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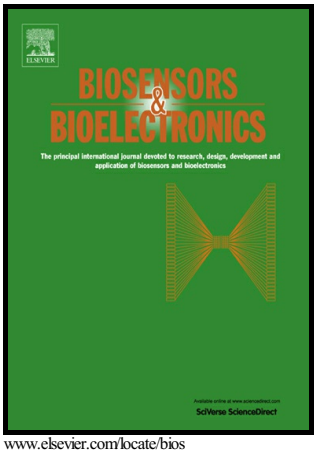

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Recent advances in biosensor based diagnosis of Urinary Tract Infection

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Abstract:

Urinary tract infections (UTIs) are potentially life threatening infections that are associated with high rates of incidence, recurrence and mortality. UTIs are characterized by several chronic infections which may lead to lethal consequences if left undiagnosed and untreated. The uropathogens are consistent across the globe. The most prevalent uropathogenic gram negative bacteria are *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*. Early detection and precise diagnosis of these infections will play a pivotal role in health care, pharmacological and biomedical sectors. A number of detection methods are available but their performances are not upto the mark. Therefore a more rapid, selective and highly sensitive technique for the detection and quantification of uropathogen levels in extremely minute concentrations need of the time. This review brings all the major concerns of UTI at one's doorstep such as clinical costs and incidence rate, several diagnostic approaches along with their advantages and disadvantages. Paying attention to detection approaches with emphasizing biosensor based recent developments in the quest for new diagnostics for UTI and the need for more sophisticated techniques in terms of selectivity and sensitivity is discussed.

Keywords: Urinary tract infections, Uropathogens, Detection, Biosensors

1. Introduction

Biosensors are biological sensing molecules that are associated with a transducer, which produces signals that are proportional to specific analyte concentration (Turner., 2013). Biosensors are used in modern biotechnology to detect cellular substrates such as enzymes (Kim et al., 2016), proteins (Chang et al., 2016), hemoglobin (Yazdanpanah et al., 2015); chemicals such as mercury (Li et al., 2016), acrylamide (Batra et al., 2016); activity of toxins (Boczek et al., 2015; Das et al., 2015a); and different pathogens such as food pathogens (Adley et al., 2015), waterborne pathogens (Bridle et al., 2014) and uropathogens (Altobelli et al., 2015; Liu et al.,

2014). Urinary tract infections (UTIs) are the second most prevalent type of infection with estimated to be nearly 25 % of all the infections (Foxman., 2003). UTIs are the fourth most prevalent type of health care associated infections (HAIs) in the United States. UTIs comprise about 13% of the total HAIs reported annually (Magill et al., 2014). It is estimated that more than 1 million cases in the hospitals are due to catheter associated UTIs, the most common nosocomial infections. In USA, the estimated cost per urinary tract infection ranges from $750-$1000 and the total cost exceeds $3.5 billion annually (Mireles et al., 2015). UTI is the third most prevalent infection in India (Saha et al., 2014) and is the common bacterial infection in infants and children (Kaur et al., 2014). Males have higher risk of developing UTI during the first year of life, but beyond 1-2 years females are more prevalent to infection than men with a ratio of 10:1 (Kaur et al., 2014; Arvind et al., 2001). The epidemiology varies with age, gender and various factors of genetic susceptibilities. The UTIs are classified into two types based upon the site of localization of pathogenic bacteria in the urinary tract. If pathogenic *E.coli* is in the urinary bladder, it is uncomplicated UTI. If left untreated, it may spread upto the ureters to the kidneys and leads to complicated UTI, which can be accompanied by urinary tract obstruction and renal failure (Noormandi et al., 2015). The effects and symptoms of UTIs are shown in fig 1. The uropathogens are consistent throughout the world. Both gram negative and gram positive bacteria have been isolated from infected urine samples. Most of the urinary tract infections associated pathogens are gram negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia, K.oxyntoca, Salmonella paratyphi, Citrobacter freundii, Vibrio cholera, Serratia marcescens, Providencia stuartii. Enterobacteriaceae* constitutes 81% of the reported cases (Soubra et al., 2014), of which *E.coli* is the most common pathogen (Bouamri et al., 2015; Bokil et al., 2011) in both community acquired and catheter associated urinary tract infections (Floyd et al., 2015). Although *E.coli* is the most common in the UTIs of both sexes, it is predominantly found with greater incidence in female patients (Linhares et al., 2013). *Pseudomonas aeruginosa* represents the fourth most common uropathogen in males, and ninth most common in females (Linhares et al., 2013). Gram positive bacteria include *Enterococcus facecalis and Staphylococcus saprophyticus. Actinobacuulum schaali,* facultative anaerobe, gram positive, rod shaped bacteria, phylogenetically related to *actinomyces* is an emerging uropathogen in human genitourinary tract. Since 1997, *A.schaalii* has been responsible for a large number of urinary tract infections

mainly in the elder people usually older than 60 years (Cattoir., 2012). Most common uropathogens are listed in Table 1. A comprehensive literature survey has been assessed over the past ten years with an emphasis on uropathogen detection (Fig. 2).

2. Transmission routes of uropathogens

Uropathogen control is of a prominent requirement regarding health and medical issues. There are various areas demanding rapid identification of uropathogen levels such as health care, pharmaceutical, agriculture, food and beverage industries, sewage treatment. Uropathogens can be transmitted or spread through various routes such as fecal, oral, contact, contaminated water, food and transplacental passage. *E.coli* are transmitted through fecal-oral route, direct contact, through food or water, and can also be foodborne (Foxman et al., 2002). *Salmonella typhimurium* is spread through contaminated food (Gebreyesus et al., 2014), through fecal-oral route (Wijburg et al., 2006) and occasionally through direct contact and contaminated water. *Treponema pallidum* can be transmitted through sexual contact and via transplacental passage (Burgess et al., 1994). Uropathogenic *E.coli* produces biofilms on polystyrene plates and glass tubes and on flint shingles from fresh water streams located in agricultural areas (Cooper et al., 2007) and may survive even after the treatment stages in sewage treatment (Anastasi et al., 2013).

3. Detection of uropathogens

Uropathogen detection has been one of the major interest and concern in global health care issues. Rapid detection, monitoring and quantification of uropathogen levels in the samples have always been a major focus. Failure of detection in the early stages frequently resulted in lethal effects. Therefore, diverse range of methods using various techniques have been discovered and tested for identification of uropathogens (Table 2).

3.1. Conventional methods

Conventional methods are established techniques that are being used from several years such as non-culturing and culturing methods, enzyme linked immunosorbent assay, polymerase chain reaction, and isothermal microcalorimetry.

3.1.1. Non-culturing methods

Bacteriuria can be diagnosed microscopically by gram staining of urine specimens. The important advantage of urine Gram stain test is that, it provides rapid information of the nature of the infectious bacterium allowing the physician to select an empiric antimicrobial therapy. The most important disadvantage of this technique that limits its use in clinical settings is that it is an

insensitive technique as it cannot detect concentrations of bacteria $\leq 10^5$ cfu/ml of urine (Wilson et al., 2004). Bacteriuria is detected when the bacteria produce nitrite from nitrate, the characteristic feature of *Enterobacteriaceae* (John et al., 2006). The drawback is that nitrite production is not a characteristic of other uropathogens such as *Pseudomonas* species, *S.saprophyticus* (Wilson et al., 2004). Even pyuria can be detected microscopically by measuring urinary leukocyte excretion rate. An alternative for microscopic leukocyte counts is leukocyte esterase test that is based on the hydrolysis of ester substrates by esterolytic activity of the proteins (Rangaiahagari et al., 2015). This test may yield false positive results if the urine is contaminated with bacteria present in vaginal fluid (Pezzlo., 2014). Overall, these non-culturing methods are time consuming with less sensitivity, selectivity and low positive predictive values (Nostrand et al., 2000).

3.1.2. Culturing method

Culturing method is the oldest known traditional method that has been employed as a standard technique for the detection of uropathogens for many years. The major disadvantages involve, a separate culture media and particular supplements are required by each and every microbe for its optimum growth (Graham et al., 2001). As most of the uropathogens are gram negative bacteria, blood and MacConkey agar are often used for culturing. Moreover, the incubation time is different for every microorganism. The time of incubation for uropathogens is a controversial issue as some studies suggest that most of the uropathogens can be detected in 6 hours or less, while some studies conclude that 24 hours is adequate, but many laboratories perform for 48 hours (Cavagnolo., 1995).

3.1.3. Polymerase Chain Reaction (PCR)

PCR based molecular methods have been developed for identification of pathogens and diagnosis of infections (Kubista et al., 2006). PCR methods have been developed for the detection of Bacteriuria, pyuria from blood, urine and other clinical samples with the ability to obtain results in a much shorter time compared to other conventional methods (Dark et al., 2009). Universal primers or selective primers that amplify specific DNA are generally used for the detection. However, universal primers have an advantage of detecting a large number of bacterial species in the sample. Most of the researchers use primers for 16S rRNA gene amplification. The uropathogen detection and analysis is achieved in a single vessel. The most

important PCR variant may be real-time PCR, which allows monitoring the products being formed continuously as the reaction proceeds (Catanzaro et al., 2012). To monitor the amplification of products, either DNA binding nonspecific fluorescent dyes or DNA binding specific fluorescent probes are used (Parks et al., 2001). Several different sequence specific probes with different fluorescent labels can be used in the same reaction that allows simultaneous determination of amplification of multiple products. A multiplex PCR assay has been developed for the detection of *E.coli, P.mirabilis, P.aeruginosa* and *K.pneumonia* (Padmavathy et al., 2012). This technique would be more convenient when the clinical sample contains several pathogen species.

3.1.4. Isothermal microcalorimetry

Isothermal microcalorimetry (IMC) is based on the principle of that heat produced by any growing microorganism is proportional to their metabolism and replication rate. This heat is recorded by IMC and plotted as heat flow versus time. The mean detection time for fast replicating and more virulent uropathogens such as *Escherichia coli* and *Proteus mirabilis* is shorter as compared to slower replicating and less virulent organisms such as *Staphylococcus aureus* and *Enterococcus faecalis* (Bonkat et al., 2013). This technique is a non-destructive method and can measure heat flow less than a microwatt. As few as 10,000 bacterial cells in culture are adequate to produce a real-time signal (Braissant et al., 2010). Quantification of microbial activity over periods of hours to days has been done using IMC. Most of the isothermal microcalorimeters are of heat conduction type, where heat liberated in the reaction vessel flows to a heat sink. A thermopile placed between vessel and sink acts as a sensor for heat flow (Wadso et al., 2002). The limitations include: the samples need to be equilibrated for approximately 1 hour before measurements to achieve high sensitivity, chemical factors like oxygen depletion have to be considered for measurements, and heat flow is not specific as several other phenomena may produce heat (Braissant et al., 2010).

