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DIAGNOSIS OF PARASITIC: THEN AND NOW

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ABSTRACT

Objective: The geographic location of patient and clinical and travel history beside the clinical symptoms is the first step for diagnosis of parasitic disease. Also, the microscopic examination is main step for diagnosis of parasites. The parasites are divided to intestinal, blood, on skin, cerebral, hepatic, or ocular. So, the samples, which is taken from patient may be stool, blood, sputum, skin scrub, urine, saliva, vaginal swap, or biopsy. The test for diagnosis of human parasites may be need serum to detect the antibodies or antigen to know the kind of parasite. Also, sometimes, need the X-ray, CT, or animal inoculation. **Methods:** The diagnosis of parasitic diseases take many steps. -At first: managing the sample administrative as laboratory identification number and an outbreak identification label, storage by refrigeration or freeze. -Second: the methods of laboratory were divided into two types, direct and indirect. **Results:** The most recent techniques are Proteomic fingerprints, Nanotechnology, Biosensor. The recent tests are RT-PCR, LAMP, Luminex, that allows the detection of various targets simultaneously. RARD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), RFLP (restriction fragment length polymorphism), ELISA: FAS-ELISA, RIPA-ELISA and Tri-combo. Since lot a years ago the diagnosis of parasitic diseases was taken several steps, initially from macroscopic examination, ending by molecular techniques. The direct method – look for /detect the agent, Indirect method – detect host response to the agent. Numbers of immunological/serological techniques have been emerged such as (CFT), (ID), (IHA), Direct or Indirect immunofluorescent antibody test (IFA). **Conclusion:** Today the tests is improved to give most accurate result and specific. Also, to short the time of diagnosis for accelerate the treatment. In future study most focused on identifying of a specific proteins which it circulate in patient' serum. Also, in future, the physicians are will change there vision for using medical nanotechnology.

KEYWARDS: Parasites, diagnosis, diseases, ELISA, Nano-scale, biosensor, fingerprinting.

INTRODUCTION

The geographic location of patient is the first step for diagnosis of parasitic disease as well as clinical and travel history beside the clinical symptoms. In past century the microscopic examination is main step for diagnosis of parasites. The parasites are divided to endoparastie which live in or on the host (protozoa and heminthes) and ectoparasite as arthropods. So, the parasites may causes infection in digestive tract by food and drink transmission; skin, through pentation of larvae when patient exposed to infected soil or water, and blood infected by insects bite the skin and sucking blood and inject parasites into human blood. The specimens, which are taken from patient may be stool, blood, plasma, sputum, urine, saliva, CSF, vaginal swap, or biopsy, anal swab, duodenal aspirate, and skin scrub, tissues and aspirates may be needed to diagnosed. Serum are needed to detect the antibodies or antigen to know the kind of parasite. Also, sometimes, need the X-ray, CT, or animal inoculation. Since past century and lot a years ago, the diagnosis of parasitic diseases was taken several steps, initially from macroscopic examination, ending by molecular techniques, and still most laboratory used these tests. The diagnostic methods may be direct – look for /detect the agent; or Indirect – detect host response to the agent.

Samples are arrived to laboratory Soon, the samples reached to laboratory,

-At First: managing the sample administrative as laboratory identification number and an outbreak identification label, storage by refrigeration or freeze.

-Second: the methods of laboratory were divided into two types, direct and indirect.

Direct Methods

Macroscopic evaluation (Consistency rice water stools for Cholera, Blood, Visible parasites, helminths segments); **Direct microscopy** (Wet mount technique, Hanging drop, Dark background microscope to fragile organisms e.g. spirochetes, Viability maintained mobility may be observed) [Observations white blood cells (denotes invasion), red blood cells, parasites protozoa, helminths eggs, moving bacteria]; **Electron microscopy** Being replaced by antigen detection; **Staining** Aspecific staining as Gram staining, Specific staining with chemicals as Ziehl Neelsen staining (Mycobacteria), Modified Ziehl Neelsen staining *(Cryptosporidium*). labelled antibodies like Immunofluorescence is considered Specific staining, used when gram stain cannot confirm diagnosis; Schaudinn's fluid **used as Fixatives** for the concentration as well as merthiolate iodine-formalin, sodium acetate-acetic

acid-formalin (SAF), or 5% or 10% formalin. The fixatives for the permanently stained smears include iron hematoxylin, trichrome, modified polyvinyl alcohol (PVA) (containing mercury compounds), Ziehl-Neelsen stains and SAF. Parasites and ova are examined in a minimum of three stool samples over no more than 10 days is recommended, Main techniques:- direct agglutination: cards, slides; -latex agglutination: slides, cards; -immuno-chromatography: dipsticks, Molecular methods and Propagate the agent.

