APPLICATION OF IMMUNO CHROMATOGRAPHIC METHODS IN PLEURAL TUBERCULOSIS

Hadizadeh Tasbiti .AR, Yari.SH, Bahrmand.AR, Karimi.A ,Fateh.A, Sayfi.M

Tuberculosis Dept.Pasteur Institute of Iran.Tehran.Iran

INTRODUCTION

- Tuberculous pleuritis is a common manifestation of extrapulmonary tuberculosis and is the most common cause of pleural effusion in many countries.

- Conventional diagnostic tests, such as microscopic examination of the pleural fluid (fluid in the lung) , biochemical tests, culture of pleural fluid, sputum or pleural tissue, and histopathological examination of pleural tissue, have known limitations.

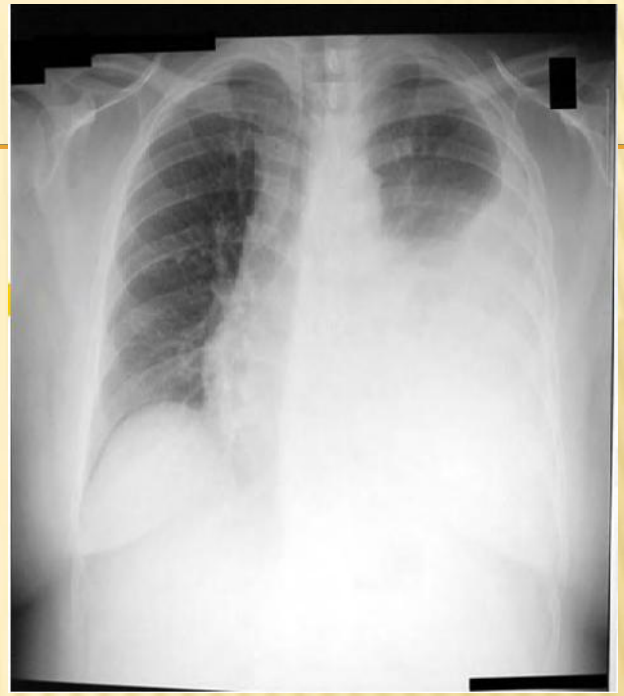
- Due to these limitations, newer and more rapid diagnostic tests have been evaluated.

WWW.THELANCET.COM/INFECTION VOL 13 APRIL 2013

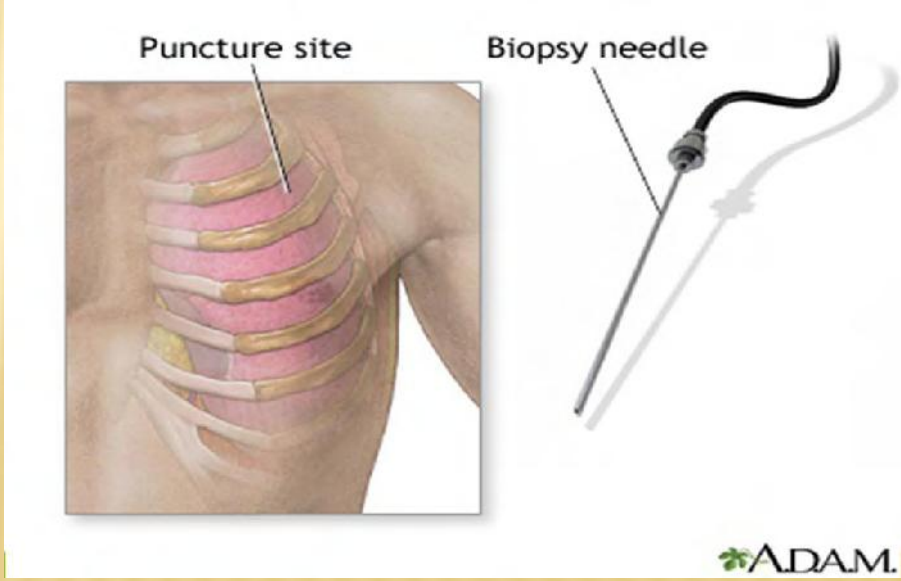
- ***Panel 1: The estimated global burden of tuberculosis in 2011***