3.1.5. Enzyme linked immunosorbent assay

Due to its high reproducibility, feasibility to conduct a large number of assays simultaneously and versatility to apply to medical devices, pharmaceuticals and blood derived products, ELISA is being used widely to determine the bacterial components present in the sample (Mohanan et al., 2011; Ivnitski et al., 1999). Sandwich ELISA, which involves two antibodies, is considered as the most efficient form among all variants (Zhao et al., 2014). A 96 well plate is the

commonly used where the first wells are used to draw the standard curve. The samples are added in duplicate or triplicate and then the mean results are calculated. A paper-based ELISA has been developed recently that can detect *E.coli* or bacteriuria in 5 hours (Shih et al., 2015). However, the disadvantages include less sample volume of the microtitration plate, long incubation time required for each step (Ivnitski et al., 1999), requires extensive sample cleaning and biomolecule purification and are label dependent (Skottrup et al., 2008).

*3.2.*Biosensor based detection methods

The development of rapid and sensitive biosensor technologies for uropathogen detection has been one of the major focuses in the current research. Although the traditional culture-based tests and conventional methods are confirmative to identify uropathogens, these methodologies require considerable time and skilled persons to perform, and a requirement in the increase in sensitivity (Yamada et al., 2016). Hence development of rapid molecular approaches for uropathogen detection is key to improving the treatment of urinary tract infections (Sun et al., 2005). Advances in biosensor based detection methods have the capability to push away the general practices in medicine (Gaster et al., 2009).

3.2.1. Dual signal amplification via enzyme-based target recycling

Dual signal amplification process has been coupled to various biosensor based methods to increase the sensitivity (Hou et al., 2014; Fang et al., 2015; Cui et al., 2016). Similarly, Most of the methods use the target recycling process or primer regeneration process that helps in lesser usage of primer (Ye et al., 2016; Su et al., 2011). Su et al. developed a nucleic acid based biosensing detection method that involves a dual signal amplification process, which combines the enzymatic target recycling technique with the quantum dot (QD) layer-by-layer (LBL) assembled labels to acquire signal enhancement and sensitivity simultaneously. The enzyme based target DNA recycling aids to use each target DNA sequence multiple times and results in direct amplification and signal improvement (Chen et al., 2016). The sensitivity is further enhanced by the LBL assembled QD labels (Deshmukh et al., 2013). The linker DNA is designed specifically to bind to the target DNA to form double stranded duplex. Exonuclease III enzyme exclusively cleaves linker DNA when present along with target DNA in duplex (Xu et al., 2016). In the absence of target DNA (uropathogens), Exo III is inactive against single stranded linker, so that the linker hybridizes with complementary DNAs present on polystyrene (PS)-$(CdS)_2$ assemblies and magnetic beads. Upon magnetic separation, PS-$(CdS)_2$-linker-

magnetic bead complexes are dissolved to release Cd^{2+}. The released Cd^{2+} ions exhibit large voltammetric response. On the other hand, in the presence of target DNA, linker binds to the target to form duplex. Exo III digests the linker and the released target can bind to other linkers, this is called enzyme based target recycling. As the concentration of the linker reduces, the level of PS-$(CdS)_2$-linker-magnetic bead complexes also decreases and thereby lesser Cd^{2+}, that ultimately results in the suppression of voltammetric response. The overall process is showed in Fig. 3. The stripping current of Cd^{2+} is inversely proportional to the amount of target DNA. The sensitivity of this technique is as high as 5 femto molar (fM) of target DNA can be detected (Su et al., 2011).

3.2.2. Magnetoelastic sensor based technology

Using electrochemical aptamers to detect targets has some limitations such as limited aptamer loading density due to lesser surface to volume ratio of its planar substrates, long incubation period for binding step and difficulty in removing nonspecific targets during washing step (Yoon et al., 2013). Therefore researchers developed aptasensors that employ magnetic beads to detect the targets and overcome the limitations. Use of magnetic beads has been reported by several researchers in biosensing methods in conjugation with immunocomplexes and aptamers (Reverte et al., 2015). Magnetic beads have been employed for the detection of *E.*coli (Geng et al., 2011), measurement of bacterial growth and susceptibility (Sinn et al., 2011). Zuo et al. developed magnetoelastic sensor based method that employs two aptamers against endotoxin, magnetic beads (MB), a fluorophore labeled single strand DNA that is complementary to one of the aptamers. This method involves primarily of three steps: MB-aptamer B2 complex formation, characterization of MB-B2 complex and endotoxin conjugated sandwich complex formation. Magnetic beads were added to a solution containing carboxyl groups and the magnetic beads covered by carboxyl groups were separated with a magnetic stirrer from the solution. NH_3-modifed aptamer-B2 were then added to suspension of carboxyl groups covered magnetic beads in morpholinoethane sulfonic acid (Mes) buffer. MB-B2 complexes were separated using a magnetic separator (Fig. 4a). Characterization of MB-B2 was performed by adding carboxy fluorescein fluorophore labeled complementary single stranded DNA to solution of MB-B2 complexes. Bare magnetic beads are used as controls and flow cytometry was used for detection. Carboxyfluorescein labeled aptamer-B9 was then added to endotoxin solution so that endotoxin-B9 complexes were formed. MB-B2 complexes were added to the solution containing endotoxin-

B9 complexes (Fig. 4b). The formed endotoxin conjugated sandwich complexes were observed under scanned confocal laser microscopy (Papp et al., 2003). The interaction between MB-B2 with other substances like glucose, bovine serum albumin protein, RNA and sucrose was found to be absent. This method had detected endotoxin within a range of 10^{-2} to 10^{6} ng/ml. MB-B2 complexes were regenerated seven times and the deviation in detection was 5.5% after all regenerations. Detection of endotoxin required only 2 µl of MB-B2 complexes (Zuo et al., 2014). This assay is thought to provide an excellent approach for performing rapid endotoxin detection in medical and food sectors.

Ong et al. developed a technique based on the same principle that monitors the change in viscosity in the LAL assay as a function of time by measuring resonance amplitude of the immersed magnetoelastic sensor. The maximum clot rate and the time-to-clot have showed a strong correlation to endotoxin concentration (Miao et al., 2013). The maximum clot rate is proportional to the power of endotoxin concentration whereas the clot time is inversely proportional. The sensitivity of this technique is very high as it can detect the presence of endotoxin at 0.0105 EU/ml and the detection time is several tens of minutes (Ong et al., 2006).

3.2.3. Electrochemical endotoxin sensors based on TLR-4/MD-2 complexes

Electrochemical sensors are widely used for detection of pathogens (Dechtrirat et al., 2014; Liao et al., 2007), bacterial DNA and RNA (Sun et al., 2005; Tosar et al., 2010), lactoferrin in urine (Pan et al., 2010). Yeo et al. developed an electrochemical sensor based approach that is based on immobilization of a human recombinant toll-like receptor 4 (rhTLR4) and myeloid differentiation-2(MD-2) complex which specifically bind to endotoxin (Shen et al., 2014), on gold electrodes through a self-assembled monolayer (SAM) technique. The electrochemical signals generated from interactions between the complex and endotoxins were measured by cyclic voltammetry and differential pulse voltammetry (Yadav et al., 2016; Guerreiro et al., 2014). The detailed view of this technique includes fabrication of a gold electrode on a glass slide, fabrication of endotoxin sensors on the gold electrode, electrochemical characterization and testing specificity to endotoxin. Titanium deposition on a glass slide at the rate of 2A/s, and then gold deposition at the rate of 2A/s was performed. Nanosurface profiler was used to measure the thickness of the gold electrode. Scanning electron microscopy (SEM) and Atomic force microscopy (AFM) was used to check the surface morphology (Zeng et al., 2016; Medina et al., 2015). In the next step, the gold electrode was immersed in Tetrahydrofuran solution

containing Dithio bis(succinimidyl undecanoate) (DSU) (Zhu et al., 2015). DSU/gold electrode was dipped in reaction buffer containing rhTLR4/MD-2 complexes. rhTLR4/MD-2/DSU/gold electrode was then dipped in bovine serum albumin solution to prevent non-specific binding (Kovalchuk et al., 2015) of endotoxin (Fig. 5). Electrochemical analysis was done through cyclic voltammetry and differential pulse voltammetry with an electrochemical analyzer. To analyze the specificity of this sensor, Dipalmitoyl phosphatidyl choline was used as a substitute for endotoxin as its structure resembles the lipid chain of endotoxin. The detection limit was fairly low as it can detect 0.0002 EU/ml. The binding of endotoxins on the electrodes requires 30 mins (Yeo et al., 2011). The electrochemical sensors have some limitations such as weak stability or shelf life of the electrode and less selectivity (Grieshaber et al., 2008; Mehrvar et al., 2004).

3.2.4. **Aptamer based biosensors**

Aptamer is a type of synthetic oligonucleotides that can bind to various targets such as hormones, proteins, or even whole cells with a very high specificity and can be discriminated by screening in vitro (Wang et al., 2012). Systemic Evolution of Ligands by Exponential Enrichment (SELEX) is an efficient screening technique that progressively selects the aptamers by repeated cycles of separation and amplification from a huge random synthetic oligonucleic acid library (Nezlin., 2016). For the preparation of aptamers, a specific DNA/RNA that binds specifically to the target has to be obtained from the whole library, which is amplified and sequenced to get the aptamer (Fig. 6). Using aptamers as biosensors for the detection of pathogenic organisms possess many advantages like easy preparation, simple modification and high stability (Guillén et al., 2012; Bunka et al., 2006). A single aptamer may be useful and sufficient if the target is a protein or a small molecule, whereas for whole cell or whole bacterial detection, a single aptamer might produce false negative results. Cao et al. designed aptamers against *Staphylococcus aureus*, and to improve the specificity of these aptamers they used *Streptococcus* and *Staphylococcus epidermidis* for counter selection. Initially eleven sequences from eight families were chosen, out of which five aptamers were concluded to be specific to *S.aureus* and were not cross reactive to *S.epidermidis* and *Streptococcus* (Cao et al., 2009).

Ravindranath et al. reported a surface enhanced Raman spectroscopy (SERS) aptasensor to detect *Salmonella typhimurium, Staphylococcus aureus* and *E.coli* O157:H7 simultaneously. This method has higher selectivity and sensitivity with a detection limit of 10^2 cfu/ml. Chemiluminiscence technique offers high sensitivity (2 pg/ml) due to its detection of emitting

photons. Fluorescent aptasensors are based on the change in fluorescence intensity produced by binding of aptamers labeled with fluorescent probes and targets.

Su et al. developed an electrochemical biosensor using a lipopolysaccharide (LPS) specific single stranded DNA aptamer as a probe and was immobilized on gold electrode. A wide range of concentration of LPS (1 pg/ml to 1 ng/ml) could be detected by this aptamer based impedance biosensor without any cross reactivity to plasmid DNA, RNA and bovine serum albumin used in this experiment. This method offered higher specificity to LPS, lower detection limit of 1 pg/ml, lesser cost and easy operation (Su et al., 2012).