INDIRECT METHODS

Detect by **antibodies** against the agent (Precipitation, Agglutination, Haemagglutination **(IHA**) and haemagglutination inhibition, Radio-immunoassays, Immunoblotting, Immunochromatography, Indirect latex agglutination, Immunoelectrophoresis, the amebic gel diffusion test, Complement fixation (**CFT)**, Indirect Immunofluorescence assay **(IFA**), and Enzyme-Linked Immunosorbent assay (**ELISA**). Many different assays have been developed for the detection of antibodies, including **Precipitation** soluble antigen combines with its specific antibody.[1] Antigen-antibody complex is too large to stay in solution and precipitates as **flocculation test** because the precipitated product is forced to remain suspended, it sensitive for antigen detection, Limited applications, Time taken-10 minutes; **immuno-diffusion test**, **counter-immuno-electrophoresis** (CIEP).[2] **Direct agglutination** combination of an insoluble particulate antigen with its soluble antibody forms antigen-antibody complex particles clump/agglutinate used for antigen detection. **Indirect agglutination precipitation reaction** converted into agglutination - coating antigen onto the surface of carrier particles like red blood cells, gelatin, latex, bentonite background clears as latex agglutination, co-agglutination passive hemagglutination (treated red blood cells made resistant). **Reverse passive agglutination** antigen binds to soluble antibody coated on carrier particles and results in agglutination detects antigens. Advantages of tests are sensitive for antibody detection, Limitations, Prozone phenomenon: requires the right combination of quantities of antigen and antibody, handled through dilution to improve the match, Time taken 10-30 minutes. **Hemagglutination** many human viruses have the ability to bind to the surface structures on red blood cells from different species thereby causing agglutination. **Hemagglutination inhibition** Antibodies to the virus in the patient serum bind to the virus; blocks binding sites on the viral surfaces prevents the virus from agglutinating the red cells. Advantages highly specific can be used as gold standard; Limitations technically demanding, time consuming, cannot distinguish IgG from IgM; Time taken 1 day.[3] **Immunoblot:** Used for discrepant analysis, Highly specific Rapid kits available, Limitations Cost, Concern

validated data, Time taken 1 day. **Radio-immunoassays** Radioactively labelled-antibody (or antigen) competes with the patient's unlabelled antibody (or antigen) for binding sites on a known amount of antigen (or antibody) Advantages: highly sensitive can be used for detection of small quantities, quantification possible; Limitations, expensive requires isotopes; Time taken 1 day**. Latex agglutination test** includes some of the advantages, they are, Ability to obtain semi quantitative results, A low individual test cost, relatively short time to obtain results as well as Immune-chromalography (dipsticks) and quantitative. **Complement fixation tests** appear to be less sensitive than others, cost more to perform, most laboratories are not used it. IHA is highly specific to perform and has been shown to be a (99.1%) diagnostic tool in human immune deficiency virus-infected patients presenting with gastrointestinal symptoms. **Immuno-chromatography**: (1) Dye-labelled antibody, specific for target antigen, is present in a plastic well provided with the strip or on the lower end of nitrocellulose strip. Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line either antibody specific for the labelled antibody, or antigen, is bound at the control line. (2) If antigen is present, some labelled antibody will be trapped on the test line. Excess-labelled antibody is founded on the control line Advantages: Commercially available single use, rapid test easy to perform, can detect antigen or antibody, can be used in the field limitations, cost concern validated data. **T-cell response** Intra-dermal injection of antigen (e.g. Tuberculin skin test), some don't consider this a laboratory test against the agent. Advantages of T- cell are very specific and sensitive assay for tuberculosis easy to perform. Disadvantages delayed response (few days), patient has to be seen twice. **Interferon.** However, this test requires experience in culture and subsequent antigen preparation, making it difficult to explain in a routine clinical laboratory. Serum IgG antibodies persist for years after like in *E. histolytica* infection[4], whereas the presence of IgM antibodies is short lived and can be detected during the present or current infection. The IgM levels become decreased time by time to become zero results at 6 months or 100% becoming negative by 46 weeks after treatment. A negative test therefore indicates that a patient never had invasive amebiasis,[5] In addition to the abovementioned clinical assays, research-based detection tests used of monoclonal antibodies against a lectin-rich surface antigen**,** a lipophospholglycan, a 170-kDaadherence lectin amebic antigen detected in saliva, and an uncharacterized antigen.

