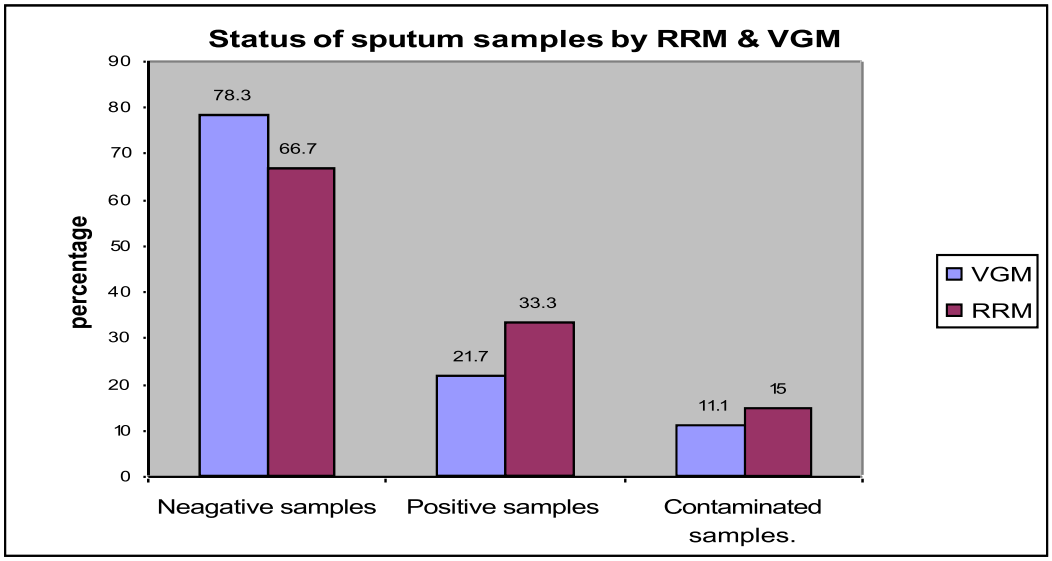

Figure 2: *Status of sputum samples by RRM and VGM technique*

Amongst two methods, RRM detected highest number of positive samples for M. tuberculosis(33.3%) and contaminated samples (15 %) compared to VGM. While, GM detected highest number of negative samples (78.3 %). [Figure 2]

Table 1: *Comparison of rate of positivity of VGM and smear for detection of M. tuberculosis in sputum samples; (n=207)*

	Positive result (VGM)	**Negative result (VGM)**	**Total (Smear)**
Positive result (Smear)	40 (19.32 %)	17 (8.21%)	57 (27.53%)
Negative Result (Smear)	05 (2.41 %)	145 (70.06%)	150 (72.47%)
Total (VGM)	45 (21.73%)	162 (78.27%)	207 (100%)

Out of 207 samples, 40(19.32 %) samples were detected positive for *M. tuberculosis* by both Smear and VGM method. While, 145 (70.06%) samples were negative by both the methods. Smear detected 17 (8.21%) samples positive which were negative by VGM, while VGM detected 5 (2.41%) samples positive which were negative by smear. VGM detected total 45 (21.73%) samples positive and smear detected 57 (27.53%) samples positive out of total 207 sputum samples. [Table.1]

Table 2: *Comparison of sensitivity, specificity, Positive Predictive Value and Negative Predictive Value of Smear and VGM method for detection of M. tuberculosis Strains in sputum samples*

Measurement	**Value**	**95% Confidence Interval**	
		Lower	**Upper**
Sensitivity	88.89%	75.93%	96.29%
Specificity	89.51%	83.72%	93.77%
Positive Predictive Value	70.18%	56.56%	81.54%
Negative Predictive Value	96.67%	92.40%	98.91%
Positive Likelihood ratio	8.471		
Negative Likelihood ratio	0.124		

Chi-square Tests	Value	Df	p-value	Difference is-
Pearson Chi-Square	104.58	1	< 0.0001	Significant
Continuity Correction	137.30	1	< 0.0001	Significant