Queiros et al. developed a DNA aptamer biosensor to detect outer membrane proteins (OMPs) of *E.coli*. ECA-I and ECA-II aptamers were immobilized on different electrodes. Thiol groups at 5' ends of aptamers were covalently bound to Au on the electrodes. The aptamer immobilized electrodes were immersed in 6-mercapto-1-hexanol (MCH) solution, a blocking agent, to prepare SAM (Garcia et al., 2014). The surface density of aptamer was estimated by chronocoulometry (Chen et al., 2012). ECA folds around the OMPs to form OMP-ECA complex, which prevent electron resistance; as a result electron transfer resistance (R_{et}) increases. A small resistance was observed even before the formation of OMP-ECA complex that has to be subtracted from the resistance increased due to interaction of OMPs. Increase in R_{et} is directly proportional to the incubation time up to 90 min. Ultraviolet-visible absorption technique was used to monitor the interaction of aptamers and proteins. The OMPs alone have showed almost no absorption spectra. Both aptamers have showed peak absorption at 260 nm, whereas the absorption peak of both the complexes (ECA-I-OMP and ECA-II-OMP) has been shifted to 250 nm and extended. BSA was used as a control, which exhibited no increase in R_{et} (Queiros et al., 2013). The detection range of this assay is 1×10^{-7} to 2×10^{-6}M of OMPs. The main drawback is that the tertiary structure of aptamers is highly dependent on solution conditions and is easily degraded in blood samples (Keague et al., 2012).

3.2.5. Microcantilever array biosensors

Cantilever biosensors can be used in three modes of operation; dynamic or resonance mode, static mode and differential static mode (Simoni et al., 2015). In dynamic mode of operation, desired analyte molecules interact with molecules on functional layers of cantilevers, changing their chemical structures, adsorption, desorption or inducing binding or release of molecules from the functional layers. The variation in the adsorbed mass could be analyzed by monitoring

the resonance frequency of cantilever (Johnson et al., 2012). In static mode of operation, the change in the analyte may cause change in chemistry of cantilever's surface and leads to surface stress on the side where reaction takes place that causes cantilever to bend. The measurement of this bending is the basic principle behind this mode of operation (Kim et al., 2015; Arlett et al., 2011). However, the bending may be caused by several phenomena other than analyte, for instance, temperature. Therefore, precise measurement of bending caused by analyte has to be measured by eliminating the bending influenced by other factors. This is achieved by differential static mode of operation in which an additional reference cantilever is used which does not react with the analyte. Hence, by comparing the measurements of both the cantilevers, it is possible to calculate the exact bending caused by the analyte (Boisen et al., 2009; Fritz., 2008).

A new cantilever based sensor technique using antibodies against LPS of *Hafnia alvei*, a prominent uropathogen, was introduced by Nieradka et al. 2014. They used differential static mode of operation to detect the endotoxins. Whole or killed cells of *H.alvei* PCM 1185 or *H.alvei* PCM 1186 were injected into six week old BALB/c male mice with incomplete Freund's adjuvant subcutaneously for immunization (Dag et al., 2004). Splenocytes were isolated from the immunized mice and fused with myeloma cells of mice to produce hybridomas (Sroubek et al., 2012; Lin et al., 2010). Anti-LPS antibodies producing hybridomas were discriminated and selected. Cell culture supernatant of hybridomas was taken and monoclonal antibodies were purified by ammonium sulfate precipitation (Mariam et al., 2015) and affinity chromatography (Zhang et al., 2015) using protein A-Sepharose column. Using mouse immunoglobulin isotyping kit, the isotype of *H.alvei 1185* antibodies were found to be IgG2a with kappa light chain and that of *H.alvei 1186* to be IgG3 with kappa light chain. SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) was performed to check the purity of the antibodies (Ganguly et al., 2015) and ELISA was done to analyze the specificity (Yang et al., 2016) (Fig. 7a). Silicon wafers were covered with a gold layer and then the sensor was dipped in water to generate OH groups on silicon surface. The sensor was then dipped in a solution of 3-aminopropyltriethoxysilane (APTS) in ethanol to form a self-assembled monolayer base on the oxide side (Lu et al., 2011; Rao., 2012). Then the sensor was immersed in solution of methyl polyethylene glycol-N-hydroxysuccinimide (mPEG-NHS) in water to create methoxy-glycol surface that prevents binding of antibodies on the oxide side. The reference cantilever was now engrossed in a solution of polyethylene glycol methyl ether thiol (mPEG-SH) and 2-

mercaptoethanol (ME) in water to generate a non-binding methoxy glycol surface on the gold surface. The test cantilevers were engrossed in a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) hydrochloride (EDC) and N-hydroxy succinimide (NHS) to generate protein binding sites. IgG antibodies against *H.alvei* 1185 and *H.alvei* 1186 were then added for measurement. Cantilevers were arranged as shown in Fig. 7b. This biosensor technique based on micromechanical cantilevers showed high specificity in detecting endotoxins (Nieradka et al., 2014). Microcantilever sensors have the potential of detecting minute concentrations of targets in parts per billion or parts per million (Vashist., 2007) but lack specificity (Hansen et al., 2005).

3.2.6. Limulus amoebocyte lysate assay

Limulus amoebocyte lysate assay was first approved in United States in the early 1980s to test parenteral drugs instead of rabbit pyrogen test and then all other nations followed (Sakata et al., 2009). In 1995, the application of this assay was spread to antibiotics and then to a wider range. LAL assay was proved rapid, accurate and highly sensitive in detecting endotoxin and used for vaccines, antibiotic products and blood products. The Limulus reaction is basically an application of the natural antibacterial defense mechanism or immune response of horseshoe crab (*Limulus polyphemus*) for the detection of endotoxins secreted by uropathogens in which the uropathogen infection leads to the clotting of its hemolymph (Noda et al., 2010; Chalupniak et al., 2014). The detection of uropathogens can be done using blood sample, urine sample and any other body fluids. When using blood samples, the hemoglobin from erythrocytes inhibits the assay system. Therefore, the erythrocytes were eliminated by adding an erythrocyte-aggregating agent, hydroxyethyl starch (HES) to blood before the assay (Kan et al., 2013). The hemolymph of limulus contains granular amebocytes that act as the major defense system in horseshoe crabs (Das et al., 2014). The lysate is the aqueous extract of amebocytes of horseshoe crab that contains all the proteins necessary for the cascade of reactions mentioned below (Miao et al., 2013). The cascade of enzymatic reactions is initiated by the reaction between factor C and the endotoxin present in the sample that results in the autocatalysis of factor C and eventually leads to the activation of factor C (Das et al., 2015b). The activated factor C cleaves factor B to activate it. Activated factor B cleaves proclotting enzyme converting it into coagulase (the clotting enzyme). Coagulase is the main enzyme in this enzymatic cascade. The cleavage pattern of the enzyme is studied for the development of several varieties of LAL assay including the

chromogenic based approach. Coagulase converts coagulogen into coagulin, which leads to either gelation of the solution or increase in the turbidity (Hurley., 1995) (Fig. 8). The gelation time is dependent upon the concentration of the endotoxins in the sample (Obata et al., 2008).

Endotoxins can induce the above mentioned coagulation reaction, but this reaction can also be induced in an independent way by β-D-glucan from fungal cell walls (Yabusaki and aoyagi., 2012), the presence of which may result in false positive results. β-D- glucan reacts with factor G to give the coagulation reaction. Therefore, to increase the specificity of the LAL assay, many variants have been developed by the removal or suppression of factor G in lysate reagents which eliminated the cross reactivity to glucan (Noda et al., 2010). However, it was proved that the pyrogenic activity of endotoxin is nearly 10,000 fold greater than that of other substances such as β-D-glucan, lipoteichoic acid and peptidoglycan. For the detection of endotoxin, gelation or turbidity or fluorescence modified peptide could be used. There are several LAL methods employing turbidimetric and chromogenic end-point assays as well as turbidimetric and chromogenic kinetic methods for endotoxin detection. The end-point methods possess some limitations regarding their sensitivities with a detection limit of about 0.01-0.1 EU/ml (Smulders et al., 2012) and the kinetic methods have limitations regarding the detection time of approximately 60 minutes. The principle of end-point methods is a protease reaction of coagulogen or between a chromogenic substrate and the lysate that leads in the increase of turbidity or color in the endotoxin presence. The time of the reaction is about 15-30 min and the sensitivity is 0.1-0.01 EU/ml. The kinetic chromogenic method depends on the time of start of turbidity increase or the color reaction and is based on the amount of endotoxin present in the sample. As the detection limit of this method is 0.002 EU/ml with a measurement time of 76 min, it is applied for detection of minute amounts of endotoxin in the sample. The detection limit of kinetic turbidimetric method is 0.0005 EU/ml with a detection time of 138 min (Marinho et al., 2015). Therefore, to check the contamination of endotoxin, end-point methods are used and for detection of minute amounts of endotoxin in the samples, kinetic methods are used.

The most common chromogenic substrate used for LAL assay is para Nitroaniline (pNA) (Inoue et al., 2012). pNA is conjugated as Benzoyl-Leu-Gly-Arg-pNA, as the clotting enzyme, a serine protease cleaves coagulogen (A-chain—peptide C—B-chain) at two terminals of peptide C at Arg-Lys and Arg-Gly linkages joining A- and B-chains. This proteolytic activity of clotting enzyme can be used on the chromogenic substrate that separates pNA that is yellow in color

(Ostronoff et al., 2015). pNA can be substituted with a luminescent substrate such as aminoluciferin (luminescent substrate method). Noda et al. used a mutant North American firefly (*Photinus pyralis*) luciferase enzyme that generated ten-fold higher luminescence intensity than that of wild type luciferase. The mutant luciferase detected aminoluciferin at concentrations of 1pM, whereas wild type luciferase could not detect concentrations lower than 10pM (Noda et al., 2010). The detection limit was 0.0005 EU/ml with a measurement time of 15 min. This assay was reported to give inaccurate results for blood samples of healthy females as serum proteins may slow down the initial phase of the enzymatic cascade (Gnauck et al., 2015). Furthermore, this test cannot distinguish between dead and living bacteria (Sushruta et al., 2011).

LAL assay has been successfully applied in detecting pathogenic toxins from snake antivenoms (Solano et al., 2015), liposomal cholesterol and monophosphoryl lipid-A (Beck et al., 2015), concentration of ATP in human blood plasma (Onodera et al., 2012) and assessing anti-lipopolysaccharide antibodies to *Vibrio cholerae* (Chang et al., 2001).

4. Commercially available biosensors

The first commercial biosensor kit was pioneered in 1970 and after seven years Food and Drug Administration (FDA) mentioned the regulations for the application of LAL assay for end product test in clinical samples and medical instruments for detection of toxin. Important companies that manufacture biosensing detection kits are Charles River, Lonza BioSciences, Seikagaku, Biomerieux, Invivogen, Pyrostar, Associates of Cape Cod Incorporated, etc (Das et al., 2014). Seikagaku manufactures endotoxin detecting reagents such as Pyrochrome®, Endospecy®, Toxicolor®, etc. PYROGENT™ kit from Lonza Company and Endosafe® kit manufactured by Charles River Company are two commercial kits for LPS detection that rely on LAL reagents. There are many other endotoxin detection kits that are not approved by the FDA, but are marketed for instance, EndoLISA® and PyroGene®. These kits are based upon recombinant factor C and a fluorescent substrate (Zuzuarrequi et al., 2015). Commercially available sensors are based on various principles and have diverse detection limits (Table 3).

