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Editorial

Commercial serological tests for the diagnosis of active tuberculosis in India: Time for introspection

India accounts for one fifth of the global TB burden - a total of 9.2 million new cases and 1.7 million deaths every year[1,2]. There are two main components of effective TB control programme: the rapid and sensitive diagnosis of the disease and containment of its spread to the uninfected population. The sputum smear microscopy, still a backbone of TB diagnosis, is less sensitive and can miss half of the pulmonary TB cases. The conventional culture method which uses egg based medium (Lowenstein-Jensen, L-J) is time consuming and lacks desirable detection level. Liquid automated culture methods, such as Bactec-MGIT (BD, USA) and MB-Bact (Biomerieux, France) have highly improved detection rates, comparatively much faster (median detection time 10-24 days) but are costly requiring air handling and infection control measures[1]. Molecular methods such as polymerase chain reaction (PCR) and its modified versions have come as boon in the diagnosis of tuberculosis. Despite the limitation of detecting dead bacilli, PCR is rapid (report can be made available on the same day) and precise, depending on the gene sequences targeted and protocols used. These methods can be used on various clinical samples such as sputum, tissue biopsies, cerebrospinal and other body fluids, lymph nodes and other tissue aspirates, urine, faeces, *etc*[1].

In resource limited settings like India, tuberculosis detection rates are suboptimal mainly because the diagnosis is usually made by less sensitive tools such as sputum microscopy and chest X-rays. The poor detection rates lead to mismanagement of infectious cases and possibility of drug resistance development. However, non-availability of affordable, rapid and precise diagnostic tools at peripheral level have led to mushrooming of commercial serological tests[2-4]. Extensive reviews and meta-analyses have concluded that the presently available antibody detection based serological tests are no good for the diagnosis of tuberculosis while helpful for other diseases. In view of this, the WHO has recently issued an advisory[2]. This editorial provides an academic overview of the issue.

Commercially available serological tests

A search showed more than 73 manufactures of TB serological test kits[4,5]. Most of these (60) are prepared in rapid test (lateral flow or flow through) format as compared to microwell ELISA (only 13). The Table shows widely publicized manufactures and trade names of these test kits.

This non-exhaustive list clearly indicates that China has over taken all other countries for rapid test marketing. There are at least 24 TB rapid test kit manufactures from China alone. However, only two manufacturers market microwell ELISA. China is followed by USA, with 15 rapid test kits and one ELISA kit, probably understanding that rapid test market is more lucrative. India has eight rapid test manufacturers and four ELISA manufacturers (Table).

In 2008, the WHO started a kit evaluation programme for various infectious diseases including HIV, hepatitis and malaria and TB rapid test kits[5]. Only 19 firms responded to WHO evaluation programme and submitted their kits (Serial no. 31-49) and six firms refused to provide their kits for WHO evaluation (serial no. 50-55), presumably because these firms do not market directly. No microwell ELISA kit was evaluated by the WHO under this programme. However, recently several scientists have analysed both rapid and ELISA formats[6,7]. The results of these analyses are scary. Beside these listed commercial tests, there are a number of in-house assays claiming high sensitivity and specificity[8]. Most of these tests are developed in unaccredited basic biology laboratories with little cross-validation by third parties.