Table 3: *Comparison of rate positivity of RRM and smear for detection of M. tuberculosis in sputum samples (n=207) of*

	Positive result (RRM)	Negative result (RRM)	Total samples (Smear)
Positive result (Smear)	55 (26.57%)	02 (0.966%)	57(27.53%)
Negative result (Smear)	14(22.70%)	136(65.70%)	150(72.47%)
Total culture (RRM)	69(33.33%)	138(66.64%)	207(100%)

Out of 207 samples, 55 (26.57%) samples were detected positive for *M. tuberculosis* by both Smear and RRM method. While, 136 (65.70%) samples were negative by both the methods. Smear detected 2 (0.966%) samples positive which were negative by RRM, while RRM detected 14 (22.70%) samples positive which were negative by smear. RRM detected total 69 (33.33%) samples positive and smear detected 57 (27.53%) samples positive out of total 207 sputum samples. [Table 3]

Table 4: *Comparison of sensitivity, specificity, Positive Predictive Value and Negative Predictive Value of Smear and RRM method for detection of M. tuberculosis Strains in sputum samples*

Measurement	Value	95% Confidence Interval		
		Lower		Upper
Sensitivity	96.49%	87.88%		99.57%
Specificity	90.67%	84.83%		94.80%
Positive Predictive Value	79.71%	68.33%		88.43%
Negative Predictive Value	98.55%	94.86%		99.82%
Positive Likelihood ratio	10.338			
Negative Likelihood ratio	0.039			
Chi-square Tests	**Value**	**Df**	**p-value**	**Difference is-**
Pearson Chi-Square	141.20	1	< 0.0001	Significant
Continuity Correction	137.30	1	< 0.0001	Significant

Table 5: *Comparison of rate of positivity of RRM and VGM for detection of M. tuberculosis in sputum samples (n=207)*

	Positive result (RRM)	**Negative result (RRM)**	**Total (VGM)**
Positive result (VGM)	30(14.5 %)	15(7.3 %)	45(21.7 %)
Negative result (VGM)	39(18.8 %)	123(59.4 %)	162(78.3 %)
Total (RRM)	69(33.3 %)	138(66.7 %)	207(100%)

Out of 207 samples, 30(14.5%) samples were detected positive for *M. tuberculosis* by both VGM and RRM method.123 (59.4%) samples were negative by both the methods. RRM detected 39 (18.8%) samples positive which were negative by VGM, while VGM detected 15 (7.3%) samples positive, which were negative by RRM. RRM detected total 69 (33.33%) samples positive and VGM detected 45 (21.7%) samples positive out of total 207 sputum samples. [Table 5]

Table 6: *Comparison of sensitivity, specificity, Positive Predictive Value and Negative Predictive Value of VGM and RRM method for detection of M. tuberculosis Strains in sputum samples*

Measurement	**Value**	**95% Confidence Interval**	
		Lower	**Upper**
Sensitivity	66.67%	51.10%	78.98%
Specificity	75.93%	68.59%	82.32%
Positive Predictive Value	43.48%	31.53%	55.95%
Negative Predictive Value	89.13%	82.72%	93.78%
Positive Likelihood ratio	2.769		
Negative Likelihood ratio	0.439		

Chi-square Tests	**Value**	**Df**	**p-value**	**Difference is-**
Pearson Chi-Square	28.750	1	< 0.0001	Significant
Continuity Correction	26.865	1	< 0.0001	Significant

Table 7: *Comparison of VGM and RRM for detection of M. tuberculosis in sputum*

Sr. No.	**Number of days**	**VGM**	**VGM**	**RRM**	**RRM**
		No. of strains evaluated	**Cumulative %**	**No. of strains evaluated**	**Cumulative %**
1	1 – 3	Not recorded	Nil	2	2.9
2	4 – 6	Not recorded	Nil	8	14.49
3	7 – 9	Nil	Nil	25	50.72
4	10 – 12	Not recorded	Nil	22	82.26
5	13 – 15	Nil	Nil	12	100
6	16 – 18	Not recorded	Nil	N. A.	N. A.
7	19 – 21	Not recorded	Nil	N. A.	N. A.
8	22 – 24	Not recorded	Nil	N. A.	N. A.
9	25 – 27	Not recorded	Nil	N. A.	N. A.
10	28 – 30	14	31.11	N. A.	N. A.
11	31-33	Not recorded	N.A.	N. A.	N. A.