5. Future prospects

The first biosensor was documented in the early 1960s (Dinh et al., 2001). Since then, there has been a tremendous research in this field. Biosensors are used in various areas such as environmental monitoring, food and beverage industries, pharmaceutical industries, agriculture and medical fields (Lévêque et al., 2015). Biosensor and biochip technologies have witnessed

many new interventions and diagnostic approaches. More than 50,000 articles have been published in the area of biosensors in the last fifteen years. The number of articles from the year 2000 (954) to 2015 (6143) clearly shows the demand and use of biosensors in the present situation. Biosensors may be bioreceptors such as antigens, antibodies, enzymes, nucleic acids, cellular structures and whole cells, or transducers such as optical, electrochemical and mass-sensitive. Bioreceptors specifically bind the analyte of interest to be monitored. Antibodies bind to the target molecules with higher affinity; therefore antibodies conjugated with fluorescent label can be used to detect the presence of a specific target. The resulting antibody-target complex produces fluorescence, which is proportional to the quantity of antigen bound. Enzymes possess catalytic activity that makes them function as biosensors. They catalyze a specific reaction or hydrolyze the substrates to change one or more parameters like pH, color, and specificity of the substrate that is monitored (Pile., 2013). Nucleic acids have the characteristic of complementary binding that forms the basis DNA biosensors, often called genosensors. Surface Plasmon resonance and SERS are used to monitor the binding of ligands to DNA. The whole cell or cell structure based biosensors are based on recognition and binding to specific species (Dinh et al., 2000). Electrochemical biosensors have the potential to recognize large number of targets in complex matrices (Kumar et al., 2012). Electrochemical strategy is relatively cost effective as it is portable and does not need much instrumentation (Queiros et al., 2013). Although, electrochemical techniques are sensitive, they should be more selective in diagnosing UTIs (Das et al., 2014). Solution based circuits have been developed for detecting uropathogens from both blood and urine, and are harnessed on a chip to boost the level of multiplexing that can even detect single base modifications in the sequence (Lam et al., 2013). In the last decade, biosensor based diagnostics have moved more than expectations, validating clinical samples for rapid recognition of pathogens (Wark et al., 2010). The next generation UTI diagnostics would use biosensor platform for the multiplex detection of DNA, RNA and proteins (Mohan et al., 2011). The biosensor array for UTIs offers a hopeful technology platform without the necessity of labor intensive and time consuming methods (Mach et al., 2011). The future integration of biosensors in UTI diagnostics will generate vast interest as this will certainly lead to emergence of miniature biosensors that would selectively detect uropathogenic components in minute concentrations in all biological samples. These biosensors will provide information regarding key parameters that

determine the quality of the sample and severity of the infection. The biosensors will be portable, sensitive, time saving, easy to use and cost effective (Mozaz et al., 2004).

6. Conclusion

This review discussed recent developments in biosensing methods for UTI diagnosis. Diverse approaches have been explored for the production of novel biosensors that use bioreceptors and transducers as sensing elements. The demand for advanced technologies having potential to detect uropathogens rapidly with high affinity and sensitivity in intricate environments helps to continue the quest for novel analytical methods. Biosensors meet the requirement of increasing demand for a rapid, highly specific, precise and cost effective analytical method to detect uropathogens. Worldwide effort is being made towards the progress of biosensors that can be employed for recognition, quantification and monitoring of specific bacterial species. The requirement for ultrasensitive biosensors and the demand towards early UTI diagnosis have made biosensing methodologies one of the most fascinating fields of research.

Current detection methods used for identification of uropathogen mainly rely on conventional culture based methods that require about 48 hours to provide consistent results. By that time infection steps forward and patients may reach critical situation before an empirical treatment could be given. The objective of this review on biosensors is not to eradicate the use of clinical laboratory methods but to accompany them for better results in relatively shorter time. Biosensors are being applied in both research and diagnostics. Various cellular and bacterial markers and transducers have been employed as biosensors to detect UTI. Research on UTI biosensors is accelerating further to construct even smaller, economical, faster and efficient tools that would eventually lead to successful amalgamation of electronic sensing and cellular systems. The future biosensor based diagnostics for UTI would be more informative in addition to being sensitive and rapid. The biosensor array for UTIs is the promising means in the management of these infections. The applications and science of biosensors will contribute to the future commercial diagnostics in the upcoming years. Ongoing interest and Progress in this field will change the future of UTI diagnostics and health care sector. Further studies are essential for expansion to other uropathogens and improving clinical management of UTIs. We foresee that development of new UTI diagnostics on biosensor platforms will feature significantly in the near future as this electrifying area of research goes on developing. We expect biosensors will

overcome the present limitations and meet the challenges to become an economical and easy-to-use UTI diagnostic approach.

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References:

1. Abbott, S.L., Moler, S., Green, N., Tran, R.K., Wainwright, K., Janda, J.M., 2011. Journal of Clinical Microbiology 49 (9), 3122-3126.
2. Adley, C.C., Ryan, M.P., 2015. High Throughput Screening for Food Safety Assessment, 315-326.
3. Alamuri, P., Eaton, K.A., Himpsl, S.D., Smith, S.N., Mobley, H.L., 2009. Infection and Immunity 77 (2), 632-641.
4. Altobelli, E., Mohan, R., Mach, K., Sin, M.L.Y., Anikst, V., 2015. The Journal of Urology 193 (4), e226-e227.
5. Anastasi, E.M., Wohlsen, T.D., Stratton, H.M., Katouli, M., 2013. Water Research 47 (17), 6670-6679.
6. Arlett, J.L., Myers, E.B., Roukes, M.L., 2011. Nature Nanotechnology 6, 203-215.
7. Arvind, B., 2001. Indian Pediatrics 38, 1105-1115.
8. Ayaka Shima., Atsushi Hinenoya., Masahiro Asakura., Norihiko Sugimoto., Teizo Tsukamoto., Hideaki Ito., Akira Nagita., Shah M.F., Shinji Yamasaki., 2012. Infection and Immunity 80 (4), 1323-1332.
9. Bank, S., Jensen, A., Hansen, T.M., Søby, K.M., Prag, J., 2010. Emerging Infectious Diseases 16 (1), 76-80.
10. Batra, B., Pundir, C.S., 2016. Acrylamide in Food, 497-505.
11. Beck, Z., Matyas, G.R., Alving, C.R., 2015. Biochimica et Biophysica Acta-Biomembranes 1848 (3), 775-780.
12. Ben Haj Khalifa, A., Khedher, M., 2012. Pathologie Biologie 60 (2), e1-5.

13. Boczek, J.M., Karwaciak, I., Ratajewski, M., Gagala, I., Jurczak, T., Zalewski, M., Pulaski, L., 2015. Aquatic Toxicology 168, 1-10.
14. Boisen, A., Thundat, T., 2009. Materialstoday 12 (9), 32-38.
15. Bokil, N.J., Totsika, M., Carey, A.J., Stacey, K.J., Hancock, V., Saunders, B.M., Ravasi, T., Ulett, G.C., Schembri, M.A., Sweet, M.J., 2011. Immunobiology 216(11), 1164-1171.
16. Bonkat, G., Braissant, O., Rieken, M., Solokhina, A., Widmer, A.F., Frei, R., van der Merwe, A., Wyler, S., Gasser, T.C., Bachmann, A., 2013. World Journal of Urology 31 (3), 553-557.
17. Bouamri, E.M.C., Arsalane, L., Zerouali, K., Katfy, K., El Kamouni, Y., Zouhair, S., 2015. African Journal of Urology 21 (3), 161-166.
18. Braissant, O., Wirz, D., Göpfert, B. and Daniels, A.U., 2010. FEMS Microbiology Letters, 303: 1–8.
19. Bridle, H., Desmulliez, M., 2014. Waterborne Pathogens, 189-229.
20. Bunka, D.H.J., Stockley, P.G., 2006. Nature Reviews Microbiology 4, 588-596.
21. Burgess, A.W., Paradise, L.J., Hilbelink, D., Friedman, H., 1994. Proceedings of the Society for Experimental Biology and Medicine 207 (2), 180-185.
22. Cahill, B.K., Seeley, K.W., Gutel, D., Ellis, T.N., 2015. Microbiological Research 180, 1-10.
23. Cao, X., Li. S., Chen. L., Ding. H., Xu. H., Huang. Y., Li. J., Liu. N., Cao. W., Zhu. Y., Shen. B., Shao. N., 2009. Nucleic Acids Research 37 (14), 4621-4628.
24. Catanzaro, M., Cirone, J., 2012. American Journal of Infection Control 40 (7), 663-666.
25. Cattoir, V., 2012. Journal of Infection 64(3), 260-267.
26. Cavagnolo, R., 1995. Journal of Clinical Microbiology 33 (7), 1954-1956.
27. Chalupniak, A., Waszczuk, K., Hałubek-Głuchowska, K., Piasecki, T., Gotszalk, T., Rybka, J., 2014. Biosensors and Bioelectronics 58, 132-137.
28. Chang, H.S., Sack, D.A., 2001. Clinica Chemical Acta 312 (1-2), 49-54.
29. Chang, Y.F., Fu, C., Chen, Y.T., Jou, A.F., Chen, C., Chou, C., Ho, J.A., 2016. Biosensors and Bioelectronics 77, 1201-1207.
30. Chen, C., Du, Y., Li, J., Yang, X., Wang, E., 2012. Analytica Chimica Acta 738, 45-50.
31. Chen, H.G., Ren, W., Jia, J., Feng, J., Gao, Z.F., Li, N.B., Luo, H.Q., 2016. Biosensors and Bioelectronics 77, 40-45.