The main recent tests are using today improved to give most accurate result and specific. Also, to short the time of diagnosis for accelerate the treatment. Initially by:

1- **Proteomic fingerprints** : this technique has been used to the study of serum biomarkers of infectious diseases such -to identify new soluble biomarkers that my improve diagnostic tests, to investigate the proteins secreted by parasites using mass spectrometric analysis of conditioned culture media, and compared the two or more sp. Of one parasites with specific antibodies using sera collected from patients, approaches of proteomic present for identifying new parasite proteins that are detectable in patients. Many proteomic analysis are already published as in case of *T. cruzi*.[6] These analysis made it possible to obtain good understand for both ways of secretion used by different stages of parasites to release several proteins through the extracellular medium and many secreted proteins identified. Only a few studies have established first and second stage of human African trypanosomiasis CSF protein resulted. The patients have first or second stage give accurate markers to two proteins (osteopontin and beta-2-microglobulin).[7] Proteomic steps can identify proteins by bottom-up and Top-down approaches. Bottom-up steps are difficult to quantitate and cannot identify modified molecules. Top-down strategies seek to identify proteins and peptides in complex biological fluids. Such studies have focused on identifying a specific configuration of circulating serum proteins that are indicative of a specific pathophysiological state, a so-called "proteomic fingerprint.

Other techniques used for the expression analysis of proteins are: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry **(MALDI-TOF MS)**[8] and Surface-enhanced laser desorption ionization time of flight mass spectrometry (**SELDI-TOF MS)**[9] to be analyzed large numbers of clinical samples at same time, Techniques are allows sample binding to chemically active ProteinChip surfaces. ProteinChip divided to different properties, may be chemical (cationic, anionic, metallic, hydrophobic, or normal phase) and biological (enzymes, antibody, receptors). Recently, **Proteomic fingerprints** most be study of serum biomarkers of infectious diseases such as acute phase of Severe Acute Respiratory Syndrome; African trypanosomiasis; fascioliasis; cysticercosis; and Chagas diseases.[10] -Liquid chromatography mixed with mass spectrometry (**LC-MS**) is now a routine technique with the development of electrospray ionization[11] **(ESI**) providing a simple and active interface**.** It can be used to a wide range of biological molecules and the use of one by one mass spectrometry and stable isotope internal standards allows highly sensitive and accurate assays to be developed although some method optimization is required to minimize ion suppression effects. When scan speeds are fast they allow a high degree of mixed and many compounds measured in a single analytical run. That is to say mass

spectrometers converted the analytic molecules to ionized state, with subsequent analysis of the ions and any fragment ions that are produced during the ionization process, on the basis of their mass to charge ratio (m/z). Isotope-coded affinity tags **(ICAT**) is an isotopic labeling method used for quantitative proteomics by mass spectrometry that uses chemical labeling reagents**.** Isobaric tags for relative and -Absolute quantification **(iTRAQ**) is an isobaric labeling method used in quantitative proteomics by tandem mass spectrometry to determine the amount of proteins from different sources in a single experiment. The real potential of proteomic fingerprinting is in its use as immunologically based antigen-detection tests that could be implemented in dipstick or cassette formats.[12]

