Developing Biosensor for rapid detection of Tuberculosis

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Abstract

Tuberculosis is one of the world's dead liest infectious diseases (about 1.7 million people die annually). The standard test for diagnosing Mycobacterium tuberculosis (Mtb) is smear sputum microscopy, which is unable to identify one-half of true infections. Several molecular-based techniques have been examined for the rapid detection of Mtb but they require specialized equipment, are expensive and otherwise difficult to implement in primary health care settings. Therefore, there is a critical need for accurate, rapid and cost-effective screening methods that are field deployable. Our objective for this research focuses on developing Biosensors and validating a hand held field-operable biosensor to quickly and cheaply detect Mtb infection. The biosensor uses magnetic nanoparticles (MNPs) to non-specifically extract bacterial cells from artificial sputum samples. Antibodies (Ab) specific to Mycobacterium species are conjugated to gold (Au) nanoparticles to form Ab-Au NPs. These Ab-Au NPs are then added to the MNP-cells to form a MNP-cell-Ab-Au complex. This complex is added to a screen-printed carbon electrode chip that is connected to a handheld p. Differential pulse voltammetry is used to generate the signal. Preliminary results show that the biosensor can detect Mtb bacterial cells, For effective TB detection in resource-poor settings the field of biosensors has very strong potential. However, key to the development of such biosensors is a focus on both the sample preparation steps from biofluids (e.g. blood, sputum).designing a biosensor with allthese advantages has not yet been completely successful, aseach attempt has specific draw backs. It is a challenge to satisfy all these needs in a single biosensing device. Nevertheless, the advances in nanosensors and other upcoming technologies reviewed here, suggest that biosensors to detect TB can be expected to play a larger role in the near future. Such platforms will also need to solve issues around sample collection and preparation. Currently most diagnosis techniques available utilize sputum samples as test sample, which due to its high viscosity and sticky nature is very difficult to work with. Hence other sources like blood or urine should also be considered as test samples Lack of reliable and tested biomarkers in those samples is however an issue that needs attention.

KEYWORDS-Sputum , blood, Tuberculosis, MDR, Biosensorsz

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INTRODUCTION

Mycobacterium tuberculosis [MTB] is the causative agent of tubercle bacillus [TB], accounting for approximately two million deaths annually, mainly in developing countries , and remains one of the leading causes of respiratory infections and has posed critical threats to public health . Currently, the global number of TB cases is rising at a rate of 2% per year. Hence, the key to the control of this infectious disease is to provide the short course therapy and the post-exposure vaccine. Moreover, the rapid detection

method with high sensitivity and specificity is essential to aid the diagnosis, assess the prognosis, and monitor the disease recurrence .

Drug Resistant TB

A person with active TB disease has drug resistant TB if the TB bacteria that the person is infected with, will not respond to, and are resistant to, at least one of the main TB drugs. Drug susceptible TB is the opposite of drug resistant TB. If someone is infected with TB bacteria that are fully susceptible, it means that all of the TB drugs will be effective so long as they are taken properly. It still means that several drugs need to be taken together to provide effective TB treatment. Drug resistant TB has frequently been encountered in India and its presence has been known virtually from the time anti TB drugs were introduced for the treatment of TB. If a person has drug resistant TB it means that their illness will not respond to at least one of the main TB drugs. The prevalence of multi drug resistant MDR TB has though been believed to be at a low level in most regions of the country. Various studies have found MDR TB levels of about 3% in new cases and around 12-17% in retreatment cases. However even if there is such a small percentage of cases it still translates in India into large absolute numbers.

Types of Drug resistant TB-

There are two main types of drug resistant TB, MDR TB and XDR TB.

