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Immunosensor techniques (Electrochemical, SPR and QCM) used in Food Safety

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ABSTRACT: The greatest challenge confronted by food processing industries in both developed as well as developing countries are the detection of pathogens and fungal toxins. Immunosensors claims to be one such analytical method that offers high potential in food safety sector due to their low cost per analysis. Immunosensors have shown fast response and reduced sample preparations as compared to other conventional techniques used like HPLC, PCR and ELISA. Also, conventional and standard bacterial detection methods such as culture and colony counting methods, immunology-based methods and polymerase chain reaction based methods take up to several hours or even a few days to yield a result for the detection of bacterial pathogen and toxin in food. Immunosensors have shown potential of online or on -field applications, which saves lengthy and tedious job of detecting pathogens and toxins. The prospect of incorporating of nanoparticles have enhanced the potential of Immunosensors for monitoring of several analytes and miniaturisation especially in Immunosensors based on Electrochemical, Surface Plasmon Resonance and Quartz Crystal Microbalance techniques. Immunosensor technology can have an impact on food safety if the developed devices will be able to produce wide-ranging data on emerging pathogens. Sensitivity of Immunosensors still remains an area of concern, but further research will surely improve upon their features without compromising the cost.

***Keywords*:** Immunosensor, Biosensor, E.coli, PCR

INTRODUCTION

According to Asian development Bank Food safety is a foremost issue in both developed and developing countries. Food safety issue is much severe in developing countries as compared to developed countries (Lin et al, 2010), so there is a need of global collaboration to solve this issue through research and development of methods that are cost affective and portable (Parker and Tothill, 2008). Besides the significant progress to strengthen food safety system achieved by many countries and to reduce and prevent food-borne disease, unacceptable rates of food borne illness still remain and a very important problem which should be addressed (FAO/WHO, 2006).Food borne risks to human health can arise from hazards that are biological, chemical or physical. Of these the biological hazards and chemical hazards are most potent and require much attention specially the contamination cause by microorganisms and chemical contaminants including biological toxins (FDA Ranking). In food industries the outdated system of end product testing rather than controlling the process during the food production process causes many Food safety problems because when a consignment of food product has been produced ,it becomes very difficult to find the contaminants in the whole batch in the end because of the difficulty of high number of samples to be analyzed by a particular analytical method, more time is also consumed when end product testing is done because of two things reasonably high number of samples to be analyzed and contaminants can be heterogeneously distributed in a batch or consignment of product. Moreover, end-product testing detects only failures and does not identify causes.

The HACCP (Hazard Analysis Critical Control Point) is a very efficient system that is being employed in the food industries .It plays an important role in identifying

precise hazards and measures for their control because rather than relying on end-product testing ,it can be applied throughout the food process chain(Akdogan & Mutlu,2011). There are certain critical control points during food production process for example refrigeration of poultry at 74^{o}C is necessary, this is a CCP (25). A, sensitive and accurate method which can do complete testing within minutes or hours need to be employed in these CCP to detect biological and chemical contaminants that can prove very beneficial for the food industries. Also, this would enable processors to take quick corrective actions when contaminants are detected (Ricci et al, 2007). Another reason for the need of a potential rapid analytical method is that it can be employed to detect contaminants in perishable food like raw meat on site without the need of storing it in inventory which is very important, because according to USDA (United States Department of Agriculture) the raw meat products should be held in inventory until test results showing presence of no pathogenic agents are confirmed, this affects the quality of this perishable food product. Therefore, the development of portable, rapid and sensitive Immunosensor technology is crucial, that can detect contamination in food in real-time (Ricci et al, 2007).

The importance of biosensors/Immunosensors is due to their high specificity and sensitivity, which allows the detection of a broad spectrum of analytes in complex samples with minimum sample pre-treatment.

Immunosensor are devices in which the molecular recognition element is an antibody or antibodies fragments for specific analytes (antigens).Depending on the nature of the transducers, Immunosensors can be classified into different types namely: piezoelectric, electrochemical and optical sensors(Shankaran et al, 2007).