2-**Nanotechnology** is a major recent technique affecting medical approaches to treatment the disease and immunity induction. Nanotechnology provides the chance to meet microbes and immune systems at a molecular level. There are most applications for nanotechnology in the area of infection and immunity, some of the earliest and potential promising developments have been in the development of antimicrobial agents and new vaccines. Nanotechnology allows materials to be custom-designed on a molecular scale and enables the design of new and different devices. In recent years, the significance of nanotoxicology has started to become recognized and studied and understanding of the effects of nanotechnology on human health. There are also major economical, political, social and environmental ramifications associated with this technology. Nanotechnology technique opened many new approaches to treating and preventing infectious disease, Nanoparticle antimicrobials and vaccines are examples of how this new technology is likely to be used in the coming decade. Physicians will have a major role in policy-setting, technology implemention and answering the questions of a concerned and curious public. Nanotechnology exactly means any technology performed on a nano-scale that has practical applications to our every-day occurring daily activities. This can offer an improvement in imaging and diagnosis of the fatal parasitic diseases as well, slipping away the limitations of some biological barriers.[13] In the parasitology field the use nanotech have only a few applications and the research have been reported on *leishmania* sp. And *plasmodium* sp. Nano-particles have also shown improvement in very complicated treatments like cerebral malaria by *Plasmodium falciparum* infection. In Cuba and Brazil some project are currently running using nanopeptides as a preliminary test against ruminant helminthes (*Fasciola hepatica*). This technology can also allow the development of adjuvants for veterinary parasite vaccines, boosting their present low efficacy. Newly, a nano-microparticulated malaria vaccine elicited long-lasting

protective antibody titers with only a single dose. Although, the nanotechnology is rapidly changing the face of medicine, most physicians are still unable to define it.

3-**Biosensor**: A biosensor is an analytical device that converts molecular recognition of a target analyte into a measurable signal via a transducer. For parasitic diseases, biosensors consider an easy-to-use, inexpensive and sensitive technology platform that can identify parasite rapidly and predict effective treatment**.** The advantages of biosensors include small fluid volume manipulation (less reagent and lower cost), low energy consumption, short assay time, high portability, high through put and multiplexing ability. For the diagnosis of malaria various biomarkers have been used among them the plasmodial lactate dehydrogenase and histidine-rich protein 11 (HRP 11) has received increasing attention.[14] A low-cost biosensor system was also made with nanostructured films containing specific *leishmania* amazonensis and *T. cruzi* antigens and employing impedance spectroscopy as the detection method. Some physicians are used favorited the next tests or techniques because the famous laboratories facilitated it. These techniques are considered Molecular tests as **-PCR** and **RT-PCR** (real-time polymerase chain reaction). Four basic components for PCR: A DNA template, Primers, A heat-stable DNA-polymerase enzyme, Free nucleotides. Real-time PCR (RT-PCR) Principle: This method utilizes an additional primer, the probe, which also binds specifically to the target DNA sequence. Probes have a fluorescent 'reporter' dye at one end and a 'quencher' dye, which inhibits fluorescence, at the other.[15] The fluorescence are measured at each cycle and increases the number of copies produced. Advantage: Quantitation, This eliminates gel electrophoresis thereby greatly reducing the risk of contamination and the introduction of false-positives. **-LAMP** (loop-mediated isothermal amplification): is a powerful innovative gene amplification technique emerging as a simple rapid diagnostic tool for early detection and identification of microbial or parasitic diseases. (LAMP) is a unique amplification method. It is considered the six different primers specifically designed to recognize eight distinct regions on a target gene, with amplification only occurring if all primers bind and Stampa product.[16] Recently, parasitologists used the LAMP approach to diagnose of several parasitic diseases as *Entamoeba*[17]; *Trypanosoma*[18]; *Taenia*; *Plasmodium*[19]; and *Cryptosporidium*[20], and *Theilera* and *Babesia* as the animal parasites and even to the identification of vector mosquitoes carrying *Plasmodium* and *Dirofilaria immitis* parasites**.** Most of these studies explain the many advantages of PCR technique. In addition, the reaction can be carried out without extracting the DNA from the collected samples as shown in the case of RIME, a nonautonomous retroelement found in

