CLINICAL SIGNIFICANCE OF FLOURESCENCE SPECTROSCOPY

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Abstract: There are number of techniques and methods available for diagnosis of bacteria, viruses, fungi and parasites. On account of its demerits, emerging techniques called fluorescence spectroscopy with its high sensitivity and specificity come into picture that significantly produces immense impact on diagnostic and research area. Fluorescence spectroscopy helps in viewing the color spectrum of porphyrins. Fluorescence spectroscopy is a convenient choice for detection of chlorophylls and in food quality evaluation and analysis, it is used for determination of numerous food components, contaminants, additives, and adulterants. Recent studies suggested that the fluorescence spectroscopy is used to evaluate antioxidant contents and antioxidant capacity in roasted coffee extract. Also to study of dissolved organic matter (DOM) in beverages, it is viably used for many purposes. The current paper is review of study conducted by researchers for clinical importance of fluorescence spectroscopy.

Keywords*: Fluorescence spectroscopy, microbial detection, clinical advancement.*

Introduction:

From the previous studies done on emerging applications of fluorescence spectroscopy in medical microbiology field, it is suggested that infectious diseases are caused by microorganisms such as bacteria, viruses, fungi and parasites. Infectious diseases are major killer around the world especially in developing nation. For which there is a need to prevent the spread by detecting it with new diagnostic technique with high sensitivity and specificity. Currently available traditional diagnostic methods and techniques for microorganisms like bacteria take normally at least one day. Also, Antibiotic sensitivity testing is also required by physicians to choose specific antibiotic for treating infection.[1]

The technique classical fluorescence spectroscopy requires fewer samples pretreatment and is noninvasive, nondestructive, and sensitive.

The technique has proven to be very promising and useful in the study of organic and inorganic as well as clinical studies of microbes. This spectroscopy is used in many fluorescent structures original in soil organic components.[2]

Fluorescence spectroscopy is being applied in therapeutic microbiology field for various determinations. There are many findings where it has been proved that fluorescence spectroscopy is highly sensitive and specific for study of microorganisms associated diseases and diagnosis with the help of spectroscopic fingerprints. Fluorescence spectroscopy and Fluorescence Correlation Spectroscopy (FCS) can be used to study various pathophysiological steps. [3]

Fluorescence correlation spectroscopy (FCS) use the basic principle in which a fluorescing molecule shows a specific free diffusion velocity which is directly correlated with its size. The diffusion will be slower if the molecule is big. Diffusion or conformational fluctuations of bio molecules or artificial particles can be studied by using statistical deviations of the fluctuations in fluorescence.

Whereas the auto correlation functions (ACF) is used to extract the number and diffusion coefficient of fluorescent particles diffusing through the focus volume.[3]

Fluorescent profiles of bacteria e.g. S. pneumonia, and H. influenzae which are responsible for otitis Media in children have been studied. It was proved that a different specific Fluorescence profile was produced by each bacterium. The data shows that it may be an excellent fluorescence based diagnostic technique for otitis media. As per as the bacterial taxonomy concern, results proved that Fluorescence spectroscopy may be an excellent tool in polyphasic approach to pseudomonad taxonomy. This approach provide more information as compared to rRNA

and DNA bacterial homology grouping as they provide more information about differentiation between strains which are difficult to differentiate on PCR identification methods.[1]

It has also a proven studies on fungal taxonomy, Diagnosis of fungal infection is made either by morphological examination of fungi or by biochemical and molecular biology techniques. For rapid diagnosis of different fungi such as yeast, Microsporum gypseum, Microsporum canis, Trichophyton schoenleinii, Trichophyton rubrum, Epidermophyton floccosum and Fusarium solani, Fluorescence spectroscopic fingerprints method was used.[1]

Fluorescence correlation spectroscopy (FCS) studies discovered different binding means for an icosahedral virus.[1] Tryptophan which present in both viruses and host bacterial protein can be detected as it is fluorophore in UV .[1]

Fluorescence Spectroscopy for the studies of Bacterial Biofilms [4]:

Another review paper suggested that The Bacterial biofilms have a complex 3D architecture composed of a single bacterial species or of multiple species. These Microbial biofilms are responsible for range of oral and other diseases. Its efficient detection is necessary.

Bacterial metabolites (porphyrins) fluoresce in the red and infrared regions of the spectrum and hence important for diagnostic purpose .For detection of biofilms, particularly in dentistry fluorescence is used as a powerful tool.

Flushing, removal with gases, treatment with nitric acid, application of biocide like sodium hypochlorite, hydrogen peroxide, ozone, iodine etc. are used to control biofilms which causes blockage of narrow tubing. The typical water content of individual bacteria is 65% as bacterial biofilm volume is 65% water.

The calculation is made as the amount of energy absorbed by bacterial biofilms will vary according to the wavelength used, the concentration of the absorbing fluorophores and their absorption coefficient. The absorption of fluorophores in biofilms can be assessed across a range of possible excitation wavelengths via spectroscopy .The current level of technology deployed in dental practice for fluorescence diagnostics includes systems with LED illumination and charge-coupled device, complementary metal oxide semiconductors (CMOS) sensors. The intra-oral cameras are also installed which uses a continuous wave emissions from multiple LEDs as the light source. [3]

Food Analysis by fluorescence spectroscopy:

Determination of numerous food components, contaminants, additives, and adulterants can be done by using Florescence spectroscopy.

Earlier, the Fluorescence spectroscopy was only used for analysis of various vitamins and polycyclic aromatic hydrocarbons. The studies on this has significantly expanded and become a diversified, now the technique can also be applied to non-fluorescent species, using several physicochemical means such as chemical or photochemical derivatization reactions. Use of fluorescence spectroscopy in coffee authenticity studies enabled the discrimination of Brazilian green coffee with the other varieties of roasted coffee. Tea fluorescence contains anthocyanins, catechins, flavonol polymers,theaflavins and thearubigins, and chlorophyll which are all pigmented elements and can be easily detected by florescence spectroscopy. A fluorescence spectroscopy was developed to detect flavonoid content in terms of concentration in green tea brews. Fluorescence spectroscopy can be used to determine caffeine, class III, class IV caramel in soft drinks and energy drinks. It has several other applications in environmental aspects. Florescence spectroscopy can be used to characterize natural water, to monitor wastewater in surface waters, to trace sources of pollution and evaluation of drinking water quality by using univariate method. [1]

Conclusion: Fluorescence spectroscopy has a significant value for laboratory assessment of complex materials and mixtures, including food analysis and biochemical clinical detection and application. Porphyrins derivatives help in detecting the bacterial morphological as main clinical studies.

This highlights the value of fluorescence as a non-invasive aid to conventional clinical examination in detection and diagnosis of infected areas.

In food industries, for the detection of various elements in coffee, adulterants ,fluorescence Spectroscopy is predominantly used. Many more studies are being performed on fluorescence spectroscopy on the light of clinical aspects.

Thus the fluorescence spectroscopy is used as dynamic tool for clinical and industrial applications with few limitations.

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