FIELD OF INVENTION:

The present invention relates to DNA diagnostic kit. The present invention in particular relates to DNA diagnostic kit for identification of different microorganisms as well as genetic diseases.

BACKGROUND AND PRIOR ART:

Identification of microorganisms is traditionally made by the combination of microbiological, biochemical, physical, in vivo animal experimentation, cell culture and other methods. This necessitates different infrastructure, biological and specialized expertise for identification of different microorganisms causing infection and diseases like cancer. These methods are expensive but are unavoidable need of human, animal and plants. The constituents are short lived and highly perishable and require cryo-preservation. All these methods involve purification of strains of microorganisms by repeated culture and subculture which is time consuming, cumbersome and expensive. In spite of all the above limitations, these methods, quite often lead to false positive or false negative detections, because of reasons like gene expression, presence of dead and debilitated microorganisms, insensitivities, interference growth, , cross reactivity etc.

Molecular methods of detections DNA probe is a well known molecular method with varied applications like identification of infection in man, animals and plants, identification of genetic diseases like cancer, identification of recombinant clone, DNA fingerprinting etc. It is also being used extensively in forensic sciences

Publication No. KR20120038430 provides a probe for detecting pathogenic microorganism causing diseases infected by sexual contact is provided to ensure high specificity and sensitivity.

Publication No. KR20110137642 provides a DNA chip containing a probe which complementarily binds to 44 types of HPV nucleic acids is provided to accurately diagnose complex infection of HPV and to predict cervical cancer

Publication No. CN101818213 discloses a gene chip for detecting human papillomavirus (HPV), comprising a solid carrier and a human papillomavirus detecting probes fixed on the solid carrier.

Publication No. KR100968360 describes a method for diagnosing breast cancer caused by variation of the number of Her-2 gene replication is provided to confirm amplification by variation of the number of Her-2 gene and to diagnose breast cancer.

Publication No. CN101240335 provides a gene chip for detecting common pathogen in dairy, which comprises a solid-phase vector and an oligonucleotide probe fixed on the solid-phase vector. It also provides a gene chip for detecting common pathogen in dairy, which comprises a solid-phase vector and an oligonucleotide probe fixed on the solid-phase vector, wherein the oligonucleotide probe includes DNA fragment

Publication No. CN101407837 describes a gene chip used for detecting blood pathogen and a kit used for detection. The gene chip includes a solid phase vector and an oligonucleotide probe fixed on the solid phase vector, wherein the oligonucleotide probe mainly comprises DNA segments selected from the 16S rRNA gene sequence.

Publication No. KR20080011257 provides a kit comprising a gene probe capable of detecting a gene of 16S rRNA commonly included in a waterborne pathogen is provided to be able to detect the waterborne pathogen contaminated by a plurality of samples and identify the detected pathogen individually, thereby being widely and effectively utilized for preventing waterborne infectious diseases.

Publication No. KR20100006282 describes a multiplex kit and chip for determining pathogen analysis and antibiotics resistance are provided to accurately confirm pathogen of respiratory infection and reduce antibiotics resistance rate.

Becton Drive,Franklin Lakes, USA provides BD Affirm™ VPIII DNA probe that offers a dependable, rapid means for the differential detection and identification of the causative agents for vaginitis: *Candida species*, *Gardnerella vaginalis* and *Trichomonas vaginalis*.. The test features an easy-to-read visible color reaction that is more accurate than current microscopic methods for detecting the causative agents of vaginitis.

PathVysion provides a HER-2 DNA probe which is designed to detect amplification of the HER-2/neu gene via fluorescence in situ hybridization (FISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens.

MetaSystems offers a wide range of high quality DNA probes routinely applied in genetics and allows nearly unlimited and targeted visualization of genomic DNA using either metaphase spreads, interphase nuclei, tissue sections, or living cells.

Life Technologies Corporation provides MicroSEQ® detection kit for simply, reliably and rapidly detecting Listeria spp. in food and environmental samples. The assay uses the polymerase chain reaction (PCR) to amplify a unique microorganism-specific DNA target sequence and a TaqMan® probe to detect the amplified sequence. This system comes with a PrepSEQ™ Nucleic Acid Extraction Kit.