32. Chua, A., Yean, C.Y., Ravichandran, M., Lim, B., Lalitha, P., 2011. Biosensors and Bioelectronics 26 (9), 3825-3831.
33. Coburn, P.S., Gilmore, M.S., 2003. Cell Microbiology 5 (10), 661-669.
34. Cogen, A.L., Nizet, V., Gallo, R.L., 2008. British Journal of Dermatology 158 (3), 442-455.
35. Cole, A.M., Tahk, S., Oren, A., Yoshioka, D., Kim, Y.H., Park, A., Ganz, T., 2001. Clinical and Diagnostic Laboratory Immunology 8 (6), 1064-1069.
36. Cooper, I.R., Taylor, H,D., Hanlon, G.W., 2007. Journal of Applied Microbiology 102 (5), 1293-1299.
37. Cui, W., Wang, L., Jiang, W., 2016. Biosensors and Bioelectronics 77, 650-655.
38. Dag, S., Niedziela, T., Dzieciatkowska, M., Lukasiewicz, J., Jachymek, W., Lugowski, C., Kenne, L., 2004. Carbohydrate Research 339 (15), 2521-2527.
39. Dark, P.M., Dean, P., Warhurst, G., 2009. Critical Care 13 (4), 217.
40. Das, A.P., Kumar, P.S., Swain, S., 2014. Biosensors and Bioelectronics 51, 62-75.
41. Das, A.P., Bal. B., Mahapatra, P.S., 2015a. Biological and Pharmaceutical Applications of Nanomaterials, CRC press, Taylor & Francis.
42. Das, A.P., Bal, B., Mahapatra, P.S., 2015b. Horseshoe Crabs in Modern Day Biotechnological Applications, in: Carmichael, R.H., Botton, M.L., Shin, P.K.S., Cheung, S.G. (Eds.), Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management, Springer International Publishing Switzerland, pp. 463-474.
43. Dechtrirat, D., Eichelmann, N.G., Wojcik, F., Hartmann, L., Bier, F.F., Scheller, F.W., 2014. Biosensors and Bioelectronics 58, 1-8.
44. Deshmukh, P.K., Ramani, K.P., Singh, S.S., Tekade, A.R., Chatap, V.K., Patil, G.B., Bari, S.B., 2013. Journal of Controlled Release 166 (3), 294-306.
45. Dinges, M.M., Orwin, P.M., Schlievert, P.M., 2000. Clinical Microbiology Reviews 13 (1), 16-34.
46. Dinh, T.V., Cullum, B., 2000. Fresenius Journal of Analytical Chemistry 366 (6-7), 540-551.
47. Dinh, T.V., Cullum, B., Stokes, D.L., 2001. Sensors and Actuators B: Chemical 74 (1-3), 2-11.
48. Fang, L.X., Huang, K.J., Liu, Y., 2015. Biosensors and Bioelectronics 71, 171-178.

49. Fey, P.D., Olson, M.E., 2010. Future Microbiology 5 (6), 917-933.
50. Floyd Kyle, A., Moore Jessica, L., Eberly Allison, R., Good James, A.D., Shaffer Carrie, L., Zaver Himesh., Almqvist Fredrik., Skaar Eric, P., Caprioli Richard, M., Hadjifrangiskou Maria., Parsek Matthew, R., 2015. PLoS Pathogens 11 (3), 7366-7374.
51. Foxman, B., 2002. American Journal of Medicine 113(Suppl 1A), 5S-13S.
52. Foxman, B., 2003. Disease-a-month 49 (2), 53-70.
53. Fritz, J., 2008. Analyst 133, 855-863.
54. Ganguly, A., Malabadi, R.B., Bhatnagar, P.K., Tang, X., Das, D., Loebenberg, R., Suresh, M.R., Sunwoo, H.H., 2015. Journal of Virological Methods 220, 5-12.
55. Garcia, M., Batalla, P., Escarpa, A., 2014. TrAC Trends in Analytical Chemistry 57, 6-22.
56. Gaster, R.S., Hall, D.A., Nielsen, C.H., Osterfeld, S.J., Yu, H., Mach, K.E., Wilson, R,J., Murmann, B., Liao, J.C., Gambhir, S.S., Wang, S.X., 2009. Nature Medicine 15 (11), 1327-1332.
57. Gebreyesus, A., Adane, K., Negash, L., Asmelash, T., Belay, S., Alemu, M., Saravanan, M., 2014. Food Control 44, 45-48.
58. Geng, P., Zhang, X., Teng, Y., Fu, Y., Xu, L., Xu, M., Jin, L., Zhang, W., 2011. Biosensors and Bioelectronics 26 (7), 3325-3330.
59. Gnauck, A., Lentle, R.G., Kruger, M.C., 2015. Journal of Immunological Methods 416, 146-156.
60. Gopal, J., Hasan, N., Wu, H.F., 2013. Biosensors and Bioelectronics 39 (1), 57-63.
61. Graham, J.C., Galloway, A., 2001. Journal of Clinical Pathology 54, 911-919.
62. Gregory, W.J., McNabb, P.C., 1986. Infection Control 7 (5), 281-284.
63. Grieshaber, D., MacKenzie, R., Vörös, J., Reimhult, E., 2008. Sensors (Basel, Switzerland) 8 (3), 1400-1458.
64. Guerreiro, G.V., Zaitouna, A.J., Lai, R.Y., 2014. Analytica Chimica Acta 810, 79-85.
65. Guillén, Z.G.A, Avila, S.J.L., Blondeau, P., Riu, J., Rius, F.X., 2012. Biosensors and Bioelectronics 31 (1), 226-232.
66. Hansen, K.M., Thundat, T., 2005. Methods 37 (1), 57-64.
67. Heras, J.Y., Pallarola, D., Battaglini, F., 2010. Biosensors and Bioelectronics 25 (11), 2470-2476.
68. Hertle, R., 2005. Current Protein & Peptide Science 6 (4), 313-325.

69. Hoffmann, S., Peterbauer, A., Schindler, S., Fennrich, S., Poole, S., Mistry, Y., Montag-Lessing, T., Spreitze,r I., Löschner, B., van Aalderen, M., Bos, R., Gommer, M., Nibbeling, R., Werner-Felmayer, G., Loitzl, P., Jungi, T., Brcic, M., Brügger, P., Frey, E., Bowe, G., Casado, J., Coecke, S., Lange, J., Mogster, B., Naess, L.M., Aaberge, I.S., Wendel, A., Hartung, T., 2005. Journal of Immunological Methods 298 (1–2), 161–173.
70. Hou, T., Wang, X., Liu, X., Pan, C., Li, F., 2014. Sensors and Actuators B: Chemical 202, 588-593.
71. Hurley, J.C., 1995. Clinical Microbiology Reviews 8 (2), 268-292.
72. Inoue, K.Y., Takahashi, S., Ino, K., Shiku, H., Matsue, T., 2012. Innate Immunology 18 (2), 343-349.
73. Ivnitski, D., Abdel-Hamid, I., Atanasov, P., Wilkins, E., 1999. Biosensors and Bioelectronics 14 (7), 599-624.
74. John, A., Boyd, J.C., Lowes, A.J., Price, C.P., 2006. American Journaal of Clinical Pathology 126, 428-436.
75. Johnson, B.N., Mutharasan, R., 2012. Biosensors and Bioelectronics 32, 1-18.
76. Kan, S., Takahashi, G., Onodera, C., Shouzushima, T., Matsumoto, N., Inada, K., Endo, S., 2013. Journal of Infection and Chemotherapy 19 (2), 299-304.
77. Kaur, N., Sharma, S., Malhotra, S., Madan, P., Hans, C., 2014. Journal of Clinical and Diagnostic Research 8(10), DC01-DC03. doi:10.7860/JCDR/2014/8772.4919.
78. Keague, M., DeRosa, M.C., 2012. Journal of Nucleic Acids 2012, doi:10.1155/2012/748913.
79. Kim, H.H., Jeon, H.J., Cho, H.K., Cheong, J.H., Moon, H.S., Go, J.S., 2015. Sensors and Actuators B: Chemical 221, 1372-1383.
80. Kim, K., Park, C., Kwon, D., Kim, D., Meyyappan, M., Jeon, S., Lee, J.S., 2016. Biosensors and Bioelectronics 77, 695-701.
81. Kovalchuk, S.I., Anikanov, N.A., Ivanova, O.M., Ziganshin, R.H., Govorun, V.M., 2015. Analytica Chimica Acta 893, 57-64.
82. Kubista, M., Andrade, J.M., Bengtsson, M., Forootan, A., Jonak, J., Lind, K., Sindelka, R., Sjoback, R., Sjogreen, B., Strombom, L., Stahlberg, A., Zoric, N., 2006. Moecular Aspects of Medicine 27, 95-125.

83. Kumar, S., Neeraj, D., Manju, B., Gaurav, B., Kumar, R., 2012. Bio Nano Science 2(4), 196-217.
84. Kushwaha, K., Babu, D., Juneja, V.K., 2014. Proteus, in: Carl A.Batt., Mary-Lou Tortorello, second ed, Encyclopedia of food microbiology. Academic press, London, pp. 238-243.
85. Lam, Brian., Das, Jagotamoy., Holmes Richard, D., Live, Ludovic., Sage, Andrew., Sargent Edward, H., Kelley Shana, O., 2013. Nature Communications 4, DOI: 10.1038/ncomms3001
86. Lévêque, C., Ferracci, G., Maulet, Y., Mazuet, C., Popoff, M.R., Blanchard, M.P., Seagar, M., El Far, O., 2015. Scientific Reports 5, 17953.
87. Li, L., Wen, Y., Xu, L., Xu, Q., Song, S., Zuo, X., Yan, J., Zhang, W., Liu, G., 2016. Biosensors and Bioelectronics 75, 433-445
88. Liao, J.C., Mastali, M., Li, Y., Gau, V., Suchard, M.A., Babbitt, J., Gombein, J., Landaw, E.M., McCabe, E.R.B., Churchill, B.M., Haake, D.A., 2007. The Journal of Molecular Diagnostics 9 (2), 158-168.
89. Lin, C.C., Ni, M.H., Chang, Y.C., Yeh, H.L., Lin, F.H., 2010. Biomaterials 31 (32), 8445-8453.
90. Lin, F.Y., Ho, V.A., Khiem, H.B., Trach, D.D., Bay, P.V., Thanh, T.C., Kossaczka, Z., Bryla, D.A., Shiloach, J., Robbins, J.B., Schneerson, R., Szu, S.C., 2001. The New England Journal of Medicine 344 (17), 1263-1269.
91. Lindsay, G.K., Roslansky, P.R., Novitsky, T.J., 1989. Journal of Clinical Microbiology 27 (5), 947-951.
92. Linhares, I., Raposo, T., Rodrigues, A., Almeida, A., 2013. BioMed Central Infectious Diseases. doi: 10.1186/1471-2334-13-19.
93. Liu, P.V., 1974. The Journal of Infectious Diseases 130, S94-S99.
94. Liu, T., Sin, M.L.Y., Pyne, J.D., Gau, V., Liao, J.C., Wong, P.K., 2014. Nanomedicine: Nanotechnology, Biology and Medicine 10 (1), 159-166.
95. Lu, Y., Meng, X., Yi, G., Jia, J., 2011. Journal of Colloid and Interface Science 356 (2), 726-733.

96. Luke, N.R., Sauberan, S.L., Russo, T.A., Beanan, J.M., Olson, R., Loehfelm, T.W., Cox, A.D., St Michael, F., Vinogradov, E.V., Campagnari, A.A., 2010. Infection and Immunity 78 (5), 2017-2023.

97. Mach, K.E., Mohan, R., Baron, E.J., Shih, M.C., Gau, V., Wong, P.K., Liao, J.C., 2011. The Journal of Urology 185 (1), 148-153.

98. Magill, Shelley S., Edwards, Jonathan R., Bamberg, Wendy., Beldavs, Zintars G., Dumyati, Ghinwa., Kainer, Marion A., Lynfield, Ruth., Maloney, Meghan., McAllister-Hollod, Laura., Nadle, Joelle., Ray, Susan M., Thompson, Deborah L., Lucy E., Fridkin, Scott K., 2014. The New England Journal of Medicine 370 (13), 1198-1208.