Trypanosoma brucei rhodesiense and *T. b. gambiense*.[21] In 35 minutes, using a simple water bath, RIME LAMP was able to detect both *T. b. rhodesiense* and *T. b. gambiense* directly from serum, blood and CSF samples. Also, LAMP reactions are easy to set up, and results can readily be assessed. This sample is interest with primers, substrates, and a DNA polymerase capable of strand displacement in a microcentrifuge tube. During the reaction, large amounts of pyrophosphate ions are produced, leading to the formation of a white precipitate. This turbidity is done by DNA synthesized as one can assess the reaction of real-time measurement of turbidity simply through the naked-eye. For example, the detection *B. bovis* and *B. bigemina* from DNA extracted from blood spotted on filter paper. Similarly, Han ET, et al. implemented a LAMP assay based on the 18S rRNA gene for the detection of the four human *Plasmodium* species (*falciparum, vivax, malariae,* and *ovale*). -**Luminex** (is a bead-based flow-cytometric assay) that allows the detection of various targets simultaneously. Luminex is a bead-based xMAP technology, a system that combines flow cytometry, fluorescent microspheres (beads), lasers and digital signal processing, and is capable of simultaneously measuring up to 100 different analytes in a single sample Luminex Corporation. It is possible to cover each set of microsphere beads by utilizing a reagent specifically designed for a particular bioassay. This procedure enables the capturing and detection of specific analytes from a given sample. The microspheres can be covalently linked to antigens, antibodies or oligonucleotides, which serve as probes in the assay. DNA extractions, using oligonucleotide-specific probes for the ML-2 regions of each species, without the need for DNA sequencing. After about five hours, being faster and less expensive than PCR followed by DNA sequencing. Luminex considered specific and sensitive than direct immunofluorescence (DFA) by 100%, Luminex is identifying species of *Cryptosporidium* and *Giardia*, but direct immunofluorescence is not able to differentiate *C. hominis* from *C. parvum*, opposed of PCR. Molecular and Luminex techniques for diagnosis of parasite infection as *Cryptosporidium* spp., *C. parvum,* and *Giardia duodenalis*, increase sensitivity and specificity. Also in relation to the *Plasmodium,* the Luminex technology could detect all kinds of the four human *Plasmodium* species (*falciparum, vivax, malariae* and *ovale*) simultaneously in blood samples. Luminex can improve the accuracy, speed, and reliability of other PCR test[22], in addition to costing less per test than other molecular techniques. -**RAPD** (random amplified polymorphic DNA): Generally, RAPD is the method used to delineate strains of microorganisms. RAPD markers determined species of genetic structure of different microorganisms. RAPD is particularly useful for studying the genetic structure of populations because it reveals polymorphisms in the noncoding regions of the

genome, Technique is able to differentiate species of *Leishmania*[23], and polymorphisms of parasites of medical importance such as *Plasmodium* and *Trypanosoma,*[24] Analysis based on**:**

a) -**AFLP** (amplified fragment length polymorphism) polymorphic markers have discovered high genetic variability among the genome species of *Leishmania major, L. donovani. and L. tropica*, which was sufficient to distinguish between, cutaneous leishmaniosis (CL) and visceral leishmaniosis (VL).[25]

b) - RFLP (restriction fragment length polymorphism) This technique is currently one of the most commonly used molecular methods for diagnosis of species and genotypes of parasites such as *Toxoplasma gondii*.[26] This technique was first used to detect variations at the DNA level. This reaction is based on the digestion of the PCR products by restriction enzymes or endonucleases. These enzymes divided DNA into peace of certain sizes, whose analysis on agarose or polyacrylamide gel results in different patterns of fragment sizes, enabling the identification. The RFLP technique is suitable for environmental samples because it permits the detection of multiple genotypes in the same sample.

-ELISA: FAST-ELISA (falcon assay screening test) consists of using synthetic and recombinant peptides to evaluate antibody responses to an antigen. In the past, this technique is applied to the study of malaria[27], schistosomiasis, fasciolosis[28], and taeniasis, and is subjected to the same drawbacks as most serology-based tests. Cross-react with proteins of different species is done when antibodies increased against a peptide from one parasite protein. No recent studies have been published on the use of the FAST-ELISA for the diagnosis of parasitic infections.