However, the kits available in prior arts have remained as a high--tech and an expensive affair dealing with highly degradable and perishable components requiring adequate expertise and laboratory facilities.

The present invention provides -stable, inexpensive DNA diagnostic kit and a technology for the identification of microorganisms as well as genetic diseases such as but not limited to cancer even under field conditions.

OBJECTS OF THE INVENTION:

The principal object of the present invention is to provide, ready to use diagnostic kit provided in the form of dry powder with minimum aqueous stable components like the DNA probe.

Another object of the present invention is to provide a diagnostic kit which detects microorganisms directly from clinical samples without the need for time consuming and cumbersome strain purifications

Yet another object of the present invention is to provide a diagnostic kit which is simple, inexpensive and uniform for all microorganisms and genetic diseases such as but not limited to cancer.

Still another object of the present invention is to provide a diagnostic kit which can be used by semi skilled persons

Yet another object of the present invention is to provide diagnostic kit having extended shelf life under ambient conditions.

Still another object of the present invention is to provide a diagnostic kit which dispenses the necessity of a large and different infrastructure and different expertise for identification of different infections and diseases.

At the outset of the description that follows, it is to be understood that the ensuing description only illustrates a particular form of this invention. However, such a particular form is only an exemplary embodiment and is not intended to be taken restrictively to imply any limitation on the scope of the present invention.

SUMMARY OF THE INVENTION:

The present invention provides a technology and a diagnostic kit in the form of dry powder with minimum aqueous stable components like the immortalized DNA probe in a ready to use state. The kit is simple, inexpensive and uniform for all microorganisms and genetic diseases such as but not limited to cancer. The kit has an extended (may be infinite) shelf life under ambient conditions. The disclosed kit is capable of detecting almost every infection and genetic diseases

such as but not limited to cancer simply by changing the DNA probe sequence. Being uniform, it can be used by semi skilled workers like the technician, in Reference Laboratories or even under Field Conditions.

The present invention dispenses the need of a large and different infrastructure and different expertise for identification of different infections and diseases in human, animal and plants.

In a preferred embodiment of the present invention, the ambient stable, inexpensive DNA diagnostic kit is provided with an immortalized aqueous stable and ready-to-use DNA probe and it follows only a single, simple and uniform method for identification.

In another embodiment of the present invention, the DNA kit with an immortalized aqueous stable and ready-to-use DNA probe can be used for the identification of microorganisms as well as genetic and metabolic diseases in human, animal and plants, such as but not limited to cancer and under field conditions.

In yet another embodiment of the present invention, the DNA kit is provided with an immortalized aqueous stable and ready-to-use DNA probe on cellulose acetate (M/S Whatman 541) membrane which is not fragile unlike the commonly used nitrocellulose and also does not require baking at 80°C under vacuum for one hour or UV cross-linking as required for different membranes to retain the DNA on the membrane.

In still another embodiment of the present invention, the DNA kit is provided with ready-to-use immortalized DNA probe on cellulose acetate membrane.

In still another embodiment of the present invention, the DNA kit dispense the need for vacuum baking or UV cross-linking also dispenses pre-hybridization before DNA-DNA hybridization of test sample and also enables rapid detection and better sensitivity and specificity of detection than other routinely used membranes and methods.

In yet another embodiment of the present invention, the present DNA kit is provided with an immortalized aqueous stable and ready-to-use DNA probe that has been labeled or bound with Quantum Dots (QDs) or nanoparticles by the functional moiety on the particle.

In still another embodiment of the present invention, the immortal DNA kit can use chemical labeling and detection of DNA-DNA hybridization in an ambient-stable manner.

In yet another embodiment of the present invention, the kit dispenses the need of sterilization and pH adjustment

In yet another embodiment of the present invention, the DNA kit with an immortalized aqueous stable and ready-to-use DNA probe detect the sample by Auto-Fluorescence of the particle that can be viewed under UV lamp/torch or by naked eye, preferably in dark.