- **8·7 million incident cases**
- • 1 · 1 million (13%) cases in people living with HIV
- • 490 000 cases in children younger than 15 years
- **1·4 million deaths**
- • 990 000 HIV-seronegative people
- • 430 000 (31%) HIV-seropositive people
- • 500 000 (36%) women
- • 64 000 children younger than 15 years
- **Multidrug-resistant cases**
- • 630 000 prevalent cases
- • 310 000 incident cases
- • 3 · 7% of new incident cases
- • 20% of previously treated incident cases
- • 9% of multidrug-resistant cases are extensively
- drug-resistant

PLEURAL TB



- **Diagnosis:**
- Imaging (CXR, sonar, CT-scan)
- Pleural tap
- Differential white cell counts
- Total protein, LDH, glucose
- Adenosine deaminase (ADA)
- Smear microscopy, TB culture
- (GeneXpert)
- Pleural biopsy, thoracoscopy



BETTER AND MORE RAPID TESTS FOR (TPE)

- Investigation the role of renin-angiotensin system (RAS) and matrix metalloproteinase (MMPs) in the pathogenesis of pleural exudates. The results indicate that increased ACE and MMP-9 activities (extracellular converting enzyme)found in the exudates were mainly contributed from a higher level of both enzyme activities in the tuberculous pleural effusions. ***Int J Biol Sci. 2012;8(8).***
- developed ELISA based on recombinantly expressed MPT64 in combination with rabbit polyclonal antibodies. The ELISA-MPT64 method was validated using MTB strains and tested against clinical samples. Nested PCR, Löwenstein-Jensen (L-J) culture and smear microscopy were employed as the comparative tools for assessing the performance of the assay. Our results demonstrate that the newly established . **Curr Microbiol. 2012 Sep;65(3).**
- The diagnostic utility of the Xpert MTB/RIF assay in 20 cases with confirmed tuberculous pleural effusion. The sensitivity and specificity of the Xpert assay in pleural fluid were 25% and 100%, respectively. All cases positive by the Xpert assay were also positive by pleural fluid culture. J Clin Microbiol. 2011 December; 49(12)

BETTER AND MORE RAPID TESTS FOR (TPE)

- Define the role of adenosine deaminase **(ADA)** and *interferon gamma* **(IFN-gamma**) in the differential diagnosis of pleural effusion with special attention to their source, mechanism of release and methods of measurement in pleural fluid. Pleural fluid ADA and IFN-gamma are **both sensitive and specific biomarkers** of tuberculous pleur**isy. Their diagnostic accuracy across the different studies shows a smaller variability than that of other tests, for example NAATs**. ***Curr Opin Pulm Med. 2010 Jul;16(4).***
- The QuantiFERON-TB Gold In-Tube (QFT-GIT) test was performed on whole blood and pleural fluid from 43 patients with TPE and 29 control subjects (non-TPE). The sensitivity and specificity using the QFT-GIT for the diagnosis of TPE were 48.8% and 79.3%, respectively, in pleural fluid. **QFT-GIT test or its components have poor accuracy in the diagnosis of TPE. Respiration. 2011;82(4)**
- The 3525 patients who took sputum **Real Time PCR** and/or bronchoscopic PCR were reviewed retrospectively. In sputum PCR, **sensitivity was 45%; specificity 99.6% , accuracy 87% .Chest 2012.142(4).**

MATERIAL AND METHODS(1)

- **Crude *mycobacterium* antigens extract:**Bacteria was harvested by loop from the surface of the solid culture (LJ medium), (PBS) pH 7.4 containing (PMSF) 1mM, (EDTA) 20 mM, Sodium azide 0.02 %, Triton X114 0.5%, Glycerol 10%, Sucrose 12.5mM, DNAse 1µg/ml ,(DTT) 10 mM . The bacilli were subjected to sonication for 1 h at 50 Hz, using a cell sonicator.