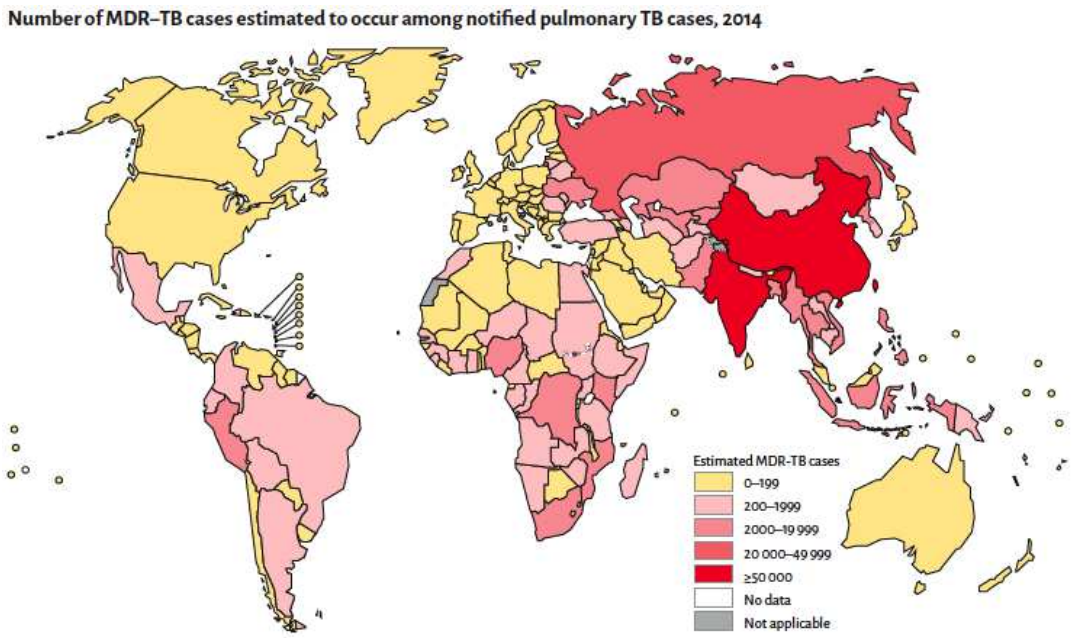
MDR TB is the type of drug resistant TB, when the bacteria are resistant to the TB drugs rifampicin and isoniazid. MDR (multi drug resistant) TB is the name given to TB when the bacteria that are causing it are resistant to at least isoniazid and rifampicin, two of the most effective TB drugs.MDR TB and XDR TB do not respond to the standard six months of TB treatment with “first line” anti TB drugs. Treatment for them can take two years or more and requires treatment with other drugs that are less potent, more toxic and much more expensive. Worldwide only a few thousand patients with MDR TB and XDR TB are treated each year.

XDR TB (extensively drug resistant TB) is defined as strains resistant to at least rifampicin and isoniazid. This is in addition to strains being resistant to one of the fluoroquinolones, as well as resistant to at least one of the second line injectable TB drugs amikacin, kanamycin or capreomycin.2

How do you get MDR TB-

There are two main ways that you can get MDR TB. Firstly you can get MDR TB if you don’t take your drugs exactly as you have been instructed to by your health care provider. You may also get it if you are not taking the correct drugs. This could possibly be because your bacteria are resistant to more drugs than your health care provider realised. Maybe you have undiagnosed XDR TB. This is referred to as acquired TB. You can also get MDR TB if you become infected with TB bacteria from another person who already has MDR TB. This is known as primary TB.

Number of estimated MDR TB cases in 2014



Symptoms-

The symptoms of active TB are very variable and depend on which part of the body has been infected, that is which type of TB it is. It is very difficult to diagnose TB just from the symptoms, as the symptoms are not usually ones that are just for TB. This means that the symptoms can often be the symptoms of another disease as well. So to diagnose TB it is always necessary to do at least one TB test.

General symptoms of active TB include weakness or feeling very tired, losing weight without trying, lack of appetite, chills, fever (a high temperature of 38C or above) and night sweats.

Epidemiology

The WHO Global TB Control Report 2012 reported that the WHO's Millennium Development Goal to halt the growth of theTB epidemic by 2015 is showing some success with a decrease of2.2% Observed Between 2010 and 2011 for new TB cases, albacore tunawith a 41% mortality rate decrease relative to 1990. Between1995 and 2011, 51 million people were successfully treated forTB, saving 20 million lives. Despite this progress, the globalburden of TB remains enormous with 8.7 million new cases of TB were registered in 2011, out of which 13% patients were co-infected with HIV. The mortality in 2011 due to TB was1.4 million, including almost one million deaths among HIHIV-negative individuals and 0.4 million among people who wereHIV-positive. The distribution of TB cases also shows a gender-dependence with men reporting more cases compared tofemales in all age groups and from every geographical

region ofthe world. India and China together account for 40% whereasthe African region contributes 24% of the world's TB cases. The statistics of the MDR cases in TB are alarming according to theWHO 2012 report. It reported resistance to more than one anti-TB drugs among 3.7% of new cases and 20% previouslytreated cases. In recognition of the problem, 26 nations havealready listed MDR-TB as a top priority health programme andfounded a global fund to support Directly Observed Therapy(DOT) to ensure the effectiveness of given medications.