12	34-36	21	46.67	N. A.	N. A.
13	37-39	Not recorded	N.A.	N. A.	N. A.
14	40-42	10	22.22	N. A.	N. A.
Total	45	100	69	100	Total
Average reporting time		34. 47 days		9.48 days	

Average reporting time of VGM is higher (34.47 days) compared to RRM (9.48 days). The difference between reporting time of two methods is statistically significant ($p>0.0001$) [Table 7]

Table 8: *Comparison of gradation of smear positivity of sputum samples & number of days required to detect the positive samples for M. tuberculosis by VGM & RRM*

	Number of days	**VGM (n = 207)**		**RRM (n = 207)**	
		No. of strains of M. tuberculosis evaluated	**No. in bracket indicates the gradation of smear positivity of VGM positive samples**	**No. of strains of M. tuberculosis evaluated**	**No. in bracket indicates the gradation of smear positivity of RRM positive samples**
1	01 – 03	Nil	Nil	2	2(3+)
2	04 – 06	Nil	Nil	8	6 (3+), 2 (2+)
3	07 – 09	Nil	Nil	22	6 (-ve), 4 (3+), 8 (2+), 4 (+ 1)
4	10 – 12	Nil	Nil	21	5 (- ve), 7 (+ 3), 7(2+), 2 (1+)
5	13 - 15	Nil	Nil	12	4 (- ve), 1 (3+), 5 (2+), 2 (1+)
6	16 – 18	02	2 (3+)	03	1(3+), 1(2+), 1 (1+)
7	19 - 21	03	3 (3+)	01	01 (-ve)
8	22 – 24	04	2 (3+), 2 (2+)	Nil	Nil
9	25 – 27	05	2 (3+) , 3 (2+)	Nil	Nil
10	28 – 30	11	1 (-ve), 3 (3+), 5 (2+), 2 (1+)	Nil	Nil
11	31-33	07	1 (-ve), 2 (3+), 2 (2+), 2 (1+)	Nil	Nil

12	34 -36	03	1 (-ve), 1 (2+), 1 (1+)	Nil	Nil
13	37-39	06	1 (-ve), 1 (3+), 2 (2+), 2 (1+)	Nil	Nil
14	40-42	04	1 (-ve), 1 (2+), 2 (1+)	Nil	Nil
Total		45	45	69	69

Key: Grade-Ve **:** No acid-fast bacilli (AFB) in 100 oil immersion fields.

1+ **:** 0–99 AFB per 100 oil immersion fields**,**

2+ **:**1–10 AFB per oil immersion field**,**

3+ **:** > 10 AFB per oil immersion field (Fisher & Kirchheimer, 1952) (Grade-Technical and operational guidelines for TB control (Chauhan& Tonsing, 2005)

4. Discussion

In the present study, RRM technique was used for detection of tubercle bacilli from the clinical specimens. The biphasic scintillation vial (BSV) was employed to monitor $^{14}CO_2$ produced from the metabolism of 14C- labeled substrate by tubercle bacilli. Initial experiments were carried out (Deodhar et al., 1983) to standardize conditions for maximum growth and hence

$^{14}CO_2$ production by tubercle bacilli.

M. tuberculosis is an obligate aerobe. The growth of *M. tuberculosis* is dependent on oxygen availability. It has been reported that as the oxygen tension decreases, the replication time of *M. tuberculosis* increases. In the complete absence of oxygen, growth ceases. The effect of aeration on growth of *M. tuberculosis* H37Rv strain has been studied in the biphasic vial system by many workers (Ganatra et al., 1980).In our radiometric system depth of media in inner vial was less than 1 cm. It is therefore plausible that free oxygen exchange could occur at this depth of media and hence similar $^{14}CO_2$ count rates could be obtained with stationary and shaking culture.

