# *RELIABILITY OF OCCULT BLOOD STRIPS IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS AMONG SUSPECTED INDIVIDUALS*

MOGAHID M. EL HASSAN [1,*], EMAN O. ABD ELAZIZ [1], YOUSIF M. AL OBAID [2], YASSIR A. TUMSAH [3], MISK ELYEMEN A. ABD ALLA [1] AND NAGEEB S. SAEED [3]

[1]*Department of Microbiology, College of Medical Laboratory Technology, Sudan University of Science and Technology*
[2]*Department of Microbiology, College of Medical Laboratory Science, Imam El Mahadi University*
[3]*Tuberculosis Reference Laboratory, National Health Laboratory, Federal Ministry of health, Khartoum, Sudan*
*Corresponding author: *Dr. Mogahid M. El Hassan: College of Medical Laboratory Science, Sudan University of Science and Technology. e-mail: mogahielhassan@yahoo.com*

Received 12/10/2009 Accepted 10/1/2010

***ABSTRACT: This study aimed to develop and establish a new rapid screening test for pulmonary tuberculosis among suspected individuals. A total of one hundred and seventy four (174) patients, who attended Al-Shaab Teaching Hospital and Abu-Anga Hospital for chest diseases (Sudan) during the period from September to December 2007, and had clinical signs of pulmonary tuberculosis, were examined for the presence of acid fast bacilli in their sputa using Ziehl-Neelsen (ZN) staining technique. The sputum samples were also examined for the presence of occult blood (OB) using a commercial test kit. Eighteen sputum samples, that were positive for the presence of occult blood, but negative for ZN, were analyzed using PCR technique to amplify IS 6110 conservative region in Mycobacterium tuberculosis complex. Results showed that 38 samples (21.8%) were ZN positive whereas 136 samples (78.2%) were ZN negative. For the presence of OB, 139 samples (79.9%) were positive whereas 35 (20.1%) gave negative result. Ten of 18 samples (55.6%) tested with PCR were positive. The study concluded that use of OB strips is a promising technique which may be used beside the conventional tedious techniques, but requires some modifications.***

**Keywords:** Pulmonary tuberculosis, Occult blood, Sudan.

## INTRODUCTION

Tuberculosis and other forms of mycobacteriosis are chronic granulomatous diseases affecting man, animals, birds, fishes, amphibians and reptiles. Mammalian tuberculosis is caused by five closely related species, collectively termed *Mycobacterium tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum, BCG* and *M. microti*), and other mycobacteria that may be associated with human diseases. The later ones have been given several collective names: atypical, anonymous, non-tuberculous, tuberculoid, opportunists and mycobacteria other than *Mycobacterium tuberculosis* (Grange, 1992; Mackie and McCartney 1996; Betty *et al*., 1998).

Tuberculosis is a major cause of morbidity and mortality and it has been for centuries, one of the most prevalent infectious diseases for human worldwide (Bloom and Murray, 1992). Tuberculosis was resurrected as a major public health problem worldwide. New evidence, and consequent estimates, suggest that the situation in developing countries, especially in sub-Saharan Africa, is deteriorating rapidly. It is estimated that some 7-8 million new cases and 2-3 million deaths occurs annually in the world, thus tuberculosis is still a major cause of disease and death, and its elimination will be impossible as long as poverty, overpopulation, and malnutrition

characterize large portions of the earth (Abel and Casanova, 2000; Ahkee *et al*., 1981).

In Sudan the tuberculosis epidemic is an outgrowth of the longstanding wars, which have resulted in poverty, malnutrition, and a large number of displaced populations and refugees. Destruction of health infrastructure, lack of microscopic services, displacement or lack of health personnel have also contributed to the epidemic. In 2004, tuberculosis incidence was estimated to be 220 per 100,000 people in Sudan (WHO, 2006).

Tuberculosis is primarily a disease of the lungs. It affects the apparently healthy people as well as being a serious disease of the immunocompromised, as has become particularly obvious in patients with AIDS (Mims et al., 2005). Tuberculosis is distributed worldwide, but is particularly common in Africa and Asia. Nearly 2 billion people, a third of the world's population, is infected. The prevalence of tuberculosis increases with poor social conditions, inadequate nutrition and overcrowding (Kumar and Clark, 2002).

The diagnosis of this disease depends on the isolation and identification of the causal organisms in an appropriate clinical setting. The traditional methods used in diagnosis of tuberculosis are microscopy using Ziehl-Neelsen and/or auramine fluorescent staining; this method produces rapid results but has low level of sensitivity and specificity. Another reliable method is culture on special solid and special liquid media, time required to this method is 4-8 weeks followed by the identification using biochemical tests with pure culture, and the time required for this is 2-3 weeks. Finally, DNA probes which are used to identify *Mycobacterium tuberculosis* complex are used also in the diagnosis of tuberculosis and it takes several hours (Kayser *et al*., 2005). Thus, a timely diagnosis of these conditions is needed. So, improving and establishing of new, ultra rapid diagnostic techniques are deeply needed.