Diagnosis-

Biosensors in TB detection

Considering the fact that 98% of all TB cases occur in developing countries without access to specialized laboratories, thereis a strong need to develop alternative, simpler and lower costtechniques for TB diagnosis. Biosensors are analytical devicesthat transduce biochemical reactions interactions of isolated enzymes, receptor proteins, antibodies, nucleic acids, organ-they, whole cells or tissues with specifc chemical compounds into an optical, thermal or electrical signal, which can be more easily measured and quantied. The main advantages of biosensors over conventional diagnostic techniques can bestated as follows:

(1) Technical advantage: in biosensors often a high level ofdevice and capture detection integration is achieved allowing single step detection.

(2) Ease of use: many of the designed biosensors are tailoredwith user-friendly interfaces connecting them to advanced instrumentation.

(3) Quick response: response time is Typically a few minutes for most biosensors enabling rapid and better control over the measurement.

Electrochemical and electrical biosensors-

Electrochemical and electrical biosensors are among the most popular biosensors that are used today in detection of not onlyTB but also a large number of other diseases.

Electronic nose-based biosensors-

Electronic “nose”type biosensors are designed to recognize volatile substancesproduced by M. tb. in liquid medium 67–70,80It basically consists of three main building blocks i.e. (i) a volatile gaschamber that passes the volatile molecule products over. sensor array, (ii) the pattern of more or less specific Responses by the sensor array and (iii) a data analysis system to interpret theoutput pattern of the detection system.

Electronicnose appliances have been used successfully to detect the presence of M.tb. 68 and efforts are also been made to develop it into commercial product. " Aeonose " is a commercial device based on the electronic nose concept.

Nanowire-based biosensors-

Biosensors built withnano-sized transducing elements are most prominently repre-sented

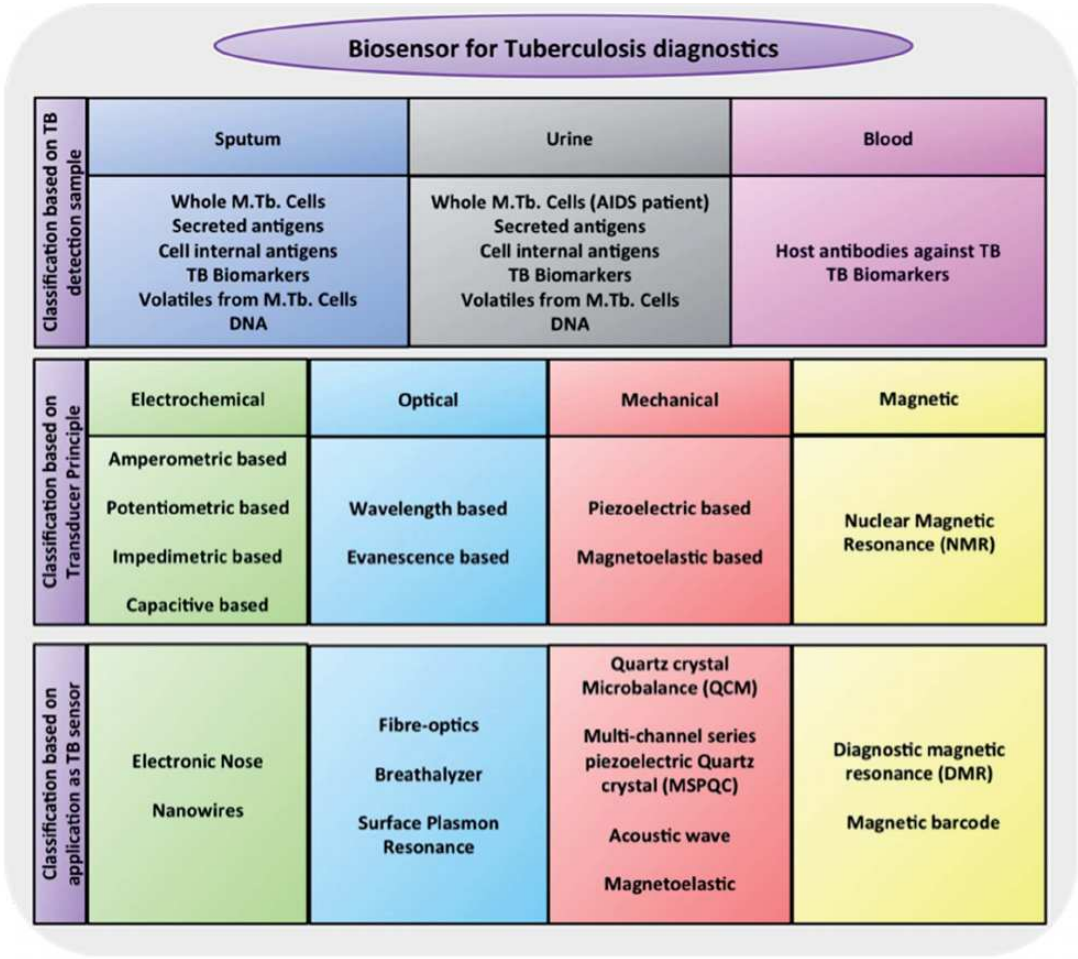
by silicon nanowires that operate as held effect transistors. carbon nanotubes (both single and multi-walled) have been used to detect various antigens including M. tb. antigens or ssDNA specific for M. tb.