This report discusses some of the potential Immunosensors used in bacterial pathogen and Aflatoxin detection, the advantages of biosensors/Immunosensors over conventional methods, applications, market potential and the commercial biosensors available in the market. An insight of the ongoing research to improve SPR, QCM and electrochemical Immunosensors sensors has also been discussed.

2. Biosensors

Biosensors are chemical sensors which converts a biological response into electrical signals. A biosensor consists of two components:

- The receptor that composes of biological recognition element such as enzyme, antibody, or microorganism. The recognition element is immobilized on a support material or a biointerface (Youssef et al, 2006).
- The detector or the transducer (electrochemical, piezoelectric and optical) which translates the biochemical signal produced by the Immunological reaction (antigen combines with antibody) into electrical signals. Transducer is the key part of a biosensor.

Biosensors work when the antibody and antigen interact with each other (biomolecular interaction). This causes a physical or chemical change at the biointerface which is converted by the transducer to an electrical signal .Output from the transducer is then amplified, processed and finally displayed as a measurable digital signal (Leonard et al, 2003).

3. Immunosensors

Immunosensor is a biosensor that uses antibodies as the biological element. The transducers used in an Immunosensor are largely based on optical, electrochemical and piezoelectric signals. An Immunosensor should possess certain properties, that makes it a good analytical method, it should be able to detect difference false negative results, able to distinguish between analyte of interest and other contaminations, biointerface should be compatible with the transduction principle, the LOD obtained should be validated using current standard techniques, user friendly, lower response time as compared to other analytical methods used, robust, provides real-time response ,and the data should be reproducible or replicable(Kyprianou,2009;Leonard et al,2003).

Immunosensors are based on the following immunological reaction that takes place on the surface of a biointerface. The support for the Immunological reactions in case of electrochemical SPR and QCM sensors is a working electrode and a quartz crystal, respectively**.**

In Direct assay (for SPR and QCM) assay either the immobilised antibody or the immobilised antigen will react with its corresponding antigen or antibody respectively. No label is required for direct assay that is applied to SPR and QCM sensors (Mutlu, 2011). Direct methods are useful for detection of only large molecules having molecular weight > 10 kDa (Ricci et al, 2007). Competitive assays are Applied for the detection of low molecular weight molecules like toxins. The labelling is done to increase the sensitivity of Immunosensors especially Electrochemical (Ricci et al, 2007; Mutlu, 2011). Competitive assays are of two types:

Direct competitive immunoassay where either the immobilized antibodies or antigens react with free antigens or antibodies respectively in competition with labelled antigens (Ag*) or antibodies (Ab*). Protein like BSA is conjugated with the low molecular weight antigens, to increases the interaction with the antibodies (Mutlu, 2011). Indirect competitive immunoassay applied where enzyme conjugates to primary antibodies are not available for the selected analyte .In that case a labelled secondary antibody IgG is used which attaches to the Fc (tail part of an antibody) region of the primary antibody after it interacts with the immobilized antigen (Ricci et al, 2007; Mutlu, 2011).Sandwich assay format can be used to detect the

presence of bacteria. In this assay first the interaction between immobilised antibodies (Ab) and free antigens takes place, labelled antibodies are then added directed toward a second binding site of the antigen. The antigen is sandwiched between two antibodies (Ricci et al, 2007;Mutlu, 2011).

For competitive and sandwich assays label is not used for SPR and QCM measurements.

SPR-based sandwich assay without the use of labels have been used for detection of *E. coli* and are revealed to be more sensitive than the direct assay which has a detection limit of 10^6 CFU/ml (Shankaran et al, 2007)

Indirect competitive inhibition assay applied to QCM and SPR immunoanalytical methods and is capable for highly sensitive detection of low molecular weight analytes, since most of them of food interest are small in size. In this assay the low-molecular weight antigen is immobilized on the sensor surface by using an analyte carrier protein (BSA) conjugate. The antigen is then mixed with particular antibody and introduced over the antigen immobilized surface. There is an increase in the resonance angle noticed when the antibody binds with the conjugate immobilized on the surface but if the quantity of antigen and antibody added is equal then the antibody available to bind with the conjugate will be less ,this causes inhibition. (Shankaran et al, 2007).

