

## Assessment of Newer Commercial Serological (ICT) Test For the Diagnosis of Active Pulmonary Tuberculosis in TB Endemic Areas

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**Abstract:** We assessed the efficacy of the diagnostic serological test (CTK Biotech Inc and ACON Laboratories, Inc, USA) for the detection of *Mycobacterium tuberculosis* in TB endemic area of Quetta Pakistan. Among 223 suspected PTB respondents, forty patients were declared as having tuberculosis on the basis of *Mycobacterium tuberculosis* positive culture being the Gold standard. IgG/IgA testing in a high prevalence area may not be entirely useful. We were more interested in the presence of IgM antibodies based on which patients were classified as positive. The sensitivity of the serological test was very poor than that of sputum culture (70% versus IgM 0%, IgG 55%, IgA 15%).

**Key words:** Antibodies, Efficacy, Endemic, Pulmonary TB, Serological test

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### I. Introduction

Tuberculosis (TB) remains a major health problem. In estimation about one third of the world population is infected with *Mycobacterium tuberculosis* (1, 2, 3). The incidence of tuberculosis in developing countries has risen up to 10 million each year, with at least 3 million deaths (4, 5, 6). It is one of the leading causes of mortality among infectious diseases worldwide, 95% of TB cases and 98% of TB deaths occur in developing countries (7, 8, 9, 10, 11). Once a person infected, active disease develops in about 10% of cases, usually within 1-2 years after exposure. Remaining individuals enter in to a state of latency (Latent tuberculosis infection LTB), which can reactivate at a later stage, particularly if the individual becomes immuno-compromised (13). South East Asia seems to be most affected, that is 44% of its population is infected with *Mycobacterium Tuberculosis* among the developing countries (1, 2).

As majority of the tuberculosis patients occupy in low and middle socioeconomic countries (12), where the identification of tuberculosis usually depends on the recognition of tuberculosis of acid fast bacilli (AFB) on sputum smear using a routine bright field microscopy. Sputum smear microscopy is still the only rapid, technically simple, inexpensive and easily available test for the conventional diagnosis of tuberculosis in most developing countries of the world. However its sensitivity even in good microscopic centers for pulmonary tuberculosis (PTB), is only about 60 – 70% with reference to sputum culture being used as Gold standard (21).

The specificity of the AFB microscopy in TB endemic areas is high, but the sensitivity differs among laboratories (ranging 20-80%). While the sensitivity is deficient for paucibacillary cases i.e. pediatric, HIV associated tuberculosis and having and/or made up of few bacilli. This lack of sensitivity of the sole diagnostic test, delays in diagnostic results in most region of the world, enabling the disease to progress and become severe and increase the potential for transmission of mycobacterium tuberculosis(17).

According to the study of Weir and Thornton (1985) the sensitivity of the culture in the diagnosis of *Mycobacterium tuberculosis* is better 71% (20). But due to significant delay of diagnostic procedure (Average two months) (18), causes a remarkable increase in morbidity or mortality. Methods based on amplification of mycobacterial DNA technique are expensive and qualified and expert staff is needed and the specimen for analysis must be taken by invasive procedures; their sensitivity and specificity is not absolute(19).

Given the infectious nature of pulmonary tuberculosis fast, simple, accurate and inexpensive diagnostic tools for tuberculosis are needed to assure the appropriate care of patients and to modify Global TB epidemic control (15). Immune based tests are seem likely to improve case detection, as some of the test formats for example, immuno-chromatographic (ICT) test are suitable for resource limited areas. The most common of this test rely on detection of the humeral (antibody) immune response to *Mycobacterium tuberculosis*.

The objective of our study was to assess the efficacy, sensitivity and specificity of the newer serological testing by chromatographic method with the Gold standard of sputum culture.

## II. Materials And Methods

### 2.1 Study site & population

We conducted a cross sectional study at Fatima Jinnah General & Chest Hospital Quetta, Pakistan in 2011-12. It serves as the principal tertiary care hospital and the Provincial Referral Laboratory (PRL) of TB for Baluchistan Province. The hospital provides indoor and outdoor medical services, including care and treatment for tuberculosis and general patients.

We assessed the efficacy of the diagnostic serological kit of the CTK Biotech Inc and ACON Laboratories Inc, a novel and commercially available serological test for the detection of *Mycobacterium tuberculosis*. The study participants were recruited from the indoor and out door department of the hospital.

