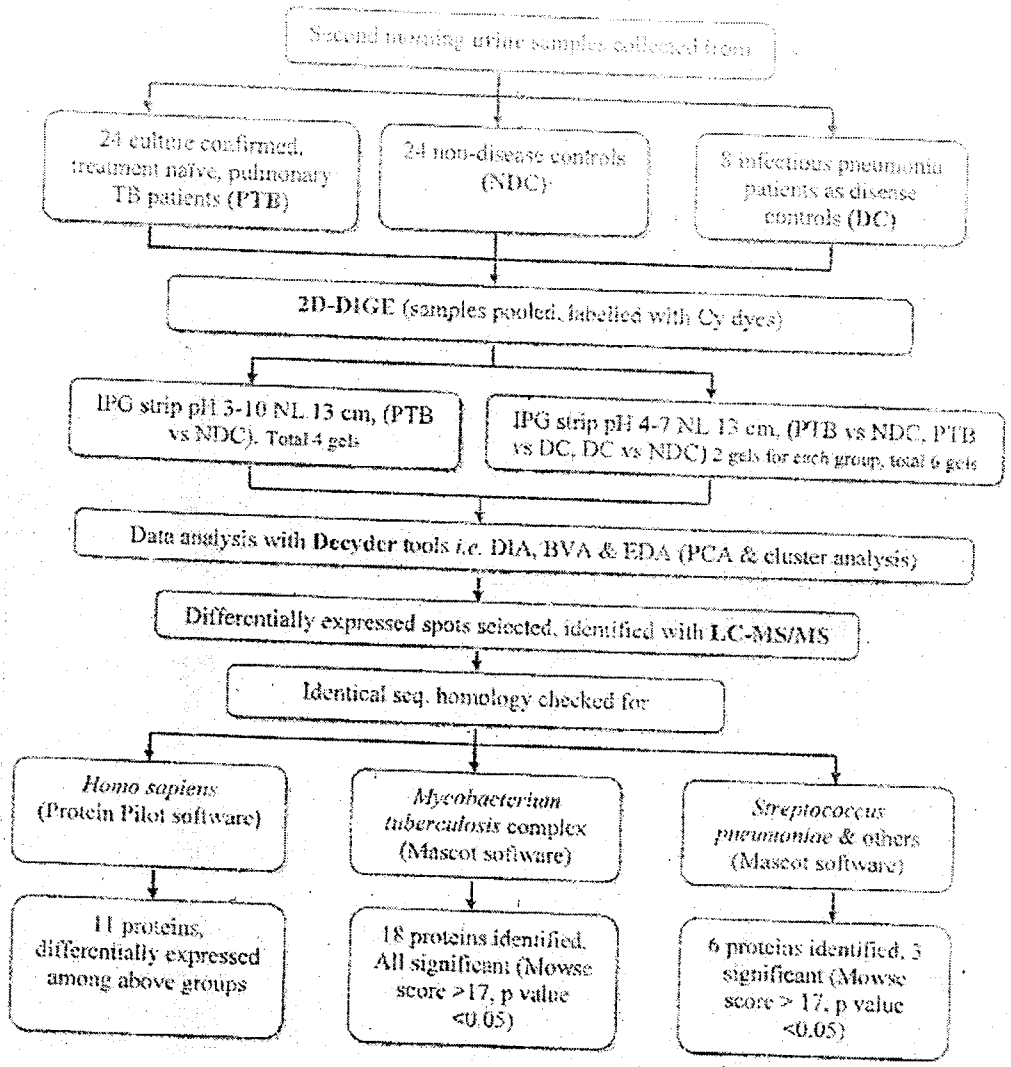

Figure 1

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FIELD OF THE INVENTION

The present invention relates to an assay for identification of mycobacterial antigens in urine of pulmonary TB patients.

BACKGROUND & **PRIOR ART OF THE INVENTION**

More than one-third of the world population is infected with *Mycobacterium tuberculosis,* the bacterium that causes the *Tuberculosis* (TB) disease. Each year, 8 million people become infected with TB, and 2 million people die from the disease. TB significantly affects developing countries and is also becoming an increasing problem in developed areas of the world. Persons infected with TB can be asymptomatic for a considerable period of time, and can be in a latent stage of the disease. In its active state, the disease is often manifested with an acute inflammation of the lungs, resulting in fever and a nonproductive cough. If untreated, serious complications and death typically result. Present diagnostic assays are often inaccurate, and are unable to distinguish between persons in the latent stage of the disease and those in the active stage.

Currently, vaccination with live bacteria is one method for immunizing persons against the disease. However, TB vaccination with certain live bacteria has often been the source of controversy in some countries. Additionally, current diagnostic tests are many times unable to distinguish between persons who have been immunized, and persons infected with TB. Effective vaccination and accurate early ***diagnosis*** of the disease are important to control the disease. Consequently, a need exists for effective diagnostic assays that detect active infection by the TB bacteria. A further need exits for a vaccine that does not use a live bacteria and provides a protective immunogenic response to the disease.