## PATIENTS AND METHODS

### Sample selection criteria

The 174 patients were suspected to have pulmonary tuberculosis (PTB) and referred to the lab for the detection of acid fast bacilli (AFB) in sputum. Data were obtained with informed consented questionnaire.

### Testing for Occult Blood in Sputum Samples

A Umedic (Karmannsstrasse 57. 41061 Moenchengladbach, Germany) one step cassette style occult blood test which is a direct binding immunoassay was used to test specimens as follows, one to 5 loopful of sputum (according to the consistency of the specimen), using microbiological loop, was transfered to the sample collection device provided with the test kit and mixed well with 1-2 ml of the sample solution, then 3 to 4 drops of the mixture was squeezed on the test sample pad and result was read after 5-10 minutes. A negative result was reported if only one pink colored band appeared on the control region and no apparent band on the test region, while a positive result was reported if a distinct pink colored band appeared in the test region in addition to the control band. If no colored bands appeared in both regions, the result was reported as invalid.

## RESULTS

The aim of the presented study was to find a new rapid and reliable screening test for the diagnosis of pulmonary tuberculosis. Sputum specimens were collected from 174 subjects; each sample was tested by ZN technique and for the presence of occult blood.

Results of ZN technique for the 174 patients showed that 38 of 174 (21.8%) were ZN +ve and 136 of 174 (78.2%) were ZN –ve, whereas 139 of 174 (79.9%) were positive for the presence of occult blood in the sputum samples and 35 of 174 (20.1%) gave –ve results (Table 1). Figure 1 shows the positive (A) and negative (B) results as seen on the ICT device.

Eighteen sputum samples, which were ZN –ve and OB +ve at the same time, were analyzed using PCR and showed that 10 of 18 (55.6 %) were positive for IS6110 sequence and the rest was –ve as shown in table (1) and figure (2).

*Table 1. Cross tabulation between results of Ziehl-Neelsen and Sputum Occult Blood test*

| ***Total*** | ***Sputum Occult Blood*** | | |
|---|---|---|---|
| | Negative | Positive | |
| ***36*** | *4* | *32* | ZN positive |
| ***138*** | *31* | *107* | ZN negative |

| | | | |
|---|---|---|---|
| **174** | *35* | *139* | Total |

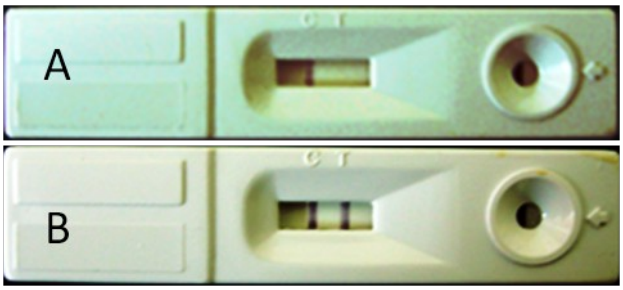


*Figure 1. Occult blood strips in the diagnosis of pulmonary tuberculosis: strip (A) shows negative result; strip (B) shows positive result.*

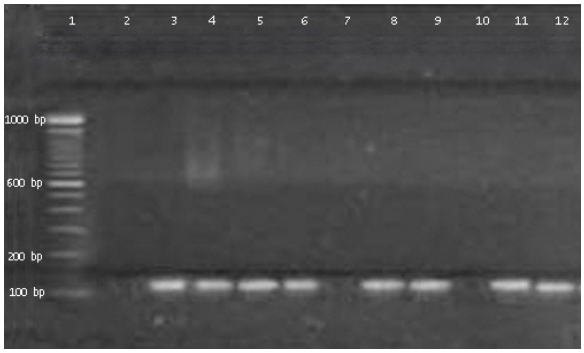


*Figure 2. Ethidium-bromide stained gel containing amplicons of IS6110 produced by PCR; Lane 1 is MW marker, lane 2 is control -ve, lanes 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 show different patients' samples 32*

## DISCUSSION

Tuberculosis is one of the leading infectious diseases in the world and is responsible for more than 2 million deaths and 8 million new cases annually (Soini and Musser, 2001). Because of the slow growth rate of the causative agent *Mycobacterium tuberculosis*, isolation, identification, and drug susceptibility testing of this organism and other clinically important mycobacteria can take several weeks or longer. During the past several years, many molecular and serological methods have been developed for direct detection, species identification, and drug susceptibility testing of mycobacteria. These methods can potentially reduce the diagnostic time from weeks to days (Soini and Musser, 2001).