In still another embodiment of the present invention, the present DNA kit can use fluorescent dye &/or enhancers to enhance the signal enabling easy detection of DNA-DNA hybridization on white membrane.

Yet another embodiment of the present invention is that the quantum dots or nanoparticles can be prevented from joining up by silver, silica etc. coating.

In still another embodiment of the present invention, the DNA kit can be used on a solid matrix or in liquid medium for hybridization.Most of the Quantum Dots used are fluorescent material. Fluorescent dye like Poly-fluorine, Rhod-5N etc. further amplifies the signal. Silver, Silica etc coating containing Fluor chrome, Luminol etc. prevent joining up of the Quantum Dots and also increases the signal intensity.

BRIEF DESCRIPTION OF THE DRAWINGS:

It is to be noted, however, that the appended drawings illustrate only typical embodiments of this invention and are therefore not to be considered for limiting of its scope, for the invention may admit to other equally effective embodiments.

Fig. 1: illustrates the ZnS - Mn+2 doped nanoparticles viewed under UV lamp before binding with DNA for a probe.

DESCRIPTION OF THE PREFERRED EMBODIMENTS:

Accordingly, the present invention relates to a durable, ambient-stable, inexpensive DNA diagnostic kit with an immortalized aqueous stable and ready-to-use DNA probe and a simple method for the identification of microorganisms as well as genetic and metabolic diseases in human, animal and plants, such as but not limited to cancer and under field conditions, as a sustainable technology. The present invention provides a diagnostic kit in the form of dry powder with minimum aqueous stable components like the DNA probe in a ready to use state. The method involved is simple, rapid, inexpensive and uniform for all microorganisms and genetic/ metabolic diseases such as but not limited to cancer in human, animal and plants.

DNA probe:

DNA diagnostic kit uses immortalized aqueous stable and ready-to-use DNA probe on cellulose acetate (M/S Whatman 541) membrane and performs DNA-DNA hybridization with DNA probe for detecting or identifying test or clinical samples.

Solid Matrix for DNA-DNA hybridization:

The present DNA diagnostic kit uses cellulose acetate (M/S Whatman 541) membrane which is not fragile unlike the commonly used nitrocellulose. This membrane has the added advantage that even intact microbial cells or their DNA or intact cells can be spotted directly or cells briefly contacted from a agar culture plate (by Dot blot or Colony hybridization) on the membrane and lysed.

Also the double stranded DNA is denatured to single stranded form and the single stranded DNA then again is bound to the membrane easily by a rapid and single step of exposure to alkali

and steam. This membrane gets hydrated very fast and also dispenses the need for baking at 80°C under vacuum for one hour, UV cross-linking etc. to retain the DNA on the membrane. It also dispenses the need for pre-hybridization before hybridization. It enables rapid detection and the sensitivity of detection is better than other routinely used membranes.

Fluorescent Dye:

The present DNA diagnostic kit can also use fluorescent dye &/or enhancers to enhance the detection signal. The oligonucleotide / polynucleotide or the DNA has been bound/labeled by Quantum Dots or nanoparticles by the functional moiety on the particle. Auto-Fluorescence of the particle can be viewed under UV lamp/torch (Fig.1) or by naked eye preferably in the dark. DNA-DNA hybridization on white membrane can be detected easily. The signal can be enhanced by enhancers or fluorescent dye which will further improve the detection.

The present DNA diagnostic kit has an added advantage of detection of microorganisms directly from clinical and *in situ* (biopsy/ autopsy) samples without requiring any time consuming and cumbersome strain or gene purifications. It also dispenses the need for animal experimentations which is discouraged due to ethical considerations. The present diagnostic kit also does not require large and different infrastructure and different expertise for identification of different infections and diseases in human, animal or plants. It enables rapid, simple and inexpensive detections of any infection or genetic or metabolic diseases in human, animal and plants.

Numerous modifications and adaptations of the system of the present invention will be apparent to those skilled in the art, and thus it is intended by the appended claims to cover all such modifications and adaptations which fall within the true spirit and scope of this invention.