3.1 Types of transducers

According to the transducers used the Immunosensors can be divided into

- Electrochemical
- Piezoelectric
- Optical

4. Electrochemical immunosensors

Electrochemical immunosenosrs are of two types:

Potentiometric Immunosensors - This sensor measures the change in voltage between electrodes induced by the reaction of antibody and an antigen on the surface of a sensor (Thakur et al, 2011).

Amperometric Immunosensors- This sensor measures the current induced by the reaction of antibody and an antigen on the surface of a sensor, either the immobilised antibody reacts with the analyte or the immobilized antigen reacts with the analyte on the working electrode surface at an certain voltage applied, causes transfer of electrons and produces current in micro or nano Ampere range, which is proportional to the concentration of the electroactive species (analyte) at the electrode surface(Ricci et al, 2007. Amperometric biosensors can work in two- or three-electrode configurations. The two electrode configuration consists of reference and working electrodes. (Pohanka & Skládal, 2008).The drawback of this kind configuration is that there is limitation in controlling of the potential on the working electrode with higher currents which has the biorecognition component on its surface. A third auxiliary or supporting electrode was employed to solve this problem, here the voltage is applied between the reference and the working electrodes, and current flows between the working and the auxiliary electrodes. This system of electrode is known as screen-printed three electrode sensor.

4.1SPE

Single use disposable (Screen Printed Electrodes) has been utilized in the development of Immunosensors because they are characterized by low-cost of fabrication. The body of the sensor is a planar ceramic supports which consists of working electrode made from gold surrounded by Ag/AgCl reference electrode and an auxillary electrode also made from gold ,the output contacts are silver .All immunological steps can be performed in drop using only micro litres of solution due to its miniaturised dimensions which results in less reagent consumption and it also provides the advantage of decreased diffusion distances between the analyte and its receptor which saves incubation time and results in more rapid assay (Ricci, Volpe, Micheli & Palleschi,2007; Pohanka & Skládal, 2008).

5. Piezoelectric Immunosensors

These are devices based on quartz crystals. These quartz crystals have either antibody or antigen immobilized to their surfaces. When analyte in a sample interacts with the immobilized antibody, there is an increase in overall mass on crystal surface; which results in change in the frequency of oscillation this is correlated to the concentration of target analyte (Thakur et al, 2011).

5.1 QCM

QCM (Quartz crystal Microbalance) sensors compose of thin piezoelectric quartz wafer with two gold electrodes attached to it, this acts as a transducer. The gold electrode surface of crystal is coated with an antibody which is upside and the crystal was placed in a holder of a flow cell which provides the advantage of continuous flow of materials. The flow-type antibody sensor system consist peristaltic pump through which first the reaction buffer flows which is followed by analyte addition through the injection; the flow cell is connected to an oscillator and a quartz crystal analyzer. The change in mass, which occurs when analyte accumulates on the surface of the disc, causes a change in resonance frequency which can be directly related to biomolecular interactions on the gold surface, which is then used to measure the final frequency shift (Adanyi et al, 2006).The analog frequency signals from the quartz crystal analyzer are then converted to the digital ones by a processor. No labelling is required for the study of analyte concentration in the solution.