- **Protein estimation;** The protein content (mycobacterium proteins) was measured by Bradford's method.

- **Rabbits:** were immunized with 250 µg of purified antigen . The booster injection was carried out by subcutaneous. The rabbits were re-immunized (boosted) at 21-day.

MATERIAL AND METHODS(2)

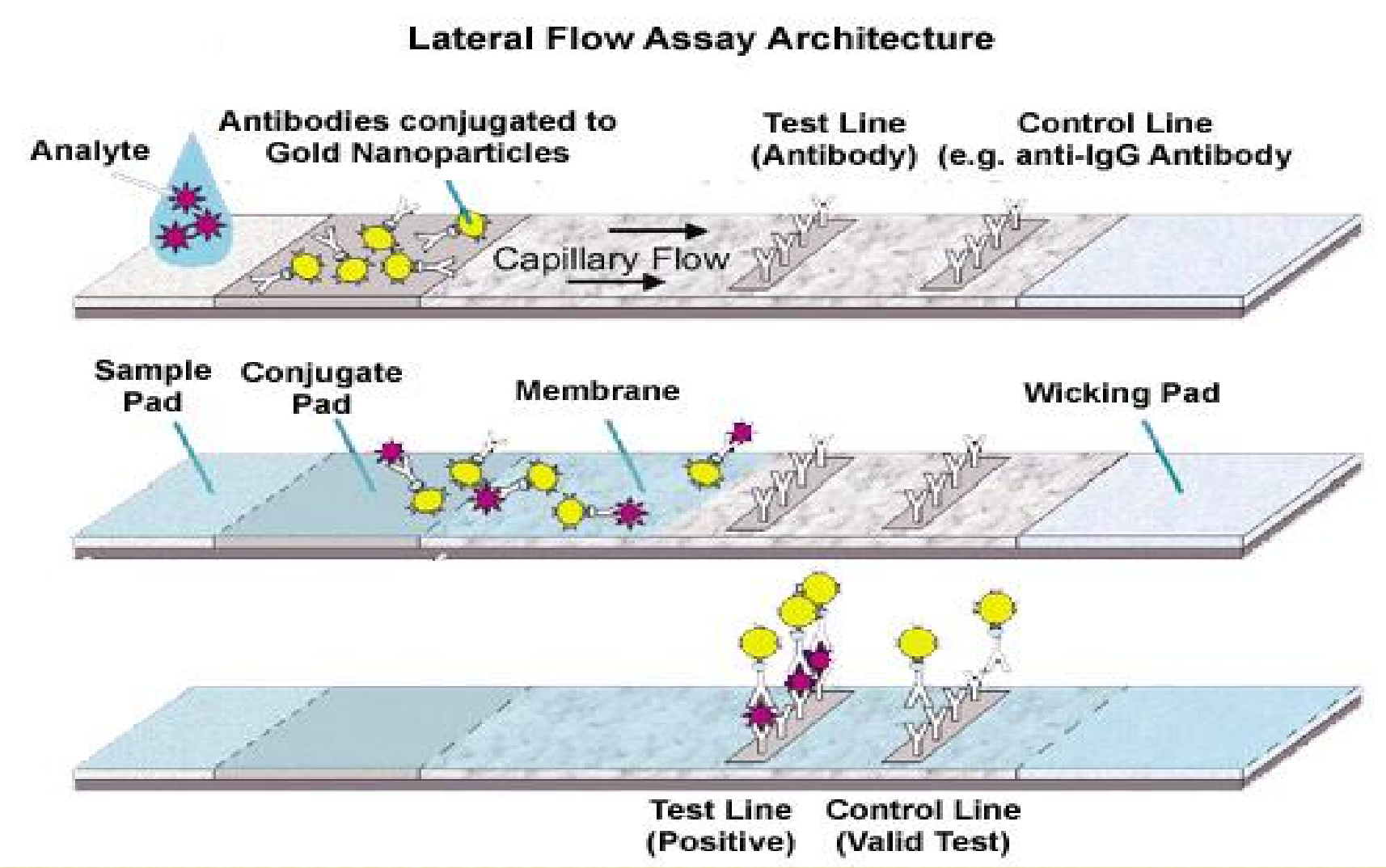
- **Antibody purification procedure :**Affinity chromatography was used to purify specific polyclonal antibodies against MTB. This step was carried out on an affinity column based on the use of MTB covalently coupled to CNBr-activated Sepharose 4B.