Total of 223 patients were included in the study suspected as PTB. Sputum culture used as Gold standard in the study to assess the diagnostic performance of the onsite TB IgG/IgM and IgA Rapid test-cassette (CTK Biotech Inc and ACON Laboratories, Inc USA) a commercially available serological test for the detection of PTB by evaluation of IgM, IgG and IgA antibodies activity against *Mycobacterium tuberculosis*. Patients were confirmed as having PTB disease on the basis of positive sputum culture for *Mycobacterium tuberculosis*.

### 2.2 Laboratory Procedure

Early morning sputum samples were collected from all suspected patients in wide mouth translucent plastic containers of volume 50 ml, incubated for AFB culture on Lowenstein Jensen (L. J medium) Media.

Five ml of whole blood also collected at the same time from the participants for serological testing. The test utilizes the principal of immunochromatography, is a sandwich lateral flow chromatographic immunoassay for the detection and differentiation of IgM anti-*Mycobacterium tuberculosis* (MTB) and IgG anti-*Mycobacterium tuberculosis* in human serum or plasma. The test band T1 is precoated with monoclonal anti human IgM for the detection of IgM anti MTB, and the test band T2 band is precoated with reagents for the detection of IgG anti MTB and the Control band C is pre-coated with goat anti rabbit IgG. When the test sample is dispensed in the sample well, the sample migrates by capillary action. If IgM anti MTB is present in the specimen, will bind with the MTB conjugate. The immunocomplex is then captured on the membrane by the precoated anti human IgM antibody, forming a burgundy colored T1 band, indicating MTB IgM positive result.

IgG anti MTB if present in the specimen, the immunocomplex is then captured by the precoated reagents on the membrane, forming a burgundy colored T2 band indicating a MTB IgG positive test result.

The test device for the detection of IgA anti MTB is a qualitative, solid phase, two site sandwich immunoassay for the detection of anti TB antibodies. The membrane is precoated with TB recombinant antigen on the test line region of the device. If the anti TB antibodies present in specimen react with the particles coated with TB recombinant antigen. The mixture migrates up word and reacts with TB recombinant antigen on the membrane and generates a colored line.

The control band exhibit a burgundy color of the immunocomplex of goat anti rabbit IgG/rabbit IgG gold conjugate regardless of the color development on any of the T bands.

## III. Results

### 3.1 Basic Sociodemographic Profile

Total of 223 patients consented to participate in the study. The basic Sociodemographic characteristics of the patients were asked in a questioner (TABLE II). The mean age of the respondents was 39 years (range 18-60). 94 (42.15%) of the patients were married, with 59 (26.45%) of the patients having weight 50-60 (Kg) while 164 (73.54) patients were weighted over 60 kg. More than half 160 (71.74%) were Illiterate, and literacy rate was 28.25% with no formal education was reported. 44 (19.73%) were interviewed as unemployed, 39 (17.48%) were employed, and the number of patients working on daily wages were 140 (62.78%). A BCG Vaccination scare was evident only in 08 cases (3.58%) and non-vaccinated were 215 (96.41%). Fifty two (23.31%) patients were currently non smokers, 49 (21.97%) were reported as smoker of 1-5 cigrates /day, 25 (11.21%) smoked 6-10 cigrates /day and 97 (43.49%) smoked > 10 cigarettes /day. 04 (1.79%) cases were reported with the family history of tuberculosis, while others 219 (98.20%) cases were free from the previous family history of tuberculosis.

### 3.2 Serological testing and AFB culture

The numbers of positive cultures samples were 40 and the sample noted to be negative were 183. Twelve samples were contaminated and were excluded from final analysis. The serological testing was done for IgG (sensitivity 94.6%, specificity 97.6%), IgM (Sensitivity 93.3%, specificity 97%) as well as for IgA (sensitivity 83.0%, specificity 98.9%, and accuracy 95.6%) antibodies. No patients found tested positive for IgM antibodies (Table I). Twenty two patients tested were true positive for IgG and 06 for IgA anti TB antibodies. IgG/IgA testing in a high prevalence area may not be entirely useful. We were more interested in the presence of

IgM antibodies based on which patients were classified as positive. Four numbers of cases were false positive for IgM, 39 for IgG and 10 for IgA. The sensitivity found for IgM 0%, IgG 55% and IgA 15%.

Result of the study as compared to the gold standard gave no positive patients for IgM (sensitivity 0%) TABLE III. The specificity, however, is noted to be 97.8% for IgM, 78.6% for IgG and 94.5% for IgA % i.e. it was able to identify correctly the patients who were negative for the disease. True negative cases for IgM were 179, IgG 144 and for IgA 173.