Table. List of commercially available TB serology kits and their manufacturers

S. No.	Name of the test	Manufactured by	Country
Rapid test formats:			
1	One step TB test	Hangzhou Clongene Biotech Co. Ltd.	China
2	TB Rapid diagnostic test	NewScen Coast Bio-Pharmaceutical Co. Ltd.	China
3	TB serum test and TB whole blood test	AccuBioTech Co. Ltd.	China
4	One Step Easy Use Accurate TB Rapid Test	Inter-Chemical Ltd.	China
5	TB Test Cassette	World of Health Biotech Co. Ltd.	China
6	Diagnostic TB strip test	Beijing Easysweet Biomedicine Science And Technology Co. Ltd.	China
7	TB Test kits	Shanghai Huaguan Biochip Co. Ltd.	China
8	One Step TB (recombinant) Rapid Tests	Orient Innovation E.I. (Beijing) Co. Ltd.	China
9	Anti-tuberculosis test	Beijing Kangruibo Imp & Exp Co. Ltd.	China
10	TB test	Core Technology Co. Ltd.	China
11	TB test/ Tuberculosis test	Nanjing Kim-Lehman Medical Instrument Company Ltd.	China
12	TB rapid Test	Hangzhou Clongene Biotech Co. Ltd.	China
13	One-Step accurate TB Rapid Cassette Test	Inter-Chemical Ltd.	China
14	TB tuberculosis rapid test kits (cassette)	Bioneovan Co. Ltd.	China
15	iCARE TB Rapid Screen Diagnostic Test Kit	JAL Innovation (s) Pte Ltd.	Singapore
16	TB test Cassette	Nantong Egens Biotechnology Co. Ltd.	China
17	TB Rapid test Cassette	Zhejiang Orient Gene Biotech Co. Ltd.	China
18	Rapid TB test	Ningbo Qihao International Trade Co. Ltd.	China
19	M.TB Ab Rapid Test Kit	Shanghai Eugene Biotech Co. Ltd.	China
20	One Step Anti-TB (*M.Tuberculosis*)	K & K-Chemospecialty Ltd.	Taiwan
21	TB rapid test	Debao Biotechnological Co. Ltd.	Canada
22	One Step Tuberculosis (TB) Antibody rapid Test kit	Wkea Med Supplies Corp.	China
23	One Step *Mycobacterium tuberculosis* (TB) Antigen Rapid Test Kit (Strip/Cassette)	Jei Daniel Biotech Corp.	China
24	EZ-TRUST Anti-Tuberculosis (TB) Rapid Screen Test Kit	CS Innovation Pte Ltd.	Singapore
25	Rapid TB test	Xeniss Life Science Co. Ltd.	South Korea
26	Bhat Bio-Scan- TB Card Test	Bhat Bio-Tech India (P) Ltd.	India
27	Accucare Rapid TB test	Lab-care Diagnostics Pvt Ltd.	India
28	SD BIOLINE Rapid TB Rapid Test	S.D. Bio Standard Diagnostic	India
29	Serocheck-MTB Rapid Test	Tulip Group (Zephyr Biomedicals)	India
30	TB SCREEN TEST Rapid	Bisen Biotech	India
31	TB Spot Ver 2.0 Rapid test	Span Diagnostics	India
32	TB Rapid Screen Test	ABP Diagnostics Ltd.	USA
33	Tuberculosis Rapid test	Advanced Diagnostics Inc.	UK
34	ABI rapid TB test	American Bionostica Inc.	USA
35	dBest One step Tuberculosis test	Ameritek	USA

S. No.	Name of the test	Manufactured by	Country
36	Rapid TB test	Bio-Medical Products Corporation	USA
37	TB Stat-Pak II	Chembio Diagnostic Systems Inc.	USA
38	Onsite Rapid test	CTK Biotech Inc.	USA
39	Rapid 1-2-3-HEMA Tuberculosis test	Hema Diagnostic Systems, LLC.	USA
40	TB Instantest	Laboratorios Silanes	Mexico
41	Immuno-Sure TB Plus	Millennium Biotechnology Inc.	USA
42	V Scan	Minerva BioTech Corporation	Canada
43	MycoDot's 9 Easy Steps	Mossman Associates Inc.	USA
44	Bioline Tuberculosis test	Pacific Biotech Co. Ltd.	Thailand
45	First Response Rapid TB Test	Premier Medical Corporation	USA
46	BioSign *M. tuberculosis* test	Princeton BioMeditech Corp	USA
47	SD TB Rapid Test	Standard Diagnostics Inc.	South Korea
48	First Sign MTB Test	Unimed International Inc.	USA
49	TB Rapid test	VEDA.LAB	France
50	Rapid *M. tuberculosis* device	Clinotech Diagnostics and Pharmaceuticals Inc.	Canada
51	DiaQuick Tuberculosis IgG/IgM Cassette	Dialab GmbH	Austria
52	TB Rapid Test	JAJ International Inc.	USA
53	TB Rapid test device	NUBENCO Medical International	USA
54	TB antibody Rapid Test	Oncoprobe Biotech Inc.	Taiwan
55	TB-Spot Version 2.0	VicTorch Meditek Inc.	USA
56	TB IgG/IgM 3 Line Rapid test	Tashima Inc, Bangalore	India
57	Accucare Rapid TB test	Lab-care Diagnostics Pvt Ltd.	India
58	Hexagon TB	Human Gesellschaft fur Biochemica und Diagnostica	Germany
59	Assure TB	Genelabs Diagnostics	Singapore
60	SDHO MTB	SDHO Laboratories	Canada
Microwell ELISA based tests:			
61	TB IgM ELISA Immunoassay Test Kit	Weifang Kanghua Biotech Co. Ltd.	China
62	Anda TB ELISA	Anda Biologicals, Strasbourg	France
63	Pathozyme TB Complex Plus and Pathozyme MYCO IgM, IgA, IgG	Omega Diag. Ltd. Alva	Scotland
64	Detect TB	Adaltis—Advanced Laboratory Diagnostics Systems	Italy
65	Tuberculosis Specific Antigen	Chengdu Pharmaceutical	China
66	*Mycobacterium tuberculosis* IgG	IBL, Hamburg	Germany
67	Active TB Detect	InBios International, Seattle	USA
68	TB Enzyme Immunoassay	Kreatech, Amsterdam	Netherlands
69	Determiner TB Glycolipid Assay	Kyowa Medex, Tokyo	Japan
70	Mycowell ELISA test	Span Diagnostics	India
71	Qualisa TB	Tulip Group	India
72	TB IgG, IgM, IgA ELISA	J Mitra	India
73	SEVA TB ELISA	JB TDR Centre, Sevagram	India