6. Optical Immunosensors

Optical immunosensors are based on the measure of the absorption or emission of light by the immunoreactants. The interactions between light and the immunoreactants can be measured as changes in refractive index (Shankaran et al, 2006)

6.1 SPR

A SPR (Surface Plasmon resonance) sensor is a label free technique that provides a suitable platform to study real time interaction between antigen and an immobilized antibody. This interaction between the biomolecules is monitored at a very close vicinity of the transducer(made up of gold) surface .An SPR immunosensor is composed of a light source, a detector, a transduction surface (usually gold-film), a prism. The transduction surface is a thin gold-film (gold is preferred due to its chemical stability and free electron behaviour) on a glass slide optically coupled to a glass prism. Plane polarized light is directed through a glass prism, the resulting reflected light is measured against the incident light angle with a detector. The resonance conditions are influenced by the biomolecules immobilized on the gold layer and when a surface immobilized antibody binds with an analyte, the change in the interfacial (point where two things meet) refractive index can be detected as a shift in the resonance angle. These changes are monitored and converted into a sensorgram. The resonance angle shift can provide information on the amount of bound analyte and the affinity of analyte for the antibody (Shankaran et al, 2006).

7. Applications

7.1 Detection of microbiological pathogens and fungal toxin (boon for developing countries)

The situation of food safety in developing countries is in dismal state since there is little or no regulation. The urgent need to attend to food safety in these countries is required since there are often hunger and malnutrition problems with the poorer sections of the society. But due to the economic and societal problems in these countries, the developed nations have to play a major role in providing their technology and experience in food safety. To sustain the growing global trades, developed as well as developing countries require cooperation and planning to address the issues related to food safety (Alocilja & Radke, 2003). One of the problems faced by both developed and developing nations is the problem of food contamination by microbiological pathogens like *Salmonella, Listeria and E.coli* and Fungal toxins such as *Aflatoxin*. These microbiological and fungal toxins are the most potent form of food contamination and have been ranked by FDA. In USA it is estimated that 5000 people die each year due to food borne illness caused by pathogens (Alocilja & Radke, 2003).In Canada between 100 and140 Listeriosis cases are reported each year (Public Health Agency of Canada)

Currently, it is expensive and takes long time to detect food borne pathogens and toxins using conventional techniques like PCR, HPLC or ELISA. So, the development of Immunosensors will play a vital role in carrying out the tests quickly and with high sensitivity.

Detection of pathogens

1. Salmonella

Salmonella may present in foodstuffs like Poultry and Eggs, Milk and Dairy Products, Raw Meats, Fish, Shrimp, Peanut Butter and can cause Salmonellosis.

Amperometric sensors have the ability to detect Salmonella in chicken carcass in the range of 10^3- 10^7 cfu/ml, in case of other food stuff it is 50-100 cells/ml (Mello & Kubota, 2002) and SPR sensors have the ability to detect salmonella in apple juice in the range of 3.4 * 10^3- $1.2*10^5$ cfu/ml (Moises & Schäferling, 2009), the infectious dose of *Salmonella* is around 10^4-10^7 cfu/ml.

2. Listeria monocytogens

Listeria may present in foodstuffs like Dairy Products, Raw Vegetables, Raw Meats, and Smoked Fish and can cause Listeriosis.

QCM based sensors have the ability to detect *Listeria monocytogenes* in food stuff in the range of 3.19 cells/ml (Moises &Schäferling ,2009) , SPR base sensors have the ability to detect *Listeria monocytogenes* in food stuff like in the range of 10^5cells/ml and Amperometric based sensors using screen printed electrodes and sandwich assay format(Ricci et al, 2007)can detect in the range $9.3*10^3$ cells/ml(Moises &Schäferling ,2009) the infectious dose of *Listeria* around 400-10^3 CFU

3. E.coli

Escherichia coli O157:H7 is strain of bacterium *Escherichia coli* and a cause of foodborne illness. Infection often leads to hemorrhagic diarrhoea, and occasionally to kidney failure, especially in young children and elderly. Most illness has been associated with eating undercooked, contaminated ground beef, and eating contaminated vegetables.