99. Mandar, R., 2013. Pharmacological Research 69 (1), 32-41.

100. Mariam, S.H.S., Ooi, C.W., Tan, W.S., Janna, O.A., Arbakariya, A., Tey, B.T., 2015. Separation and Purification Technology 144, 133-138.

101. Marinho, A.C.S., Polay, A.R.O., Gomes, B.P.F.A., 2015. Journal of Endodontics 41 (10), 1653-1659.

102. Mateusz Wojtusik., Alberto Rodríguez., Vanessa Ripoll., Victoria E. Santos., , José L. García., Félix García-Ochoa., 2015. Biotechnology Reports 6, 100-107.

103. Medina, P.S., Katsenovich, Y., Musaramthota, V., Lee, M., Lee, B., Dua, R., Lagos, L., 2015. Research in Microbiology 166 (5), 419-427.

104. Mehrvar, M., Abdi, M., 2004. Analytical Sciences 20 (8), 1113-1126.

105. Miao, P., Han, K., Qi, J., Zhang, C., Liu, T., 2013. Electrochemistry Communications 26, 29-32.

106. Michael Otto., 2009. Nature Reviews Microbiology 7 (8), 555-567.

107. Mireles, A.L.F., Walker, J.N., Caparon, M., Hultgren, S.J., 2015. Nature Reviews Microbiology 13 (5), 269-284.

108. Mishra, M.P., Debata, N.K., Padhy, R.N., 2013. Asian Pacific Journal of Tropical Biomedicine 3 (4), 315-324.

109. Mohan, R., Mach, K.E., Bercovici, M., Pan, Y., Dhulipala, L., Wong, P.K., Liao, J.C., 2011. PLoS ONE 6 (10), e26846.

110. Mohanan, P.V., Banerjee,S., Geetha,C.S., 2011. Journal of Pharmaceutical and Biomedical Analysis 55 (5), 1170–1174.

111. Mozaz, R.S., Marco, M.P., Alda, M.J., Barcelo, D., 2004. Pure and Applied Chemistry 76 (4), 723-752.

112. Nezlin, R., 2016. Molecular Immunology 70, 149-154.

113. Nieradka, K., Kapczynska, K., Rybka, J., Lipinski, T., Grabiec, P., Skowicki, M., Gotszalk, T., 2014. Sensors and Actuators B: Chemical 198, 114-124.

114. Noda, K., Goto, H., Murakami, Y., Ahmed, A.B., Kuroda, A., 2010. Analytical Biochemistry 397 (2), 152-155.

115. Noormandi, A., Dabaghzadeh, F., 2015. Journal of Traditional and Complementary medicine 5, 15-20.

116. Noriea, N.F., Johnson, C.N., Griffitt, K.J., Grimes, D.J., 2010. Journal of Applied Microbiology 109 (3), 953-962.

117. Nostrand, J.D.V., Junkins, A.D., Bartholdi, R.K., 2000. American Journal of Clinical Pathology 113, 709-713.

118. Obata, T., Nomura, M., Kase, Y., Sasaki, H., Shirasawa, Y., 2008. Analytical Biochemistry 373 (2), 281-286.

119. Ong, K.G., Leland, J.M., Zeng, K., Barrett, G., Zourob, M., Grimes, C.A., 2006. Biosensors and Bioelectronics 21 (12), 2270-2274.

120. Onodera, C., Takahashi, G., Kan, S., Shozushima, T., Matsumoto, N., Inada, K., Endo, S., 2012. Journal of Infection and Chemotherapy 18 (3), 370-377.

121. Ostronoff, C.S., Lourenco, F.R., 2015. Journal of AOAC International 98 (1), 51-55.

122. Padmavathy, B., Vinoth Kumar, R., Patel, A., Deepika, S.S., Vaidehi, T., Jaffar Ali, B.M., 2012. Current Microbiology 65 (1), 44-53.

123. Pan, Y., Sonn, G.A., Sin, M.L., Mach, K.E., Shih, M.C., Gau, V., Wong, P.K., Liao, J.C., 2010. Biosensors and Bioelectronics 26 (2), 649-654.

124. Papp, Z.F., Demel, U., Tilz, G.P., 2003. International Immunopharmacology 3 (13-14), 1715-1729.

125. Parks, S.B., Popovich, B.W., Press, R.D., 2001. American Journal of Clinical Pathology 115 (3), 439-447.

126. Patrice Vigeant., Jack Mendelson., Mark A Miller., 1995. Canadian Journal of Infectious Diseases & Medical Microbiology 6 (3), 153-155.

127. Pezzlo, M., 2014. Clinical Microbiology Newsletter 36, 87-93.

128. Pile, D., 2013. Nature Photonics 7 (1), 3.

129. Praveen, A., Mobley, H.L., 2008. Molecular Microbiology 68(4), 997-1017.

130. Queipo-Ortuño, M.I., Colmenero, J.D., Muñoz, N., Baeza, G., Clavijo, E., Morata, P., 2006. The Journal of Urology 176 (5), 2290-2293.

131. Queiros, R.B., de-los-Santos-Álvarez, N., Noronha, J.P., Sales, M.G.F., 2013. Sensors and Actuators B: Chemical 181, 766-772.

132. Rahman, S.R., Ahmed, M.F., Begum, A., 2014. Ethiopian Journal of Health Sciences 24 (2), 145-152.

133. Rangaiahagari, A., Nyirabanzi, J., Uwizeyimana, J.P., Ngoga, E., Wane, J., 2015. Rwanda Medical Journal 72 (1), 5-7.

134. Rao, S.G., 2012. Applied Surface Science 258 (13), 5195-5199.

135. Rath, S., Padhy, R.N., 2014. Integrative Medicine Research 3 (3), 133-141.

136. Ravindranath, S.P., Wang, Y., Joseph, I., 2011. Sensors and Actuators B: Chemical 152 (2), 183-190.

137. Reverte, L., Prieto-Simon, B., Campas, M., 2015. Analytica Chimica Acta (In Press). doi:10.1016/j.bios.2011.01.007

138. Rôças, I.N., Siqueira, J.F.Jr., Santos, K.R., 2004. Journal of Endodontics 30 (5), 315-320.

139. Ryall, B., Lee, X., Zlosnik, J.E., Hoshino, S., Williams, H.D., 2008. BioMed Central Microbiology 8. doi:10.1186/1471-2180-8-108.

140. Saha, S., Nayak, S., Bhattacharyya, I., Saha, S., Mandal, A. K., Chakraborty, S., Bhattacharya, R., Chakraborty, R., Franco, O, L., Mandal, S, M., Basak, A., 2014. Frontiers in Microbiology 5, 463. http://doi.org/10.3389/fmicb.2014.00463.

141. Sakata, M., Fukuma, Y., Todokoro, M., Kunitake, M., 2009. Analytical Biochemistry 385 (2), 368-370.

142. Shen, W., Gao, Y., Lu, B., Zhang, Q., Hu, Y., Chen, Y., 2014. Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease 1842 (7), 1109-1120.

143. Shih, C.M., Chang, C.L., Hsu, M.Y., Lin, J.Y., Kuan, C.M., Wang, H.K., Huang, C.T., Chung, M.C., Huang, K.C., Hsu, C.E., Wang, C.Y., Shen, Y.C., Cheng, C.M., 2015. Talanta 145, 2-5.

144. Simoni, G.D., Signore, G., Agostini, M., Beltram, F., Piazza, V., 2015. Biosensors and Bioelectronics 68, 570-576.

145. Singla, N., Kaistha, N., Gulati, N., Chander, J., 2010. Indian Journal of Critical Care Medicine 14 (3), 154-155.

146. Sinn, I., Kinnunen, P., Albertson, T., McNaughton, B.H., Newton, D.W., Burns, M.A., Kopelman, R., 2011. Lab on a chip (15), 2604-2611.

147. Skottrup, P.D., Nicolaisen, M., Justesen, A.F., 2008. Biosensors and Bioelectronics 24 (3), 339-348.

148. Smulders, S., Kaiser, J.P., Zuin, S., Landuyt, K.L.V., Golanski, L., Vanoirbeek, J., Wick, P., Hoet, P.H.M., 2012. Particle and Fibre Toxicology. doi:10.1186/1743-8977-9-41

149. Solano, G., Gómez, A., León, G., 2015. Toxicon 105, 13-18.

150. Soubra, L., Kabbani, S., Anwar, M.F., Dbouk, R., 2014. Journal of Global Antimicrobial Resistance 2, 173-178.

151. Sroubek, J., Krishnan, Y., Chinai, J., Buhl, S., Scharff, M.D., Donald, T.V., 2012. Journal of Immunological Methods 375 (1-2), 215-222.

152. Su, J., Zhang, H., Jiang, B., Zheng, H., Chai, Y., Yuan, R., Xiang, Y., 2011. Biosensors and Bioelectronics 29 (1), 184-188.

153. Su, W., Lin, M., Lee, H., Cho, M., Choe, W.S., Lee, Y., 2012. Biosensors and Bioelectronics 32 (1), 32-36.

154. Sun, C.P., Liao, J.C., Zhang, Y.H., Gau, V., Mastali, M., Babbitt, J.T., Grundfest, W.S., Churchill, B.M., McCabe, E.R., Haake, D.A., 2005. Molecular Genetics and Metabolism 84 (1), 90-99.

155. Sushruta, M., Anubha, K., 2011. International Research Journal of Pharmacy 2 (4), 67-71.

156. Tosar, J.P., Branas, G., Laiz, J., 2010. Biosensors and Bioelectronics 26 (4), 1205-1217.

157. Thomson, E.L.S., Dennis, J.J., 2012. Virulence 3 (3), 286-298.

158. Tu, N., Tu, Q., Tung, H., Hieu, D., Romero-Jovel, S., 2014. World Journal of Microbiology and Biotechnology 30 (6), 1829-1835.

159. Tufariello, J.M., Lowy, F.D., 2015. Uptodate. http://www.uptodate.com/contents/treatment-of-infections-due-to-coagulase-negative-*staphylococci*

160. Turner, A.P.F., 2013. Chemical Society Reviews 42 (8), 3175–3648.

161. Unger, R.E., Peters, K., Sartoris, A., Freese, C., Kirkpatrick, C.J., 2014. Biomaterials 35 (10), 3180-3187.

162. Vashist, S.K., 2007. A to Z of Journal of Nanotechnology Online 3, DOI : 10.2240/azojono0115.

163. Wadso, I., 2002. Thermochimica Acta 394 (1-2), 305-311.

164. Wang, J.T., Chang, S.C., Chen, Y.C., Luh, K.T., 2000. Journal of Microbiology, Immunology and Infection 33 (4), 258-262.

165. Wang, Y., Zhang, D., Liu, W., Zhang, X., Yu, S., Liu, T., Zhang, W., Zhu, W., Wang J., 2014. Biosensors and Bioelectronics 55, 242-248.

166. Wang, Y.X., Zun-Zhong, Y.E., Cheng-Yan, Si., Ying, Y.B., 2012. Chinese Journal of Analytical Biochemistry 40 (4), 634-642.