-Dot-ELISA: The main difference between the regular ELISA and the dot-ELISA lies in the surface used to bind the antigen of choice. In the dot-ELISA, the nitrocellulose or other paper membrane used instead of plastic plate onto which a small amount of sample volume is applied. The dotted membrane is incubated first with an antigen-specific antibody followed by an enzyme-conjugated anti-antibody, then chromogenic substrate causes the formation of a colored dot on the membrane which can be visually read. The benefits of this technique include its ease of use, its rapidity, and the ease of result interpretation. It is fast, and high cost-effective (e.g., as a dipstick). For all these reasons, the Dot-ELISA has been and still is extensively used in the detection of human and animal parasitic diseases. In the last few years, some studies used of the dot-ELISA for the detection of *Haemonchus contortus*,

Fasciola gigantica, *Theileria equi*, *Trypanosoma cruzi* and *Trypanosoma brucei*. Due to the better sensitivity and specificity of dot-ELISA, the researchers were able to demonstrate that ELISA in the detection of antineurofilament and antigalactocerebrosides antibodies in cerebrospinal fluid of subjects infected with African trypanosomes. They considered the most sensitivity and specificity of the dot-ELISA to the use of the nitrocellulose membrane and showed that their assay was successfully reproducible in the field.[29]

-RIPA-ELISA (radioimmunoprecipitation assay) is a lysis buffer used to lyse cells and tissue, for radioimmunoprecipitation assay (RIPA**).** The RIPA buffer gives low background but can denature.

-The **TRI-COMBO PARASITE SCREEN** test is an enzyme immunoassay for the simultaneous qualitative detection of *Giardia* spp., *Cryptosporidium* spp. and/or *E. histolytica* antigen in human fecal samples. The test is done for use as a screen for fecal samples from patients with gastrointestinal illness including dysentery may be giardiasis, cryptosporidiosis or amebiasis. In the technique, divided of a diluted fecal samples is transferred to a microassay well. The immobilized monoclonal antibodies bind the *E. histolytica, Giardia,* and/or *Cryptosporidium* antigens if they are present. Conjugate is added to the antigen-antibody complex. Any unbound materials are removed during the washing steps. After Substrate add, a color develops in the presence of enzyme-antibody-antigen complexes that formed in the presence of antigens and Conjugate. The Tri-Combo ELISA provided by TechLab, Inc (Blacksburg, VA) is a conventional two-step ELISA format with HRPconjugated detecting antibodies for colorimetric development was designed to simultaneously screen stool specimens for, *Cryptosporidium spp., Giardia lamblia. and E. histolytica* using a single assay well. For these specimens with discrepancy results between the Tri-Combo versus individual specific antigen test, RT-PCR analysis was conducted.[30]

-Immuno-fluorescenceDetection Direct immuno-fluorescence, used to detect antigen; Indirect and Sandwich immuno-fluorescence to Antigen detection and Antibody detection. **IFA** technique was shown to be strong, rapid and reproducible and helps to differentiate ALA from other nonamebic etiologies. Also, IFA tests have been shown to differentiate between treated disease and present disease. Some study following of immunoglobulin M (IgM) levels of clinical value in cases of invasive amebiasis by using the IFA test.[31]

-Buffy Coat Test (QBC) Quantitative buffy coat smear: Parasites may be concentrated by micro-haematocrit centrifugation using glass capillary tube and closely fitting plastic insert

(QBC malaria blood tubes, Becton Dickinson, Sparks, Md, USA). The QBC (quantitative buffy coat) tube is a specially prepared glass haematocrit tube, pre-coated internally with acridine orange stain and potassium oxalate. A volume of 56-65 micro-litres of blood collected from finger, ear or heel puncture and centrifuged at 12,000 rpm for 5 minutes. Normal red cells are more dense than RBCs containing malarial parasites and concentrate just below the leucocytes at the top of the erythrocytic column. The parasite contains DNA but the mature RBCs do not contain DNA and RNA. Parasitic DNA appear as bright specks of light among the non-fluorescing erythrocytes when is stained by acridine orange stain and When the QBC malaria blood tubes are rotated under a special type of lens, almost all the plasmodia in the blood sample can be visualized. So the direct methods in parasitology of monocellular organisms can be propagated by culture media. The indirect diagnosis are molecular methods, detecting antibodies against the agent, T-cell response against the agent, and interferon.[32]

CONCLUSION

Today the tests is improved to give most accurate result and specific. Also, to short the time of diagnosis for accelerate the treatment. In future, study most focused on identifying of a specific proteins which it circulate in patient' serum. Also, in future, the physicians are will change there vision for using medical nanotechnology.

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