Surface plasmon resonance (SPR) based biosensors-

Surface plasmon resonance (SPR) is an optical principlefrequently used in biosensors. Surface plasmons (evanescentwaves) are produced when a polarized light is incident at the back of a thin film of noble metals like gold and silver.

Breathalyzer biosensors-

Breathalyzer sensors have specifically been developed to diagnose pulmonary TB in patients.



Material & Methods-

Biological materials used enzyme/substrate, antibody/antigen, nucleic acid,/ complementary sequence , microorganism, animals or plant whole cells and tissue

slices,sensing element, electrochemical,optical thermometric, biorecogenation element.etc

EQUIPMENT: Rinse all glassware in DI water, coplin jars, microwave oven.

REAGENTS: Ziehl-Neelsen Carbol-Fuchsin Solution: Basic fuchsin, Distilled water 100% alcohol, Phenol crystals, melted Mix well, filter into brown bottle. Label bottle with date and initials, solution is stable for 1 year. 1% Acid Alcohol, Hydrochloric acid, 70% Alcohol

Immunological and microbiological tests Equipment For the diagnosis of TB - (1) Smear microscopy: microscopy based smear tests arerapid, in expensive, simple and relatively easy to perform methods for the detection of acid-fast bacterium such as M. tb.bacterium. Conventionally the Ziehl-Neelsen staining is utilized requiring a minimum of 1104 bacterium per ml of sputum. Fluorescence microscopy utilizing auramine-rhodamine staining was found to be more sensitive though expensive, as it requires a fluorescence microscope.The fluorescence based method is more sensitive as slides can be examined at lower imagnig cation. Results from this method can be obtained within hrs.

(2) Immunological assays like latex agglutination, ELISA, and Mantoux tests: in these tests, typically the binding of antibodies in the serum to M. tb. antigens is tested. 26,27For example, in latex agglutination tests, the polystyrene (latex) beads are function-alized with antigens extracted from a pathogenic Mycobacterium, which are then reacted with serum samples.

(3)The TB Breathalyzer device (Rapid Biosensor Systems Ltd) was field tested in the outpatient clinic of Adama Hospital, Ethiopia. Adults seeking diagnosis for respiratory complaints were tested. Following nebulization with 0.9% saline patients were asked to cough into a disposable collection device where cough aerosols were deposited. Devices were then inserted into a portable instrument to assess whether antigen was present in the sample. Demographic and clinical data were recorded and all patients were subjected to chest radiogram and examination of sputum by Ziehl-Nielsen microscopy. In the absence of culture treatment decisions were based on smear microscopy, chest x-ray and clinical assessment. Breathalyzer testing was undertaken by a separate physician to triage and diagnostic assessment.