167. Wark, A.W., Lee, J., Kim, S., Faisal, S.N., Lee, H.J., 2010. Journal of Industrial and Engineering Chemistry 16 (2), 169-177.

168. Wijburg, O.L., Uren, T.K., Simpfendorfer, K., Johansen, F.E., Brandtzaeg, P., Strugnell, R.A., 2006. The Journal of Experimental Medicine 203 (1), 21-26.

169. Wilson, M.L., Gaido, L., 2004. Clinical Infectious Diseases 38 (8), 1150-1158.

170. Xu, M., Gao, Z., Wei, Q., Chen, G., Tang, D., 2016. Biosensors and Bioelectronics 79, 411-415.

171. Yabusaki, K., Aoyagi, H., 2012. Journal of Bioscience and Bioengineering 113 (3), 406-411.

172. Yadav, P., Pandey, K., Bhatt, P., Tripathi, B., Pandey, M.K., Kumar, M., 2016. Materials Science and Engineering: B. doi:10.1016/j.mseb.2015.12.007

173. Yamada, K., Choi, W., Lee, I., Cho, B.K., Jun, S., 2016. Biosensors and Bioelectronics 77, 137-143.

174. Yang, J., Dai, X., Chen, H., Teng, Q., Li, X., Rong, G., Yan, L., Liu, Q., Li, Z., 2016. Journal of Virological Methods 229, 40-47.

175. Yazdanpanah, S., Rabiee, M., Tahriri, M., Abdolrahim, M., Tayebi, L., 2015. TrAC Trends in Analytical Chemistry 72, 53-67.

176. Ye, S.J., Zhai, X., Wu, Y.Y., Kuang, S.P., 2016. Biosensors and Bioelectronics 79, 130-135.

177. Yeo, T.Y., Choi, J.S., Lee, B.K., Kim, B.S., Yoon, H.I., Lee, H.Y., Cho, Y.W., 2011. Biosensors and Bioelectronics 28 (1), 139-145.

178. Yoon, J., Choi, N., Ko, J., Kim, K., Lee, S., Choo, J., 2013. Biosensors and Bioelectronics 47, 62-67.

179. Zeng, Y., Liu, Z., Wu, W., Xu, F., Shi, J., 2016. Microporous and Mesoporous Materials 220, 163-167.

180. Zhao, X., Lin, C.W., Wang, J., Oh, D.H., 2014. Journal of Microbiology and Biotechnology 24 (3), 297-312.

181. Zhang, C., Fredericks, D., Campi, E.M., Florio, P., Jespersgaard, C., Schiødt, C.B., Hearn, M.T.W., 2015. Separation and Purification Technology 142, 332-339.

182. Zhu, H., Xu, Y., Liu, A., Kong, N., Shan, F., Yang, W., Barrow, C.J., Liu, J., 2015. Sensors and Actuators B: Chemical 206, 592-600.

183. Zuo, M.Y., Chen, L.J., Jiang, H., Tan, L., Luo, Z.F., Wang, Y.M., 2014. Analytical Biochemistry 466, 38-43.

184. Zuzuarregui, A., Souto, D., Pérez-Lorenzo, E., Arizti, F., Sánchez-Gómez, S., Martínez de Tejada, G., Brandenburg, K., Arana, S., Mujika, M., 2015. Analyst 140 (2), 654-660.

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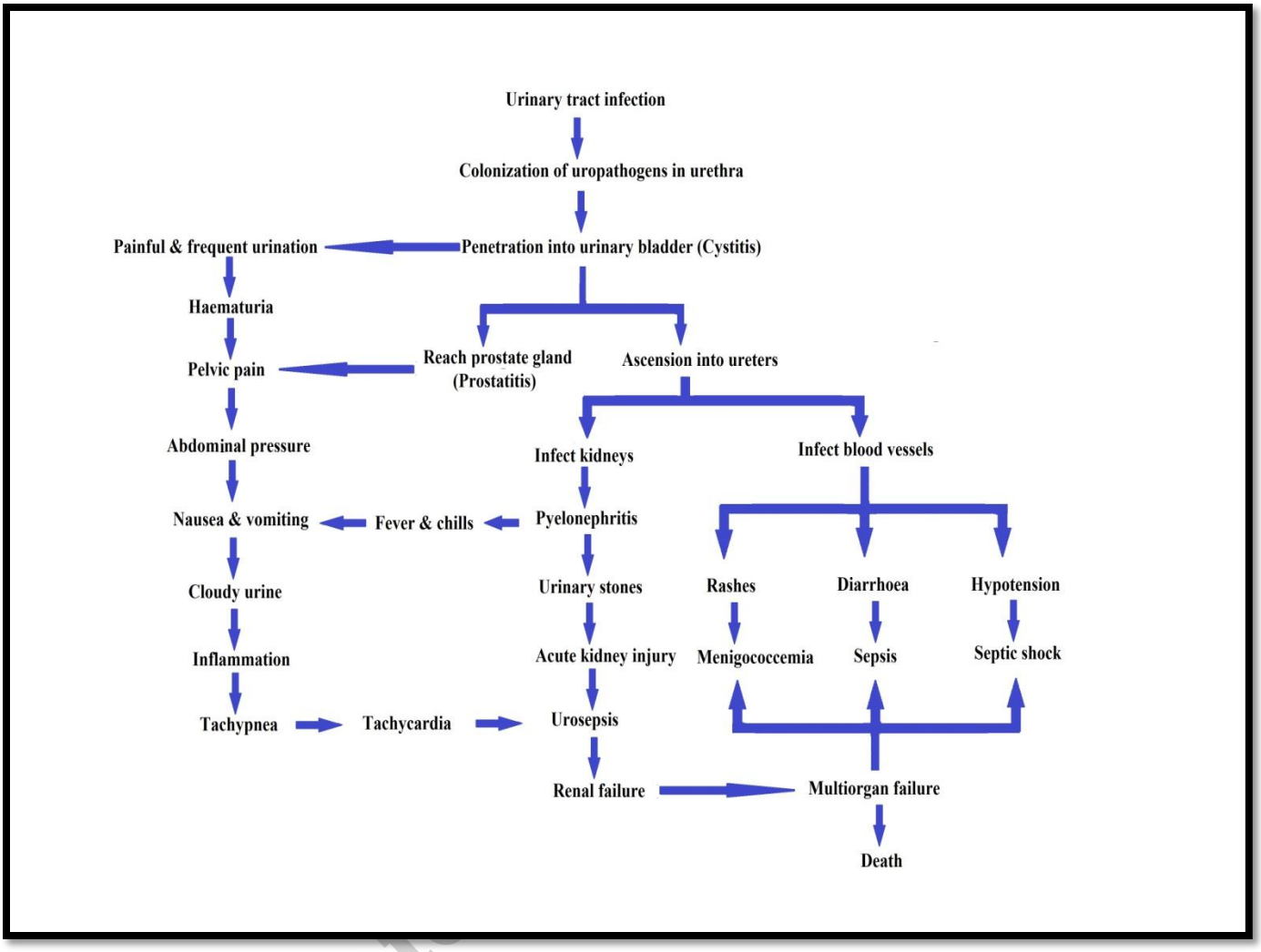

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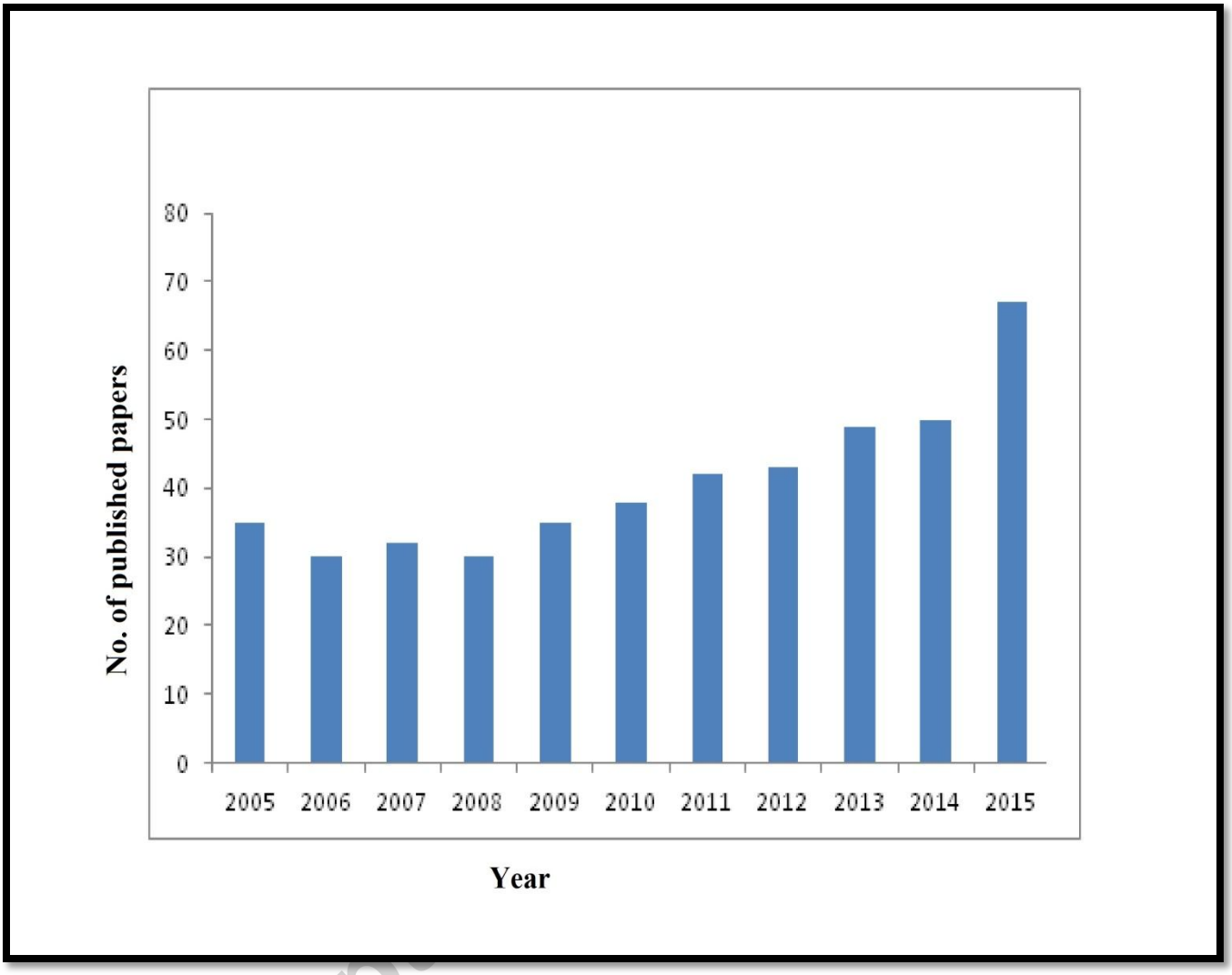