Sensitivity of commercially available serological tests

The claims of every manufacturer that their product is better are extremely tall and misguiding. For example, manufacture no. 8 [Orient Innovation E.I. (Beijing) Co., Ltd.] (Table) claims 99 per cent sensitivity and 99 per cent specificity; manufacture no. 28 (Standard Diagnostics) claims 98 per cent sensitivity and 99 per cent specificity; Tulip Group (manufacturer no. 29) goes further ahead in claiming 100 per cent sensitivity and 99 per cent specificity; manufacturer no. 30 claims 94 per cent sensitivity and 98 per cent specificity[4]. Of the 13 commercial kits based on ELISA, two are from China, one each from France, Scotland, Germany, Netherlands, Italy, USA and Japan while four are from India. All Indian manufacturers have claimed high accuracy. Tulip Group for its Qualisa TB kit claims 100 per cent sensitivity and 100 per cent specificity; J Mitra claims only 80 per cent sensitivity and 97 per cent specificity. A new entrant in the Indian market claims 97 per cent sensitivity and 99 per cent specificity for its in-house SEVA-ELISA test[8]. Indeed all these claims are based on in-house or small studies with no proper validation.

Sensitivity or ability to diagnose true TB cases is very critical and any test which has lesser detection rate than sputum microscopy does not warrant serious attention. The WHO advisory[2] is based on exhaustive literature search and evaluation of most commercially available kits by third party mandated by WHO. Pottumarthy *et al*[3], evaluated seven commercially available serological tests, and found that the diagnostic sensitivities of these tests with patients with active tuberculosis ranged from as low as 16 per cent and maximum up to to 57 per cent. The Pathozyme Tuberculosis IgA EIA had the highest sensitivity (57%) and the immunochromatographic rapid tests had a sensitivity of 41 per cent. We also carried out an extensive study (442 microbiologically proven cases) and found that a commercially available ELISA kit [Pathozyme MYCO IgM, IgA, IgG (Omega Diag., Ltd, Scotland)] had dismally poor sensitivity. Among the culture proven pulmonary TB cases, the sensitivity of Pathozyme Myco IgM was only 50.23 per cent, IgA 26.36 per cent and IgG 24.5 per cent (Singh *et al*, unpublished data). Steingart *et al*[6], while analysing the published data found that compared with ELISA which had pooled sensitivity of 60 per cent, rapid assays yielded lower pooled sensitivity (53%). The situation was worst in HIV-TB co-infected patients in whom the performance of one rapid test had poorer detection rate than smear microscopy. It could detect (sensitivity) only 68 per cent smear positive and culture proven cases and the overall sensitivity was only 16 per cent, that too in Africa[6]. The WHO expert group while deciding to issue a policy to ban all serological tests for TB diagnosis after analysing the data of 67 publications, observed that even for pulmonary tuberculosis, the sensitivity was highly variable ranging from as low as 0 to 100 per cent[2]. The 76 per cent pooled sensitivity of Anda-TB IgG, which is the most commonly evaluated test in smear-positive patients and 59 per cent in smear-negative patients. Based on another set of data analysis Dowdy *et al*[7] estimated that even the best kit (Anda60) has poorer impact on TB diagnosis than the most cost-effective and rapid smear microscopy. They also concluded that due to poor sensitivity even in open pulmonary TB cases smear microscopy will be capable of averting more number of secondary cases (containing spread from infected patients to uninfected population) than serology. The sensitivity was no better for extra-pulmonary cases, an argument most often put forward in support of serology. Indeed the liquid culture and molecular techniques take priority for pulmonary as well as extra-pulmonary cases, both in terms of detection rate and cost-effectiveness.