(4)Microcantilever device is utilized in several biosensors for detection of bio-molecule of interest. Biosensors task is to identify presence of targeted molecule and supply result into a measurable signal. Comparing with conventional biological equipments, micro scale biosensors are very fast, reliable and price effective. Microelectromechanical systems (MEMS)/nanoelectromechanical systems are used for designing of such biosensors, which might be utilized in several biological applications. Sensing mechanism of biosensor is varied with the application. This paper is focused on detection of bio-molecule using microcantilever beam. Biosensor has shown here uses piezo-resistive method for detection of targeted bio-molecule. This Bio-MEMS device is designed and simulated using coventorware software. This biosensor wants to identify a presence of T.B. in a very suspected patient.

Conclusion-

In this project we found that economic affordable diagnosis and treatment in time along with recently arising issues like multiple drug resistance and other allied infections that decrease body immunity, like tb. Current technologies for diagnosis are either too insensitive, too labo-ratory intensive or utilize expensive detection modules, which are all challenges in resource poor settings. Most of biosensors (except Electronic nose and Breathalyzer) discussed in the present review are still at the developmental stage and lack clinical validation with real TB samples from patients. All sensor methods have their own merits and potential problems with respect to sample preparation, requirement of skilled personnel to handle the sample, sensitivity or cost. An overview of the analyzed sample and detection limits of different biosensors.

Future -

biosensors to detect TB can be expected to play a larger role in the near future. search platforms will also need to solve issues around sample collection and preparation.aim to completely eliminate TB by 2050, development of techniques for early and accurate detection of TB is crucial. To build an effective biosensor for TB detection, criteria that need to be fulfilled are: (1) cost-effectiveness (2) highsensitivity (3) reliability (no false positives) (4) portability and (5)disposability.

References-

1.Soo PC, Horng YT, Chang KC, Wang JY, Hsueh PR, Chuang CY, Lu CC, Lai HC: A simple gold nanoparticle probes assay for identification of Mycobacterium tuberculosis and Mycobacterium tuberculosis complex from clinical specimens. Mol Cell Probes 2009, 23: 240–246. 10.1016/j.mcp.2009.04.006

2.Dye C, Lonnroth K, Jaramillo E, Williams BG, Raviglione M: Trends in tuberculosis incidence and their determinants in 134 countries. Bull World Health Organ 2009, 87: 683–691. 10.2471/BLT.08.058453

3.Soo PC, Horng YT, Hsueh PR, Shen BJ, Wang JY, Tu HH, Wei JR, Hsieh SC, Huang CC, Lai HC: Direct and simultaneous identification of Mycobacterium tuberculosis complex (MTBC) and Mycobacterium tuberculosis (MTB) by rapid multiplex nested PCR-ICT assay. J Microbiol Methods 2006, 66: 440–448. 10.1016/j.mimet.2006.01.010

4.M. Gandy and A. Zumla, Soc. Sci. Med., 2002, 55, 385.

5. J. M. Grange, M. Gandy, P. Farmer and A. Zumla,International Journal of Tuberculosis and Lung Disease,2001, 5, 208.

6. N. S. Shah, A. Wright, G. H. Bai, L. Barrera, F. Boulahbal,N. Martin-Casabona, F. Drobniewski, C. Gilpin,M. Havelkova, R. Lepe, R. Lumb, B. Metchock, F. Portaels,M. F. Rodrigues, S. Rusch-Gerdes, A. Van Deun,V. Vincent, K. Laserson, C. Wells and J. P. Cegielski,Emerging Infect. Dis., 2007, 13(3), 380–387.

7.J. C. Palomino, A. Martin, M. Camacho, H. Guerra, J. Swingsand F. Portaels,

Antimicrob. Agents Chemother., 2002, 46(8),2720–2722.