The aim of the present study was to use the OB strips and compare them with polymerase chain reaction (PCR), as a rapid tool for the diagnosis of pulmonary tuberculosis from direct sputum.

Among the total of 174 specimens studied, smears were positive only in 38 of 174 (21.8%) samples, this may due to the low sensitivity of ZN stain to detect AFB which need more than 500 bacilli/ml (Sajjad, 2003). This finding is also supported by (Kavita, 2006) who found that the sensitivity of smear was less than 50%. In comparison, OB was positive in 139 of 174 (79.9%), this determined the high sensitivity of OB to detect tuberculosis.

Ten of the 18 (55.6 %) samples which were smear negative and OB positive gave positive results for IS6110 with band equal in size to 123 bp suggesting that the PCR assay is more sensitive than the direct smear or culture by detection of non-viable and/or fewer viable organisms. Similar remarks were listed by Aroma, (2007) who found that PCR showed the highest sensitivity when compared to other tests.

Eight OB-positive samples were PCR negative. This may indicate the presence of PCR inhibitors in these samples, similar remarks were reported before by Maher, (1996), or may be due to other factors which may induce bleeding in buccal cavity, thus, false positive results for OB strips may be registered.

From the data obtained in this study, the following can be concluded:

1. The percentage of pulmonary tuberculosis among suspected subjects referred to the lab for the detection of AFB in sputum was 21.8%. This low sensitivity is significant enough to search for a substitutionally routine diagnostic test for pulmonary tuberculosis.
2. Results are encouraging for the development of a new rapid screening test for pulmonary tuberculosis.
3. Polymerase chain reaction is recommended for routine diagnosis as it gave more rapid and sensitive results in this study compared to the classical ZN staining.

**ACKNOWLEDGEMENTS:** Authors are grateful to Mr. Husam Osman in the Institute of Endemic Diseases, University of Khartoum for his help in the PCR. This study was sponsored by Sudan University of Science and Technology and Tuberculosis Reference Laboratory, National Health Laboratory.

## REFERENCES

Abel, L., and Casanova, J. (2000). Genetic predisposition to clinical tuberculosis: bridging the gap simple and complex inheritance. *Am. J. Human. Genet.* 67: 274-277

Ahkee, S., Srinath, L., Huang, A.K., and Ramirez, J.A . (1981) .Isolation and geographic distribution of *Mycobacterium* other than *M. tuberculosis* in British Columbia, *Cand. Med. Assoc. Journal*.133 (6): 573-576

Aroma, O., and Aruna A. (2007) Comparision of the conventional diagnostic techniques, BACTEC and PCR, *Jk Science.*. 9 (4): 123-30.

Betty, A.F., Daniel, F.S., and Alice, S.W. (1998). Diagnostic Microbiology, 10th Ed. Mosby, London. Pp. 715-781.

Bloom, B.R., Murray, J. L. (1992). Tuberculosis: Commentary on a re-emergent killer. *Science. J.* 257: 1055-1064.

Grange, J. M. (1992). Medical microbiology-A guide to microbial infection. Edited by Greenwood, D., Slack, R. C. B, and Peuther, J. F. 14th edition. Churchle living stone, New York, USA. Pp. 241-252.

Mackie and McCartney (1996). Practical medical microbiology 14th.

edition. Churchle Living Stone. New York, USA. Pp. 329-337.

Maher, M. Glennon, M., Martinazzo, G. Turchetti, E., Marcolini, S., Smith, T. and Michael T.(1996) Evaluation of a Novel PCR-Based Diagnostic Assay for Detection of Mycobacterium tuberculosis in Sputum Samples*., Journal of Clinical Microbiology*. 9: 2307–2308.

Mims, C., Dockrell, H. , Goering, R., Oitt, I., Wakelin, D. and Zukerman, M. (2005). Medical Microbiology 3rd Ed. Mosby, London, Pp 232-233.

Kumar,P. And Clark, M. (2002). Clinical Medicine 5th Ed.WB Saunders, London, Pp 298 – 302.

Kayser, F., Bienz, K., Eckert, J. and Zinkernagle, R. (2005). Medical Microbiology, Germany, Pp 262-269.

Sajjad, I. Rizwan, I. Mumtaz Khan, M. Hussain, I. Ajaz Akhtar, A. And Shabbir, I. (2003) Comparison of Two Conventional Techniques used for the Diagnosis of Tuberculosis Cases *international journal of agriculture.:* 05–4:545–547.

Soini H. and Musser J.;(2001) Molecular Diagnosis of Mycobacteria; *Clinical Chemistry* 47:5809–814.

World Health Organization (WHO), (2006). Treating TB in Southern Sudan. Global tuberculosis control manual.