SPR sensors can detect *E.coli* in apple juice, milk ground beef by sandwich assay format in the range 10^2- 10^3 cfu/ml (Ricci et al, 2007) the infectious dose of *E.coli* around 10^1 -10^2 CFU (Leonard et al ,2002)

4. Aflatoxin

Aflatoxin is ubiquitous contaminants of the human food supply throughout the economically developing world (16-32 times higher as compared to developed countries). The adverse toxicological consequences of these compounds lead to hepatocellular carcinoma (HCC) and if the person is suffering from HBV, Aflatoxin exposures multiplicatively increase the risk of liver .Aflatoxin may play a causative role in 4.6–28.2% of all global HCC cases(Farazi et al,2006).

In developing countries like India a person gets expose to 4-100 ng/kg of weight /day of *Aflatoxin*(Liu and Wu,2010). The limit of *Aflatoxin* in foodstuff specified for India is 30 micro gram /kg .SPR and electrochemical sensors can detect *Aflatoxin* in foodstuff like barley and maize 0.97 ng/g and 2ng/g (Moises & Schäferling ,2009) respectively.

7.2 Comparison with other conventional techniques available

5. Immunosensors v/s ELISA

Surface Plasmon Resonance (SPR), Quartz Crystal Microbalance (QCM) when compared to ELISA provide with label free detection and they are able to measure biochemical interactions in real time this label free detection makes it possible to study interactions without any modification of the biomolecules of interest.Moreover,ELISA include number of steps(blocking, washing, incubation of primary and secondary antibodies and substrate development), that takes several hours to complete (Skottrup et al, 2008).

6. Immunosensors v/s HPLC

The limit of detection achieved with some of the Immunosensors like SPR and Electrochemical Immunosensors were down to nanogram level and they are comparable with HPLC and LC/MS methods with the benefit of having lack of requirements for rigorous sample preparation and expensive instrumentation.

7. Immunosensors v/s PCR

The enzyme used in PCR is very sensitive to environmental contamination and sometimes this sometimes cause difficulties in quantification and generates false positives through the detection of naked nucleic acids, non-viable microorganisms which is unacceptable in food industries. But Immunosensors cause no such problems and they are not sensitive to environmental contaminations (Velusamy et al, 2010).

8. Immunosensors v/s Culture methods

Standard culture methods such as NF EN ISO11290-1 method for *L.monocytogenes* detection takes7 days to yield results and the ALOA method takes 3 days to yield results. During this period the cells may enter dormancy stage where they become Non- culturable but the bacteria are still viable (VBNC) (Velusamy et al, 2010).

8. Market potential

In 2005 the overall food-pathogen testing market was estimated to be $192 million. Recent statistics in US alone suggest that there are over 60000 food processing facilities, 250000 food retaining facilities and 600000 eating and drinking establishments which are involved in food safety practises. The need for quicker and convenient pathogen detection instrumentation will raise the demand for Immunosensors (Alocilja et al, 2003). Immunosensors have a sensitivity of 10/10 000 colony forming units (CFU)/ml. Amperometric Immunosensors can detect pathogens like *L.monocytogenes* and *E.coli* having an overall assay time of 30 min(Velusamy et al,2010) .Looking at the mushrooming growth of food industries throughout the world it has become pertinent for commercialization of biosensors for detecting quickly and accurately bacterial pathogens and naturally occurring toxins for safe food production. The total sales of biosensor based products in 2005 in food and beverages industry was $150,000 (Lin and Wang, 2005). As the world becomes more concerned with safe food, the demand for rapidly detecting Immunosensors will increase. Methods with quicker detection time and reusable features will be much coveted by those interested in real- time or on- site detection of disease causing pathogens. One major advantage of on-site testing is that during a situation of recall Immunosensor can provide rapid response saving the company to spend a lot of money (Alocilja et al, 2003) .The manufacturers should keep in mind two key technological metrics in mind while commercializing their products one is speed and the second is sensitivity. The best overall growth rate is predicted for the food segment at an estimated CAGR (annualized gain of an investment over a given time period) of 6.0, this is because the biosensors or Immunosensors used in food industry must be adapted to a wide range of diverse matrices like difference in fat, protein, carbohydrate, ash, and moisture content in food stuff. (Carlo et al, 2006).