8. N. A. Kruh, J. Troudt, A. Izzo, J. Prenni and K. M. Dobos,PLoS One , 2010, 5, e13938.6

9.J. F. Murray, Am. J. Respir. Crit. Care Med., 2004, 169, 1181.7

10.P. Nahid, M. Pai and P. C. Hopewell, Proc. Am. Thorac. Soc.,2006, 3, 103

11. Expert Group Meeting Report, WHO, 2014, p. 1.9

12.S. Murray, Can. Med. Assoc. J., 2006, 174, 33.10

13. .L. P. Ormerod, Br. Med. Bull., 2005, 73–74, 17.11

14. C. Mitnick, J. Bayona, E. Palacios, S. Shin, J. Furin,F. Alc´between, E. S´anchez, M. Sarria, M. Becerra,M. C. Fawzi, S. Kapiga, D. Neuberg, J. H. Maguire,J. Y. Kim and P. Farmer, N. Engl. J. Med., 2003, 348, 119.12

15..J. A. Caminero, International Journal of Tuberculosis andLung Disease , 2006, 10, 829.13

16.P. Isaakidis, H. S. Cox, B. Varghese, C. Montaldo, E. DaSilva, H. Mansoor, J. Ladomírske, G. Sotgiu,G. B. Migliori, E. Pontali, P. Saranchuk, C. Rodrigues andT. Reid, PLoS One, 2011, 6, e28066.14

17.C. Colijn, T. Cohen, A. Ganesh and M. Murray, PLoS One,2011, 6 , e18327.15

18. S. S. Shin, J. J. Furin, F. Alcantara, J. Bayona, E. Sanchez andC. D. Mitnick, Emerging Infect. Dis., 2006, 12, 687.16

19.C.-H. Lee, M.-C. Lee, H.-H. Lin, C.-C. Shu, J.-Y. Wang,L. N. Lee and K. M. Chao, PLoS One, 2012, 7, e37978.17

20.C. Mulenga, D. Mwakazanga, K. Vereecken, S. Khondowe,N. The Kapata, I. The C. The Shamputa, of H. Meulemans and L. The Rigouts,BMC Public Health, 2010, 10, 756.18

21..A. The Bekmurzayeva, of M. Sypabekova and D. The Kanayeva,Tuberculosis, 2013, 93, 381.19

22.A. Somoskovi, J. E. Hotaling, M. Fitzgerald, V. Jonas,D. Stasik, L. M. Parsons and M. Sal□nger, J. Clin.Microbiol., 2000, 38, 2743.20

20.S. Singh, J. Singh, S. Kumar, K. Gopinath, V. Balooni,N. Singh and K. Mani, PLoS One, 2012, 7(7), r40213.21

23. S. C. Cavalcante, E. C. C. Smith, A. G. F. Pacheco,R. E. Chaisson and B. Durovni, International Journal ofTuberculosis and Lung Disease, 2007, 11, 544.22

24.A. Anuwatnonthakate, P. Limsomboon, S. Nateniyom,W. Wattanaamornkiat, S.

Komsakorn, S. Moolphate,N. Chiengsorn, S. Kaewsa-Ard, P. Sombat, U. Siangphoe,P. A. Mock and J. K. Varma, PLoS One, 2008, 3, e3089.23

25. A. D. Harries, A. Jahn, R. Zachariah and D. Enarson, PLoSMed. , 2008, 5 , E124. 24 J.

26.C. Palomino, Eur. Respir. J., 2005, 26, 339.25 K. R. Steingart, M. Henry, V. Ng, P. C. Hopewell, A. Ramsay,J. Cunningham, R. Urbanczik, M. Perkins, M. A. Aziz andM. Pai, Lancet Infect. Dis., 2006, 6, 570.26 K. Minton, Nat. Rev. Immunol., 2003, 3, 355.27

27.J. G. Fern´Ande, I. Fern´Andez-de-Mera, L. E. Reyes,M. C. Ferreras, V. P´Erez, C. Vicente, M. Fern´andez andJ. F. Garc´New a-Mar´ın, J. Vet. Diagn. Invest., 2009, 21, 102.

28.Environmental challenge S. Bhaskar, J. N. Banavaliker and M. Hanif, Famous Immunol. Med. Microbiol., 2003, 39, 235.