- **Specimens : (Treatment 1)** Pleural fluid from 76 patients (27male and 49 female), who had been admitted to Pasteur institute (Tehran) or from TB Bank were obtained. The sample was digested and decontaminated with 4% NAOH, 2.9% sodium citrate and 0.5% N-acetyl cysteine.

  (Treatment 2):?!!!

- **Rapid test procedure:** A total of 200 µl of processed sample(pleural sample) is added to the reaction funnel and was allowed to absorb completely. After washing with the buffer, 150 µl TB antibody solution was added to the test funnel. After another wash with the buffer, 150 µl protein gold conjugate was added to the test funnel.

MATERIAL AND METHODS(3)



RESULTS(1)

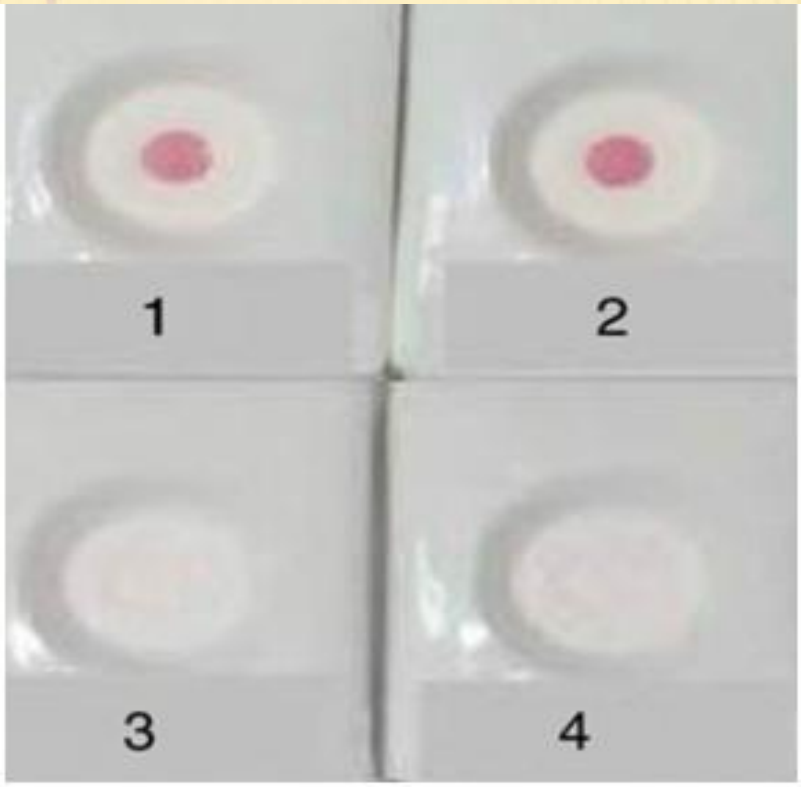


Figure 1: The results of pleural-Rapid-Test on the filtering cartridge .The presence of TB bacilli in the sample is revealed by the presence of a red-pink dot in the middle of the filter. The negative control and negative sample yielded a white central region. The treated Positive pleural fluid samples (1,2) and Negative samples (3,4).