Tuberculosis is diagnosed definitively by identifying the causative organism *{Mycobacterium tuberculosis)* in a clinical sample (for example,sputum or pus). When this is not possible, a probable—although sometimes inconclusive—diagnosis may be made using imaging (X-rays or scans), a tuberculin skin test (Mantoux test), or a, Interferon Gamma Release Assay (IGRA).

The main problem with tuberculosis diagnosis is the difficulty in culturing this slow-growing organism in the laboratory (it may take 4 to 12 weeks for blood or sputum culture). A complete

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.medical evaluation for TB must include a medical history, a physical examination, a chest X-ray, microbiological smears, and cultures. It may also include a tuberculin skin test, a serological test. The interpretation of the tuberculin skin test depends upon the person's risk factors for infection and progression to TB disease, such as exposure to other cases of TB or immunosuppression.

New TB tests have been developed that are fast and accurate. These include polymerase chain reaction assays for the detection of bacterial DNA.[40] One such molecular diagnostics test gives results in 100 minutes and is currently being offered to 116 low- and middle-income countries at a discount with support from WHO and the Bill and Melinda Gates foundation.[41]

Another such test, which was approved by the FDA in 1996, is the amplified mycobacterium tuberculosis direct test (MTD, Gen-Probe). This test yields results in 2.5 to 3.5 hours, and it is highly sensitive and specific when used to test smears positive for acid-fast bacilli (AFB).

The lack of sensitivity in sputum smear microscopy, mycobacterial culture using solid media take more than a month, X-rays has poor sensitivity, specificity & reproducibility. Many alternate technologies such as PCR and cell mediated immune response reactions require trained personnel and specific laboratory conditions.

OBJECTIVE OF THE INVENTION

A basic object of the present invention is to overcome the disadvantages/drawbacks of the known art.

Another object of the invention is to provide an assay method for identification mycobacterial antigens in urine of pulmonary TB patients.

SUMMARY OF THE INVENTION

According to the one aspect of the present invention it is provided an assay for identification of mycobacterial antigens in urine of pulmonary TB patients. It is actually an immunochromatographic based diagnosis of tuberculosis. The antigens used in the immunochromatographic based test were identified by proteomics based approaches i.e. 2-D differential gel electrophoresis (2-D DIGE) and mass spectrometry.

DETAILED DESCRIPTION OF THE INVENTION

Previously unreported unique peptides have been identified that have shown identical sequence homologies with deduced amino acid sequences of 18 different M. tuberculosis complex proteins. These antigens can be incorporated into immunochromatographic based test for detection of mycobacterial antigens in urine samples of pulmonary tuberculosis patients.

Unique peptides have been identified among urine sample of pulmonary TB, using proteomics approaches i.e. two dimentional differential in-gel electrophoresis (2D-Dige) and mass spectroscopy (reverse phase chromatography coupled to ESI-Q-TOF-MS/MS).

Most sero-diagnostic assay developed so far based on detection of antibodies in sera. Presence of antibodies varies in different stages of disease and with immunological status like in case of TB-HIV co-infection. However, an interesting alternative approach is to direct identification of M. tuberculosis antigens in the body fluids of human with active disease. In present study, the identified mycobacterial antigens using proteomics profiling of urine and this lead to identification of novel proteins. These proteins can be used as antigens (biomarkers, which can be implemented to devise rapid diagnostic tool)

Using proteomics approach, differentially expressed unique peptides have been identified that have shown sequence homologies with the deduced amino acid sequences of 18 different M. tuberculosis complex. Identification of mycobacterial proteins in urine is significant because these proteins may prove more specific and sensitive for the disease.

Example 1

Second morning urine proteins from 24 pulmonary TB (treatment naive, culture confirmed for M. tuberculosis), 24 age matched non-disease controls and 8 disease controls (infectious pneumonia) were collected betting the inclusions criteria (Fig 1). Samples were processed and proteins were precipitated with chilled acetone. Samples were subjected to two dimentional-differential gel electrophoresis. Differential expressed proteins spots were selected using DeCyder software excised and trypsin digested for mass spectrometry. Eighteen proteins have been identified which shows identical sequence homologies with the deduced amino acid

sequences of M. tuberculosis proteins. All 18 proteins show significant Mowse score. Since some of these proteins are involved in important functions, they may have imperative role in diagnosis/pathogenesis of TB. These identified maycobacterial proteins could potentially be used as a candidate biomarkers aiding in diagnosis/prognosis of tuberculosis. Confirmation of pathogen-specific protein markers in the clinical specimen will provide a proof-of-concept. Validation requires ELISA or Western blot using specific monoclonal antibodies against identified mycobacterial proteins.

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