29.Twenty-nine S. Bhaskar, J. N. Banavaliker, K. Bhardwaj and P. Upadhyay,J. Immunol. Methods, 2002, 262, 181.30

30.D. Satana, A. Y. Coban and M. Uzun, J. Clin. Microbiol.,2010, 48, 4291.31 R. Dinser, M. Fousse, U. Sester, K. Albrecht, M. Singh,H. K¨ohler, U. M¨uller-Ladner and M. Sester, Rheumatology,2008, 47, 212.32

31.L. Cosmi, L. Talbot, V. Santarlasci, F. Liotta, F. Frosali,R. Angeli, M. Mazzetti, A. Vultaggio, A. Matucci, E. Maggi,S. Romagnani and F. Annunziato, Int. Arch. AllergyImmunol., 2007, 143(1), 1–9.33

32. L. of Li, D. Qiao, X. Fu, S. Lao, X. Zhang and C. Wu,, PLoS One , 2011, 6 , e20165.Thirty-four

33.M. D. Bonecini-Almada, Mem. Inst. Oswaldo Cruz , in 2000, ninety-five , 491.35

34.M. Piuri, W. R. Jacobs Jr. and G. F. Hatfull, PLoS One , 2009, 4 , e4870.36

35. C. Pina-Vaz, S. Costa-de-Oliveira and A. G. Rodrigues, J.Med. Microbiol., 2005, 54, 77.37

36.J. J. Yan, A. H. Huang, S. H. Tsai, W. C. Ko, Y. T. Jin andJ. J. Wu, Diagn. Microbiol. Infect. Dis., 2000, 37, 25.38

35. H. Saitoh And N. Yamane, Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi , 2000, 11 , 19. 39

36.F. Alcaide, M. A. Ben´ı complexion, J. M. Escrib`a and R. Mart´ı n, J. Clin. Microbiol., 2000, 38, 398.40

37.M. Garrigo, L. M. Aragon, F. Alcaide, S. Borrell,E. Cardenosa, J. J. Gal´an, J. Gonzalez-Mart´ın, N. Martin-Casabona, C. Moreno, M. Salvado and P. Coll, J. Clin.Microbiol., 2007, 45, 1766.Forty-one

38. L. Y. Itani, M. A. Cherry and G. F. Araj, J. Med. Liban. , 2005, 53,42

39.N. Kobayashi, T. G. Fraser, T. W. Bauer, M. J. Joyce,G. S. Hall, M. J. Tuohy and G. W. Procop, Arch. Pathol.Lab. Med., 2006, 130, 1053.43

40.D. V. Cousins, S. D. Wilton, B. R. Francis and B. L. Gow, J.Clin. Microbiol., 1992, 30, 255.44

41. R. M. Shawar, F. A. el-Zaatari, A. Nataraj and J. E. Clarridge,J. Clin. Microbiol., 1993, 31, 61.45

42.G. A. Thomas, D. L. Williams and S. A. Soper, Clin. Chem.,2001, 47, 1195.46

43.A. M. Mohamed, D. R. Bastola, G. P. Morlock, R. C. Cookseyand S. H. Hinrichs, J. Clin. Microbiol., 2004, 42 , 1016.47

44.V. J. Tevere, P. L. Hewitt, A. Dare, A. Keen, J. P. Spadoro andK. K. Young, J. Clin. Microbiol., 1996, 34, 918.48

45.C. T. Michelon, F. Rosso, K. B. Schmid, R. D. Sperhacke,M. M. Oliveira, A. L. Kritski, L. Jr Rezende, E. R. Costa,A. W. Ribeiro, M. Verza, P. I. Cafrune, M. S. Silva,D. Kuhleis, A. Zaha and M. L. Rossetti, Mem. Inst. OswaldoCruz, 2011, 106, 194.49

46.C. Drost, M. Panning and S. Hug, Clin. Chem. , 2003 49, 1659.50

47. F. Broccoli, P. Scarpellini, G. Locatelli, A. Zingale,A. M. Brambilla, P. Cichero, L. A. Sechi, A. Lazzarin,P. Lusso and M. S. Malnati, J. Clin. Microbiol., 2003, 41,4565.51

48.K. Baba, S. Pathak, L. Sviland, N. Langeland, A. A. Hoosen,B. Asjo, A. M. Dyrhol-Riise and T. Mustafa, Diagn. Mol.Pathol., 2008, 17, 112.52

49.J. H. Kim, Y. J. Kim, C.-S. Ki, J.-Y. Kim and N. Y. Lee, J. Clin.Microbiol., 2011, 49, 173.53

50.S. A. Bustin, J. Mol. Endocrinol., 2002, 29, 23.54 J. Papaparaskevas, D. P. Houhoula, A. Siatelis andA. Tsakris, J. Clin. Microbiol., 2008, 46, 3177.55