8.1 Commercial Biosensors Available In Market

Sensor chips that utilize the phenomenon of SPR is manufactured by Biocore.INO which is situated in Quebec is manufacturing of biosensor based on LED illumination.
MS tech manufactures FOODSCAN 3000.A hand-held food contamination detector using High frequency-QCM sensor technology that claims to detect pathogens in 3 seconds.

NASA licensed a new nanotechnology based biosensor technology which uses ultra-sensitive carbon nanotubes which detect a biohazard by passage of electrical signal. Due to their tiny size, a single biosensor chip could carry millions of nanotubes. This fabrication makes it fast and more responsive during testing of pathogens in food, this could prevent major outbreaks of diseases which are responsible for high fatalities throughout the world. Such type of biosensor technology could provide preventive action rather than corrective actions that's being done currently (26).

9. Ongoing research

SPR and QCM sensor are two label free approaches(no requirement of enzymatic labels) for the detection of pathogens and fungal toxins. But these label - free techniques offer less sensitivity as compared to

electrochemical sensors which require label. Further, improvement is needed to increase the sensitivity of SPR and QCM techniques. Nanoparticles are being incorporated in the fabrication of these two sensors not only to improve their sensitivities but also a possible way for miniaturisation of these techniques. The sensitivity of these SPR-based immunosensors can be enhanced by using gold Nano particles tags and electronic coupling it to the SPR gold film (Muñiz, Paroloa& Merkoçia, 2010).

The development of Electronic nose with incorporating 32 conducting polymer sensors is being used in identifying contaminated meat with *S.typhimurium* .Electronic noses also have been demonstrated to be capable of detecting fungi and fungal toxins (Aflatoxin) in various types of cereal grains (Balasubramanian et al,2005).

Aptamers are highly selective receptors which are being incorporated on Carbon nanotubes to develop nanobiosensors; this will further increase the sensitivity as well as a possible way of miniaturising the Immunosensors (Guillen et al, 2009).

CONCLUSION

Immunosensors are no doubt the Holy Grail of analytical methods. Through this report, I demonstrated that how Immunosensors can be beneficial as an analytical tool in the analysis of food borne pathogens and toxins. Food safety is very important requirement in the food industries to market the food product ,this is a part of good manufacturing practice. The Immunosensors described in this report, have been supported with the applications mainly in the detection of bacterial pathogens and fungal toxins, these contaminants are posing major health problems today in both developed and developing countries. The conventional methods used are lacking sensitivity, specificity ,reproducibility and speed ,the methods used are time taking, expensive ,complicated and need trained technicians .Moreover these techniques cannot be applied on-site like Immunosensors which provides results in short time, provides with a fully automated system and hence less laborious.

ELISA and is a competitive analytical method for these Immunosensors in terms of its high sensitivity, and cost-effectiveness and this makes this technique really difficult to be replaced at the moment. SPR and QCM sensors being non label techniques have shown a decrease in sensitivity. But further improvement in SPR and QCM sensors can provide an edge over the ELISA technique .Further, SPR and QCM techniques also provide an opportunity of miniaturisation and on site detection, that ELISA doesn't.

When compared with each other the QCM sensors are very simple and less expensive than SPR ,but they are less sensitive as compared to SPR.Although, Electrochemical sensors require labelling but they offer higher sensitivities and low cost as compared to SPR and QCM. Further research is needed to make SPR and QCM sensors more sensitive and less costly. Incorporation of Nanomaterials in SPR and QCM techniques can further increase the sensitivity of these techniques.

REFERENCES

Akdogan,E.,Mutlu,M. 2011. Basic principles of Optical biosensors in Food engineering, Biosensors in food processing safety and quality control , 2 ,53.CRC Press.