Specificity of serological tests

Specificity of all diagnostic tests is very important, but it becomes critical in diseases that warrant treatment or with a social stigma. TB comes under both categories. Any test which can label an uninfected person as infected is most undesirable. Hence, WHO has justifiably taken specificity into consideration while banning all serological tests[2]. In our unpublished study we included 789 healthy family contacts of pulmonary TB patients to evaluate the usefulness of a commercially available ELISA kit [Pathozyme MYCO IgM, IgA, IgG (Omega Diag., Ltd, Scotland)]. As many as 28 per cent healthy contacts were found to be reactive to MYCO IgM, IgA, or IgG. We found a devastating cross-reactivity (up to 72%) in kala-azar patients (details not shown here). Dowdy *et al*[7], estimated that due to high false positivity of commercially available serological tests, as many as 1,57,000 false (non-diseased) cases may be wrongly treated in India alone. This costs billions of rupees to Government of India and thousands of such wrongly treated cases may develop side effects of the anti-tuberculosis treatment. The WHO in its report also slams the serology based studies by mentioning that

"a vast majority of studies were either sponsored by industry, involved commercial test manufacturers, or failed to provide information on industry sponsorship". Therefore, the WHO made a policy statement that commercial serological tests provide inconsistent and imprecise findings resulting in highly variable values for sensitivity and specificity adversely impacting patient safety. Overall data quality was graded as very low and it is strongly recommended that these tests should not be used for the diagnosis of both pulmonary and extra-pulmonary TB.

The basic premise of serological tests was ease, rapidity and ever increasing demand in TB endemic countries. Hence, these tests have always been the first choice for small time laboratories wanting to mint the easy money from poor patients. Unfortunately, unethical medical practices provided major boost to these kits in recent years, without bothering much on quality of tests and implications of false-positive and false-negative results. Few credible academic institutions have promoted use of these tests. Our own data (unpublished) and WHO evaluation unambiguously show that TB serology results confuse more than providing any diagnostic clue. The arguement that serology is a cheaper has no basis, as serology profile (IgG, IgA, IgM) costs more than even liquid culture and PCR test, combined.

However, despite WHO guidelines endorsed by TB Division of Government of India banning these serological tests, not much is expected. It is mainly because, the Central TB Division has no control over the import or manufacturing of these kits in Indian market which are licensed for marketing by Drug Controller General of India. Hence, until the import and manufacturing of these kits is banned, these kits will continue to confuse the Indian markets and interested parties making huge profits. Nevertheless, it is hoped that none of the presently marketed serological tests will be prescribed or used in India and other TB endemic countries without proper re-validation on well characterized samples, despite tall claims by the companies. This editorial is not the obituary for serology. Immunological detection with appropriate sensitivity and specificity will remain an attractive research option for developing immunodiagnosis of tuberculosis. Certainly the antigen targets have to be properly chosen and, this has not been explored so far. Techniques for antigen detection will continue to have edge over antibody detection methods due to obvious reasons. Same yardsticks also will apply to techniques based on delayed type of hypersensitivity (tuberculin, quantiferon type of assays). Finally, these aspects need to be addressed for all the diseases alike.

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