#### **IV. Discussion**

For the diagnosis of TB disease a criteria with clinical signs and symptoms of continuous cough, fever, loss of weight, loss appetite, and night sweetening along with the lab investigation of sputum for AFB and culture, chest x-rays and erythrocytes sedimentation rate are usually made.

The primary clinical suspicion and radiographic findings with subsequent laboratory confirmation by sputum smear and culture examination were the traditional methods in the diagnosis of tuberculosis (22, 23). However these methods either lack of sensitivity or time consuming particularly not suitable for patients, who are unable to produce adequate sample or/and smear negative. A rapid immuno chromatographic test (ICT) IgG, IgM and IgA were developed to alleviate these obstacles.

Serological testing promises to provide quick and an effective method in diagnosing tuberculosis. TB serodiagnosis is simple, inexpensive, and relatively non invasive procedure. It provides timely diagnosis, early initiation of therapy and limits further spread of infection. An IgM positive result indicates for fresh M.TB infection, while as IgG positive response suggests a previous or chronic infection.

To avoid false negative result due to undetectable level of AFB in the smear (paucibacillary), or poor quality of specimen collection, staining and microscopy, culture technique for AFB used as a Gold standard for the diagnosis of tuberculosis with its relative specificity of 99% and sensitivity around 80%.

In a study of Banu *et al.*, 2010 identified considerable number of smear negative, culture positive TB cases, demonstrates that sputum culture was useful diagnostic tool. (16)

In a reviewed data it is found that the serological ICT test was not sufficiently predictive for use as routine diagnostic aid for Pulmonary and/or extra pulmonary tuberculosis in TB endemic areas. The test performance was universally poor and evaluated inconsistent and imprecise estimates of sensitivities and is not enough to replace AFB smear microscopy or culture (21, 25, 26, 27, 28, 28, 29, 30, 31, 32). Despite of an acceptable specificity our study result also showed very poor performance with low sensitivity 0%, 55%, 15% for IgM, IgG and IgA respectively.

Overcrowding is one of the reasons to Dexter the transmission of TB infection (24).The main reservoirs for mycobacterium tuberculosis is infected humans. Most infections with Tuberculosis are caused by inhaling cough droplets or dust particles containing tubercle bacilli. This high prevalence of tuberculosis in patients may be related to overcrowding, closed living conditions, longer exposure to TB infected area, smoking, generally low socioeconomic status, poor nutrition and poor health of the patients.

High proportion of the patients were poorly educated and socioeconomically disadvantaged. Those with no education were more likely to have TB infection. The study revealed 160 (71.74%) patients were illiterate and 63 (28.25%) had some form of education. A formal education is needed about the disease, transmission and precautions of tuberculosis.

The research also highlights the patients, who were married and were exposed to infection; they may easily transfer the infection to their family members and to the society. Sharing of the utensils, towels and blankets etc was also noted to be higher in people who were tested positive. Poverty was stated as a major reason for acquiring the infection or disease. A man not employed always is malnourished and over worked for their survival always weakened the immune system and cannot received treatment for the reason of poverty.

In conclusion this study indicates the CTK Biotech Inc and ACON Laboratories, Inc USA MTB tests performed poorly as compared to sputum culture in the detection of PTB. This novel serological test lacks the sensitivity and specificity in TB endemic areas to replace the sputum smear and culture for the diagnosis of tuberculosis in our population.

Based on previous studies, IgG testing in a high prevalence area may not be entirely useful. We were more interested in the presence of IgM antibodies based on which patients were classified as positive. Further improvement is needed before this test could be useful in our settings. Delayed diagnosis and substandard treatment are common in our settings, resulting in prolonged transmission.

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#### **Key Messages:**

- Delay in the detection and treatment of TB cases must be minimized to reduce further transmission of infection.
- Measure to improve living condition should be implemented to reduce transmission of tuberculosis.

- Arrangement of Awareness program to improve ventilation, sanitation and overall living condition.
- Effective tuberculosis control need more funds and improves coordination between Government ministries and Non-Government agencies involved.