RESULTS(2)

Table 1: Demographic data of patients studied

Characteristic					
Age		Mean ± SD (Yr)	Range (Yr)	Minimum /maximum (Yr)	
		50.1±22.87	87.0	3 /90	Total N=76
Age group	Frequency N (%)				
0-20	10(13.2)				
21-40	20(26.3)				
41-60	15(19.8)				
≥61	31(40.7)				Total N=76
Gender	N(%) cases				
Male	27(35.5)				
Female	49(64.5)				Total N=76
Patient group					
TB-cases N (%)	Non-TB-cases N (%)	TB-cases N (%)	Non-TB-cases N (%)	TB-cases N (%)	Non-TB-cases N (%)
41(53.9)	35(46.1)	Age(mean± SD)		Gender n (%)	
		48.0±24.1	52.2±21.6	Male 10(37.0) Female 19(38.8)	Male 17(63.0) Female 30(61.2)

RESULTS(3)

Table2: The results of pleural-rapid-test performance in comparison to culture in combination with smear examination

Assay		Smear and Culture			
		Positive (n)%	Negative (n)%	Total	
Rapid test	Positive (n)%	(40) 52.6%	(2) 2.6%	(42) 55.2%	
	Negative (n)%	(1)1.3%	(33) 43.5%	(34) 44.8%	
	Total (n)%	(41) 53.9%	(35) 46.1%	(76) 100.0%	
Assay	Sensitivity	Specificity	PPV	NPV	Efficiency
Rapid test	97%	94%	95%	97%	96%

DISCUSSION

- The **pleural -Rapid -Test** (antigen detection test) **yielded 97% sensitivity and 94% specificity** for the diagnosis of EPTB.

- Findings highlighted the importance of antigen detection as a diagnostic tool.

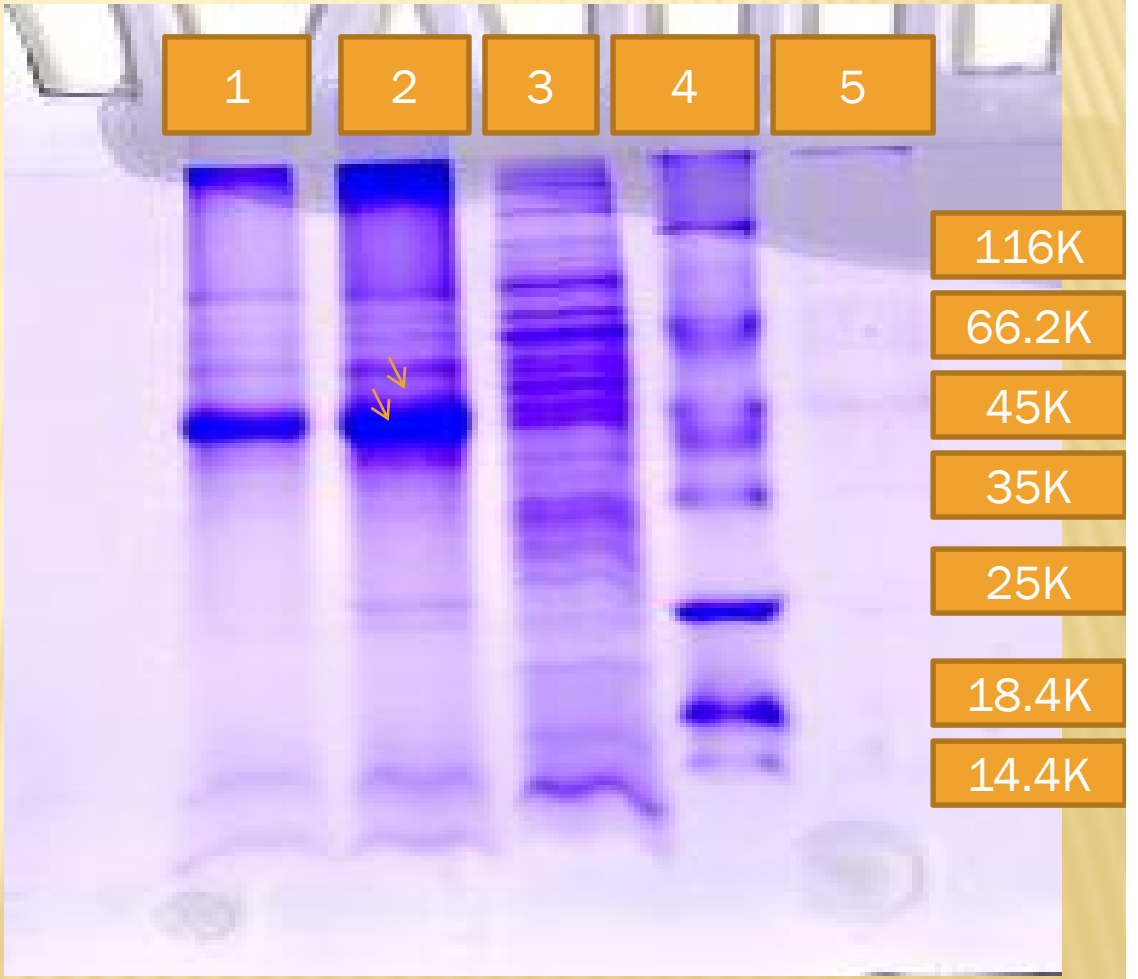
- Although currently the international standards for TB care discourages the use of serological tests in routine practice and no international guideline recommends their use, the Rapid-test evaluated in this study may be useful for diagnosis of tuberculosis and it may also have some value for diagnosis of smear negative and extra pulmonary cases of TB.