Noticeable observation is that same amount of radioactivity added, in liquid media

yielded more $^{14}CO_2$ counts than that by solid media for given substrate at any particular time. It is obvious that in the case of solid medium activity diffuses from the surface to interior of media, while in liquid it is uniformly distributed. Since the growth of organisms on solid media takes place on the surface only, total activity added is not available to the organism. It diffuses slowly from interior to surface as substrate on the surface gets metabolized.

In the case of *M. tuberculosis,* it has been reported that presence of 5-10 % CO_2 in air enhances primary growth of the organism, as seen by increase in colony counts and colony size. In fact growth of cultures with small inoculum is inhibited, when CO_2 is absorbed by alkali. To overcome these difficulties, two modifications were used which were designated by (Shah et al., 1985).Alkali content of paper was reduced to one third. Metabolic vials with inoculated sputum samples were incubated in air for three days. After three days these vials were inserted into outer detection (plastic) vial containing fluor and alkali impregnated paper.

With these two modifications it was possible to monitor $^{14}CO_2$ counts of radiometric vials up to two weeks. Above modifications significantly increased the detection rate of radiometric system. Some authors have observed that sample preparation in double –vial radiorespirometry increased counting efficiency by 58 (Coveney & Wetzel, 1984).

It was also observed by some authors that slightly modified Buddemeyer assay showed better sensitivity than ATP assay while detecting viable *Mycobacterium leprae*from clinical samples (Agrawal & Shetty, 2007). In our study, out of 207 sputum samples tested, 69 (33.3%) samples were scored positive by modified radiometric system as compared to 45 (21.7%) samples by VGM. While smear detected 57 (27.53%) positive samples. The data thus indicates that a positive case of TB missed out either, because of low sensitivity of smear and overzealous decontamination in culture, can be detected by RRM technique. Applying statistical significance test “Chi- square tests” observed difference between “RRM”, “VGM” and “smear” is statistical significant ($p < 0.0001$).

Similar findings of early detection by radiometry of M. tuberculosis from sputum samples have been reported (Middlebrook et al., 1977) using ionization chamber as $^{14}CO_2$ monitoring system and (Ganatra et al., 1980; Walawalkar et al., 2004) uses Liquid Scintillation Counter (LSC). In the studies reported earlier (Burdz et al., 2003; Middlebrook et al., 1977; Shah et al., 1985) as well as ours, visual method appears to detect few samples more than that

detected by radiometric method. The reason for this discrepancy is not known. However it is possible that occasionally organisms from sputum samples may not utilize the single ^{14}C-labeIled substrate provided. Perhaps use of mixture of ^{14}C-labeled substrate may increase the detection rate by radiometry (Ganatra et al., 1980).

In our study 138 (66.7 %) samples were found to be negative by RRM technique, while VGM recorded 162 (78.3 %) samples to be negative. 31 (14.97 %) samples were found to be contaminated by radiometric method, while 23 (11.1 %) samples by visual method. Sensitivity of radiometric system may be responsible for the higher rate of detection of contamination. The results indicated that no decontamination method is clearly superior, however a concentration of 1-2% Sodium hydroxide and an increase in the time of Sodium hydroxide-exposure of samples to 30 minutes, effectively kills contaminating bacteria without significantly affecting the viability of *M. tuberculosis (Walawalkar et al., 2004)***.**In our study Modified Petroff's method is used to decontaminate sputum samples which uses 2 % of Sodium hydroxidewith 30 minutes exposure time. The rate of positivity in our study is more by, Modified Petroff's method. This indicates high viability of tubercle bacilli after decontamination by Modified Petroff's method.