Alocilja,E.,Radke,S.M. 2003. Market analysis of biosensors for food safety, Biosensors and Bioelectronics

Alexander S., Mewhinney M. 2008. NASA Nanotechnology-based Biosensor detects Biohazards. Retrieved from http://www.nasa.gov/home/hqnews/2008/may/HQ_08131_BioSensor_Technology.txt

Balasubramanian, S., Panigrahi, S., Logue, C.M., Marchello, M., Sherwood, J.S. 2005. Identification of *salmonella* -inoculated beef using a portable electronic nose system, Journal of Rapid Methods & Automation in Microbiology

Carlo ,M.D., Nistor ,M. ,Compagnone, D., Mattiasson,B, Csöregi,E. 2006. Biosensors for Food Quality Assessment, Food Biotechnology, Second Edition

FAO/WHO. 2006. Food safety risk Analysis:A guide for national food safety authorities.

Groopman,J.D., Kensler,T.W., Wild,C.P. 2008. Protective interventions to Prevent Aflatoxin-Induced Carcinogenesis in Developing Countries, Public health.

HACCP. In Wikipedia. Retrieved March 3. 2011. from http://en.wikipedia.org/wiki/Hazard_Analysis_and_Critical_Control_Points.

Kyprianou,D. 2010. Development of novel matrices for biomolecule immobilisation on sensor surfaces.

Leonard, P., Hearty , S., Brennan ,J.,Dunne,L.,Quinn,J.,Chakraborty,T.,Kennedy,R. 2003. Advances in biosensors for detection of pathogens in food and water, Enzyme and Microbial Technology.

Lin,C.T., Wang ,S.M. 2005. Biosensor commercialization strategy – a theoretical approach, Frontiers in Bioscience.

Liu,Y. , Wu,F. 2010. Global Burden of Aflatoxin-Induced Hepatocellular Carcinoma: A Risk Assessment, Environ Health Perspect.

Lin,Y.Y. et al. 2010. Economic development is ultimate determinant of food safety: A case study of China, Environmental Pollution.

Mello,L.C.,Kubota,L.T. 2002. Review of the use of biosensors as analytical tools in the food and Drink industries, Food Chemistry.

Moises, S.S., Schäferling,M. 2009. Toxin immunosensors and sensor arrays for food quality control,Bioanalytical reviews.

Muñiz,A.E., Parolo, C., Merkoçia,A. 2010. Immunosensing using nanoparticles,www.materials today.com.

Mutlu,S. 2011. Mass sensitive biosensors Principle and application in food, Biosensors in food processing safety and quality control ,3 ,75.CRC Press.

Parker,C.O.,Tothill,I.E. 2008. Development of an electrochemical Immunosensor for Aflatoxin M1 in milk with focus on matrix interferences, Biosensors and Bioelectronics.

Pohanka,M.,Skládal,P. 2008. Electrochemical biosensors – principles and applications, Journal of applied biomedicine.

Ricci, F., Volpe , G., Micheli ,L.,& Palleschi, G. 2007 a . A review on novel developments and applications of immunosensors in food analysis,Analytica Chimica Acta.

Shankaran, D.R., Vengatajalabathy , K., Miura ,N. 2006. Recent advancements in surface plasmon resonance immunosensors for detection of small molecules of biomedical, food and environmental interest, Sensors and Actuators B: Chemical.

Skottrup,P.D., Nicolaisen,M., Justesen,A.F. 2008. Towards on-site pathogen detection using antibody-based sensors, Biosensors and Bioelectronics.

Thakur, M ., Chouhan ,R., Vinayaka A. 2011. Biosensors for pesticides and foodborne pathogens, Biosensors in food processing safety and quality control, 7,156.CRC Press.

Velusamy,V., Arshak,K., Korostynska,O., Oliwa,K., Adley,C. 2010. An overview of foodborne pathogen detection: In the perspective of biosensors, Biotechnology Advances.

Wild,C.P.,Hall,A.J. 2000. Primary prevention of hepatocellular carcinoma in Developing countries, Mutation Research/Reviews in Mutation Research, Mutation Research/Reviews in Mutation Research.

Youssef, M.M., Nessma, N. El.Haddad. 2006. Applications to biosensors to analysis and quality control of food: an overview.