51.P. Kumar, K. Nath, B. Rath, M. K. Sen, P. Vishalakshi,D. S. Chauhan, V. M. Katoch, S. Singh, S. Tyagi,V. Sreenivas and H. K. Prasad, J. Mol. Diagn., 2009, 11, 430.56

52.M. J. Taylor, M. S. Hughes, R. A. Skuce and S. D. Neill, J.Clin. Microbiol., 2001, 39, 1272.57

53. T. Luo, L. L. Jiang, W. M. the Sun, G. Fu, J. Mei and Q. Gao, J. Clin. Microbiol., 2011, 49, 3132.58

54.S. Sang, W. Zhang and Y. Zhao, Review, ON at The the Design Art of Biosensors, 2013.59

55.R. Thusu, Sensors Online (Strong Growth Predicted forBiosensors Market), 2010.60

56.Z. Dai, F. Yan, J. Chen and H. Ju, Anal. Chem., 2003, 75,5429.61

57.T. G. Drummond, M. G. Hill and J. K. Barton, Nat.Biotechnol., 2003, 21, 1192.62

58. M. Das, G. Sumana, R. Nagarajan and B. D. Malhotra, Appl.Phys. Lett., 2010, 96, 133703.63

59.M. Diaz-Gonzalez, M. B. Gonzalez-Garcia and A. Costa-Garcia, Biosens. Bioelectron., 2005, 20, 2035.64

60.C. Fernandez-Sanchez, M. B. Gonzalez-Garcia and A. Costa-Garcia, Biosens. Bioelectron., 2000, 14, 917.65

61.O. Ouerghi, A. Senillou, N. Jaffrezic-Renault, C. Martelet,H. Ben Ouada and S. Cosnier, J. Electroanal. Chem., 2001,501, 62.66

62.M. Das, C. Dhand, G. Sumana, A. K. Srivastava, N. Vijayan,R. Nagarajan and B. D. Malhotra, Appl. Phys. Lett., 2011, 99,143702.67

63.A. K. Pavlou, N. Magan, J. M. Jones, J. Brown, P. Klatser andA. P. Turner, Biosens. Bioelectron., 2004, 20, 538.68

64.K. A. Denton, M. F. Kramer and D. V. Lim, J. Rapid MethodsAutom. Microbiol., 2009, 17, 17.86 B. M. Hewitt, N. Singhal, R. G. Elliot, A. Y. H. Chen,J. Y. C. Kuo, F. Vanholsbeeck and S. Swi□, Environ. Sci.Technol., 2012, 46, 5414.87 95

65.S. C. Hsieh, C. C. Chang, C. C. Lu, C. F. Wei, C. S. Lin,H. C. Lai and C. W. Lin, Nanoscale Res. Lett., 2012, 7, 180.96

66.N. Prabhakar, K. Arora, S. K. Arya, P. R. Solanki,M. Iwamoto, H. Singh and B. D. Malhotra, Analyst, 2008,133, 1587.97 M. Duman and E. Piskin, BIOS. Bioelectron. , 2010, 26 , 908.98

67.G. N. Ferreira, A. C. da-Silva and B. Tome, TrendsBiotechnol., 2009, 27, 689.114

68.H. Mukundan, S. Kumar, D. N. Price, S. M. Ray, Y. J. Lee,S. Min, S. Eum, J. Kubicek-Sutherland, W. K. Grace,A. S. Anderson, S. H. Hwang, S. N. Cho, L. E. Via, C. IIIBarry, R. Sakamuri and B. I. Swanson, Tuberculosis, 2012,92(5), 407.128

69.A. M. Pariwono, T. Lo, C. S. Lim, S. X. Wang and Y. W. Chan,Journal of Biomedical & Pharmaceutical Engineering, 2007, 1,27.133 R. Fend, A. H. J. Kolk, C. Bessan, P. Buijtels, P. R. Klatserand A. C. Woodman, J. Clin. Microbiol., 2006, 44(6), 2039.This journal is © The Royal Society of Chemistry 2016RSCAdv.,2 016,6, 17759–17771 | 17771Review RSC Advances

: http://www.tbfacts.org/symptoms-tb/#sthash.JrzAsYxt.dpuf