Comparison of sensitivity, specificity, Positive Predictive Value and Negative Predictive Value of Smear and VGM method for detection of *M. tuberculosis* strains are 88.89%, 89.51%, 70.18% and 96.67% respectively. Comparison of sensitivity, specificity, Positive Predictive Value and Negative Predictive Value of Smear and RRM method are 96.49%, 90.67%, 79.71% and 98.55% respectively while comparison of sensitivity, specificity, Positive Predictive Value and Negative Predictive Value of VGM and RRM method are 66%, 75.93%, 43.48% and 89.13% respectively.

Comparison of these three methods shows that, RRM is the most sensitive and specific method. In our study, average detection time by RRM technique was 9.48 days compared to 34.47 days by VGM. Similar finding of early detection of MTB in 71 sputum samples from pulmonary tuberculosis cases have been reported (Middlebrook et al., 1977). These authors used ^{14}C-palmitate as substrate and M7H12 agar with oleic acid albumin enrichment as media. $^{14}CO_2$ was monitored by ionization chamber device. While studied 84 and 25 sputum samples respectively from pulmonary tuberculosis patients (Middlebrook et al., 1977;

Shah et al., 1985; Walawalkar et al., 2004). These authors used ^{14}C-acetate as substrate and LJM as media, while $^{14}CO_2$ was monitored by LSC. They observed that RRM detected the presence of bacilli in sputum samples significantly earlier as compared to the conventional culture method. 18. 8% culture negative sputum samples were positive by RRM in our study. They have shown that *M. tuberculosis* follows arithmetic growth prior to the onset of logarithmic growth (Fisher & Kirchheimer, 1952; Tsukamura, 1990). For visible colonies it is imperative that the inoculum be large enough so as to enable to initiate the exponential growth. It is possible that if the inoculum is low, the bacteria may respire without forming visible colonies. This probably explains why culture negative samples were positive by RRM. It has been observed in the study that there is correlation of number of bacilli in smear and rate of growth in RRM and VGM. Smear 3+ and 2+ samples were detected earlier in RRM and VGM than the samples with low bacillary count that is 1+ and smear negative samples.

RRM technique, as a substitute for conventional LJM is not recommended as the latter has nearly equal sensitivity. Some researchers have concluded that liquid media and LJM must both be used. According to most, liquid media allow both faster detection of certain atypical mycobacteria and increased accuracy but are not suitable for growing tubercle bacilli (Bhargava et al., 2001)**.**

Another application of this technique will be for assessing suitability of various media as well as for studying effect of different culture conditions on growth of the organisms. Particularly for slow growing organisms like tubercle bacilli, it will serve as a very useful technique for such purposes. This technique can be used for studying metabolic activities of the organism, such as utilization of different substrates by organism. Moreover the monitoring of metabolic production of $^{14}CO_2$ by the organism in presence of given compound would help in determining its effect on the growth of organisms. Application of RRM technique for studying effects of antimycobacterial drugs on tubercle bacilli forms a basis for rapid drug susceptibility test (Bhargava et al., 2001).

5. Conclusion

In conclusion, RRM technique employing biphasic vial system is a very sensitive method that can be used for early detection of tubercle bacilli, as well as drug susceptibility testing from clinical specimens. The detection of tubercle bacilli from sputum samples was much earlier with radiometry, than with classical cultural technique. The radiometric technique requires 2-4 weeks for detection as well as drug susceptibility testing, in comparison to 6-8 weeks required by visual method (Ganatra et al., 1980; Shah et al., 1984). RRM should be used along with LJM, because of its higher sensitivity and faster detection, to get best results, if the cost and maintenance of the system can be met. Still RRM is much cheaper technique than the existing radiometric methods of detection of *M tuberculosis*. And newer detection methods based on molecular level.

References

Agrawal, V., & Shetty, V. (2007). Comparison of radiorespirometric budemeyer assay with ATP assay and mouse foot pad test in detecting viable Mycobacterium leprae from clinical samples. Indian Jr Of Med Microbiol,25(4),358-363

Baily, G. (1983). Tuberculosis control in Indiacurrent problems and possible solutions. Ind J Tub, 30, 45-56.