### References

- [1]. C Dye, P. S. Scheele, V. Dolin, Pathania, and MC. Raviglion. Global burden of tuberculosis estimated, prevalence and mortality by country. *JAMA*, 282 (7), 1999, 677-686.
- [2]. S Akhtar, F. White, R. Hasan, S. Rozi, M. Younus, F. Ahmed, S. Husain, and BS. Khan. Hyper endemic pulmonary tuberculosis in peri-urban areas of Karachi, Pakistan. *BMC Public Health*, 7, 2007, 70.
- [3]. World Health Organization (WHO), 2009. What is TB? How does it spread? Stop TB Department
- [4]. W Lavenson, and E. Jawetz, Mycobacteria. In LANGE medical book\_(ed.), examination & board review, 6<sup>th</sup> edition. (Medical Microbiology and Immunology 2000) p. 135-141
- [5]. M Cheesbrough, Mycobacterium tuberculosis, 7.18.28, (In district laboratory practice in tropical countries 2000) 207-212
- [6]. Herchline, T., JK. Amorosa. 2010. Tuberculosis. eMedicine Infectious Diseases. Available at [www.papercamp.com/essay/18971/Communicable-Diseases](http://www.papercamp.com/essay/18971/Communicable-Diseases).
- [7]. PM Small, Tuberculosis Research: Balancing the Portfolio. *JAMA*, 276(18), 1996, 1512-13.
- [8]. MC Raviglione, C. Dye, S. Schmidt, and A. Kochi. Assessment of worldwide tuberculosis Controls. *Lancet*. 350, 1997, 624-29.
- [9]. Y Ahmed, P. Mwaba, C. Chintu, JM. Grange, A. Ustianowski, and A. Zulma. A study of maternal mortality at the university teaching Hospital, Lusaka, Zambia: the emergence of tuberculosis as a major non-obstetric cause of maternal cause deaths. *Int J Tuberc Lung Dis*. 3, 1999, 675-80.
- [10]. R Rajeshwari, R. Balasubramanian, M. Muniyandi, S. Geetharamani, X. Thresa, and P. Venkatesan. Socio-economic impact of tuberculosis on patients and family in india. *Int J Tuberc Lung Dis*. 3, 1999, 869-77.
- [11]. Hussain, H., S. Akhtar, and D. Nanan. Prevalence of and risk factors associated with Mycobacterium tuberculosis infection in prisoners, North West Frontier province, Pakistan. *Int J Epidemiol*. 32, 2003, 794-9.
- [12]. C Dye, CJ. Watt, DM. Bleed, SM. Hosseini, and MC. Raviglione. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence and deaths globally. *JAMA*. 293, 2005, 2767-2775.
- [13]. J Dinnes, J. Deeks, H. Kunst, A. Gibson, E. Cummins, N. Waugh, F. Drobniewski and A. Lalvani. A systemic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technology Assessment*. 11, 2007, 3.
- [14]. N Bam, and R. Karn. IgM and IgG Antibodies in Tuberculosis. *Journal of Institute of Medicine*. 31, 2009, 34-40.
- [15]. MA Garbati, H. Yusuph, MB. Kagu, and AE. Moses. Diagnosis of pulmonary tuberculosis: Utility of serology and Mantoux Reaction in a Resource limited setting. *The internet journal of infectious Disease*. 8, 2010, 1.
- [16]. S Banu, A. Hossain, MK. Mafij Uddin, M. Reaj Uddin, T. Ahmed, R. Khatun, AM. Mahmud, KA. Hyder, AB. Lutfor, S. Karim, K. Zaman, AI. Khan, PC. Barua, and SP. Luby. Pulmonary Tuberculosis and Drug Resistance in Dhaka Central Jail, the Largest Prison in Bangladesh. *Public Library of Science (PLoS ONE)*. 5, 2010, 1-5.
- [17]. MA Behr, SA. Warren, H. Salamon, PC. Hopewell, and A. Ponce de Leon. Transmission of Mycobacterium tuberculosis from patients smear negative for acid fast bacilli. *Lancet*. 353, 1999, 444-449.
- [18]. MR Weir, and GF. Thornton. Extra-pulmonary tuberculosis: experience of a community hospital and review of the literature. *Am J Med*. 79, 1985, 467-78.
- [19]. N Manjunath, P. Shankar, and L. Rajan. Evaluation of polymerase chain reaction for the diagnosis of tuberculosis. *Tubercle*. 72, 1991, 21-7.
- [20]. M Alifano, RD. Pascalis, M. Sofia, S. Faraone, MD. Pezzo, and I. Covelli. Detection of IgG and IgA against the mycobacterial antigen A60 in patients with extrapulmonary tuberculosis. *Thorax*. 53, 1998, 377-380.
- [21]. E Kassa-kelembho, E. Kassa, G. Zandanga, YB. Service, A. Ignaleamoko, and A. Talarmin. Poor performance of a novel serological test for diagnosis of pulmonary tuberculosis in Bangui. *Central African Republic Clinical and vaccine immunology*. 13, 2006, 702-703
- [22]. T.L Merlin, D.W Gibson, D.H Connor, Tuberculosis, In Rubein E and Farber JL (ed.), pathology, 2<sup>nd</sup> edition. (J.B. Lippincott Company 1994) 400-404.
- [23]. T.M Daniel, Immunodiagnosis of tuberculosis. In W.N. Rom, and S. Garay (ed.), Tuberculosis. Letter, Brown & Co., Bostan, Mass, 1996, p. 223-231.
- [24]. R Macintyre, N. Kendic, I. Kummer, S. Birage, and MH. Neil. Impact of tuberculosis control measures and crowding on the incidence of tuberculosis in Maryland Prisons. *Clin Infect Dis*. 24, 1997, 1060-67.
- [25]. K Ghadiri, B. Izadi, M. Afsharian, S. Vaziri, M. Rezaei, and S. Namdari. Diagnostic value of serological tests (IgA, IgG, IgM) against A-60 antigen in tuberculosis. *Iranian Journal of Clinical Infectious Diseases*. 3, 2008, 205-208.
- [26]. C Gounder, FC. De-Queiroz Mello, MB. Conde, WR. Bishai, AL. Kritski, RE. Chaisson, and SE. Dorman. 2002. Field evaluation of a rapid immuno-chromatographic test for tuberculosis. *Journal of clinical microbiology*. 40, 2002, 1989-1993.
- [27]. GC Ireton, R. Greenwald, H. Liang, J. Esfandiari, KP. Lyashchenko, and SG. Reed. Identification of *Mycobacterium tuberculosis* antigens of high sero-diagnostic value. *Clinical and vaccine Immunology*. 17, 2010, 1539-1547
- [28]. G Hawthorne, DR. Thickett, and AM. Wood. Serological testing for tuberculosis should be banned - W.H.O. *J Postgrad Med Inst*. 26, 2012, 117-9.
- [29]. KR Steingart, LL. Flores, N. Dendukuri, I. Schiller, S. Laa1, A. Ramsay, PC. Hopewell, and M. Pai. Commercial Serological Tests for the Diagnosis of Active Pulmonary and Extra-pulmonary Tuberculosis: An Updated Systematic Review and Meta- analysis. *PLoS Med*. 8, 2011, 8.
- [30]. KR Steingart, M. Henry, S. Laal, PC. Hopewell, A Ramsay, D. Menzies, J. Cunningham, K. Weldingh, and M. Pai. A systemic review of commercial serological antibody detection tests for the diagnosis of extra pulmonary tuberculosis. *Thorax*. 62, 2007, 911-918.
- [31]. J Dinnes, J. Deeks, H. Kunst, A. Gibson, E. Cummins, N. Waugh, F. Drobniewski, and A. Lalvani. A systemic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technology Assessment*. 11, 2007, 3.
- [32]. Steingart. KR., M. Henry, S. Laal, PC. Hopewell, A. Ramsay, D. Menzies, J. Cunningham, K. Weldingh, and M. Pai. 2007. Commercial serological antibodies detection tests for the diagnosis of pulmonary tuberculosis: A systematic review. *PLoS Medicine*. 4, 2007, 6.