REFERENCES

- 1. Sarkar S, Tang X L, Das D, Spencer J S, Lowary T L, Suresh M R. A Bispecific Antibody Based Assay Shows Potential for Detecting Tuberculosis in Resource Constrained Laboratory Settings. *PLoS ONE* 2012; 7: e 32340. www.plosone.org.
- 2. Wu X, Yang Y, Zhang J, et al. Humoral immune responses against the *Mycobacterium tuberculosis* 38-kilodalton, MTB48 and CFP-10/ESAT-6 antigens in tuberculosis. *Clin Vacci Immunol* 2010; 17: 372-375.
- 3. Brust B, Lecoufle M, Tuaillon E, et al. *Mycobacterium tuberculosis* lipolytic enzymes as potential biomarkers for diagnosis of active tuberculosis. *PLoS ONE* 2011; 6: e 25078. www.plosone.org.
- 4. Dowdy D W, Steinhart K R, Pai M. Serological testing versus other strategies for diagnosis of active tuberculosis in india: A cost-effectiveness analysis. *PLoS ONE* 2011; 8: e 1001074. www.plosone.org.
- 5. Steingart K R, Henry M, Laal S, et al. Commercial serological antibody detection tests for the diagnosis of pulmonary tuberculosis : A systematic review. *PLoS ONE* 2007; 4: e 202. www.plosone.org.
- 6. Steingart K R, Flores L, Dendukuri, N. et al. Commercial serological tests for the diagnosis of active pulmonary and extrapulmonary tuberculosis : An updated systematic review and meta-analysis. *PLoS ONE 2011*; 8: e 1001062. www.plosone.org .
- 7. Grenier J, Pinto L, Nair D, et al. . Widespread use of serological tests for tuberculosis: data from 22 high-burden countries. *Eur respire J* 2012; 39: 502-5.

NEW BATCH OF MDR AND BCG PROTEIN PURIFICATION. APR 30.2012 STAINING BY COMASSI BLUE

- 1-Isoniazid 16
  2-RIfampin400
  3-BCG
  4-Marker
  5-Isoniazid4



- Hadizadeh Tasbiti.A.R.Yari.Sh.Protein Chemistry Lab, TB Dept, Pasteur Institute-Tehran, Hadi@pasteur.ac.ir