Bhargava, A., Jain, A., & Agrawal, S. (2001). A comparison of liquid and solid culture media with radiometric system for detection of mycobacteria in clinical specimens. Indian Journal of Tuberculosis, 48(1), 9-12.

Buddemeyer, E. U. (1974). Liquid scintillation vial for cumulative and continuous radiometric measurement of in vitro metabolism. Applied microbiology, 28(2), 177-180.

Burdz, T. V., Wolfe, J., & Kabani, A. (2003). Evaluation of sputum decontamination methods for Mycobacterium tuberculosis using viable colony counts and flow cytometry. Diagnostic microbiology and infectious disease, 47(3), 503-509.

Chauhan, L., & Tonsing, J. (2005). Revised national TB control programme in India. Tuberculosis, 85(5), 271-276.

Corbett, E. L., Watt, C. J., Walker, N., Maher, D., Williams, B. G., Raviglione, M. C., & Dye, C. (2003). The growing burden of tuberculosis: global trends and interactions with the

HIV epidemic. Archives of internal medicine, 163(9), 1009-1021.

Coveney, M. F., & Wetzel, R. G. (1984). Improved double-vial radiorespirometric technique for mineralization of 14C-labeled substrates. Applied and environmental microbiology, 47(5), 1154-1157.

Deodhar, M., Shah, D., Ganatra, R., Kale, R., Virdi, S., & Deshmukh, M. (1983). A rapid radiometric biphasic vial system for detection and testing drug susceptibility of Mycobacterium tuberculosis using 14c-labelled acetate.

Fisher, M., & Kirchheimer, W. (1952). Studies on the growth of Mycobacteria. I. The occurrence of arithmetic linear growth. American review of tuberculosis, 66(6), 758.

Ganatra, R., Buddemeyer, E., Deodhar, M., Narkar, A., Powar, D., & Shah, D. (1980). Modifications in biphasic liquid-scintillation vial system for radiometry. The Journal of Nuclear Medicine, 21(5), 480-483.

Middlebrook, G., Reggiardo, Z., & Tigertt, W. D. (1977). Automatable Radiometric Detection of Growth of Mycobacterium Tuberculosis In Selective Media 1. American Review of Respiratory Disease, 115(6), 1066-1069.

Petroff, S. (1915). A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. The Journal of experimental medicine, 21(1), 38-42.

Shah, D., Deodhar, M., Ganatra, R., Narkar, A., & Buddemeyer, E. (1984). A rapid radiometric method for detection of M. tuberculosis: Optimization of experimental conditions. International journal of nuclear medicine and biology, 11(3), 283-286.

Shah, D., Devdhar, M., Ganatra, R., Kale, P., Virdi, S., & Deshmukh, M. (1985). Modified rapid radiometric method for detection of Mycobacterium tuberculosis from sputum samples. International journal of nuclear medicine and biology, 12(4), 333-335.

Stýblo, K., Meijer, J., & Sutherland, I. (1969). Tuberculosis Surveillance Research Unit Report No. 1: the transmission of tubercle bacilli; its trend in a human population. Bulletin of the International Union Against Tuberculosis, 42, 1-104.

Tsukamura, M. (1990). Growth physiology of mycobacteria in modified Dubos liquid medium.

Microbiology and immunology, 34(12), 995-1003.

Vynnycky, E., & Fine, P. (1997). The annual risk of infection with Mycobacterium tuberculosis in England and Wales since 1901. The International Journal of Tuberculosis and Lung Disease, 1(5), 389-396.

Walawalkar, N., MGR, R., Sapatnekar, S., & Deshmukh, R. (2004). Detection of M ycobacterium tuberculosis in Sputum Samples by Radiorespirometry and DNA Amplification Technology: A Comparative Analysis. Tuberculosis: Current Research Trends, 61.

WHO. (2004). Anti-tuberculosis drug resistance in the world: third global report.

Wilson, L. G. (1990). The historical decline of tuberculosis in Europe and America: its causes and significance. Journal of the history of medicine and allied sciences, 45(3), 366-396.