Table I. Performance of CTK Biotech Inc and ACON Laboratories, Inc USA MTB tests with culture-confirmed PTB patients

	Total	True pos	False pos	True Neg	False Neg	Sensitivity	Specificity	PPV	NPV	Accuracy
IgM Ab	223	0	4	179	40	0%	97.8%	0%	81.73%	0.80
IgG Ab	223	22	39	144	18	55%	78.6%	36.0%	88.8%	0.744
IgA Ab	223	6	10	173	34	15%	94.5%	37.5%	83.57%	0.80

Table II: Sociodemographic characteristics of patients tested for Tuberculosis

S#	Variables	Cases recorded	Percentage (%)
01	Age		
	18-30	128	57.39
	31-49	27	12.10
	41-50	28	12.55
	> 50	40	17.93
02	Weight		
	50-60	59	26.45
	>60	164	73.54
03	Education		
	Illiterate	160	71.74
	Literate	63	28.25
04	Previous occupation		
	Employed	39	17.48
	Unemployed	44	19.73
	Daily wages	140	62.78
05	Current smoking status		
	Non smoker	52	23.31
	1-5 cigarettes/day	49	21.97
	6-10 cigarettes/day	25	11.21
	>10 cigarettes/day	97	43.49
06	Married		
	Yes	94	42.15
	No	129	57.85
07	Family history tuberculosis		
	Yes	4	17.93
	No	219	98.20
08	H/O BCG vaccination		
	Yes	8	3.58
	No	215	96.41

Table: III Comparison of serological IgM antibodies to the Gold standard

Serological testing IgM	Culture positive	Culture negative
Positive	00	4
Negative	40	179
Total	40	183