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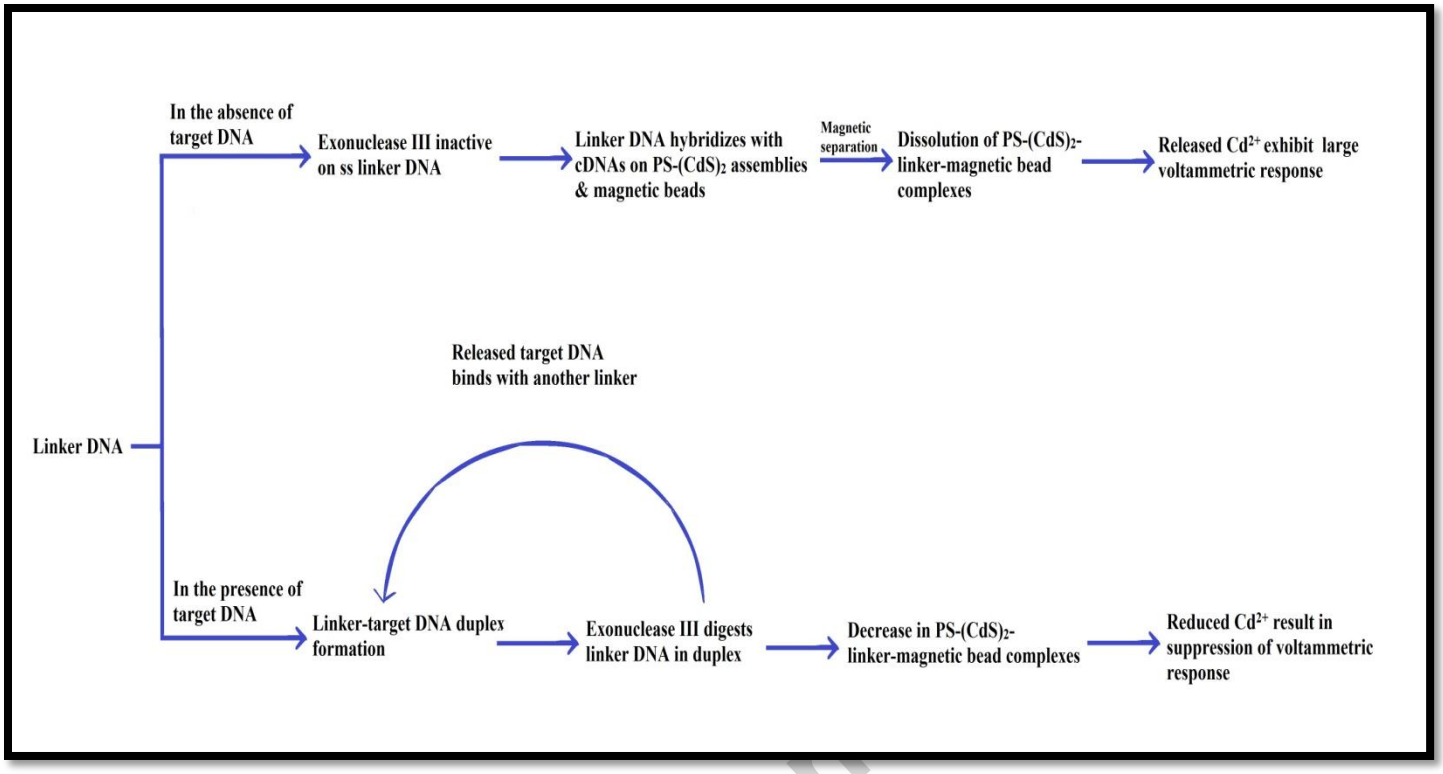

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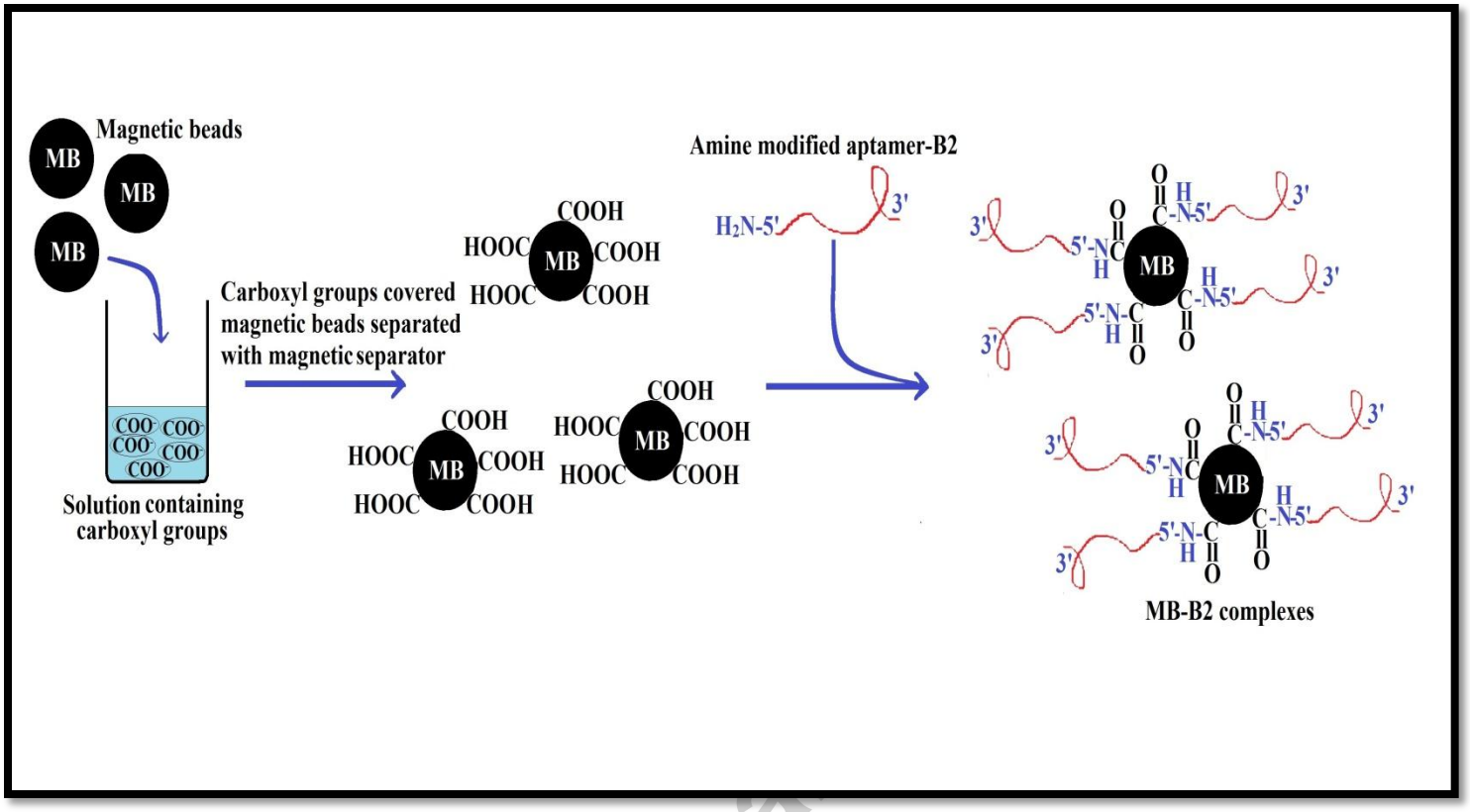

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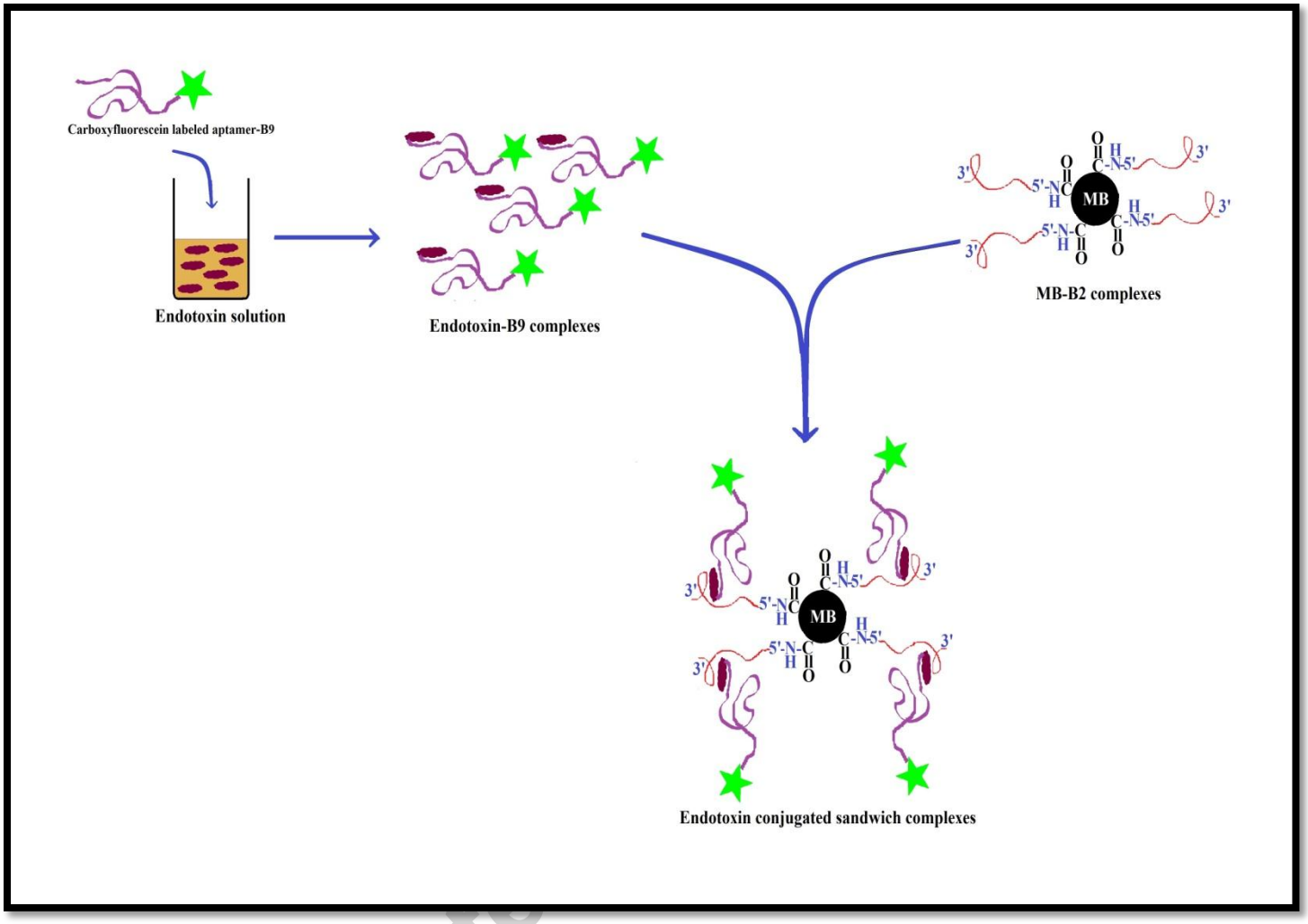

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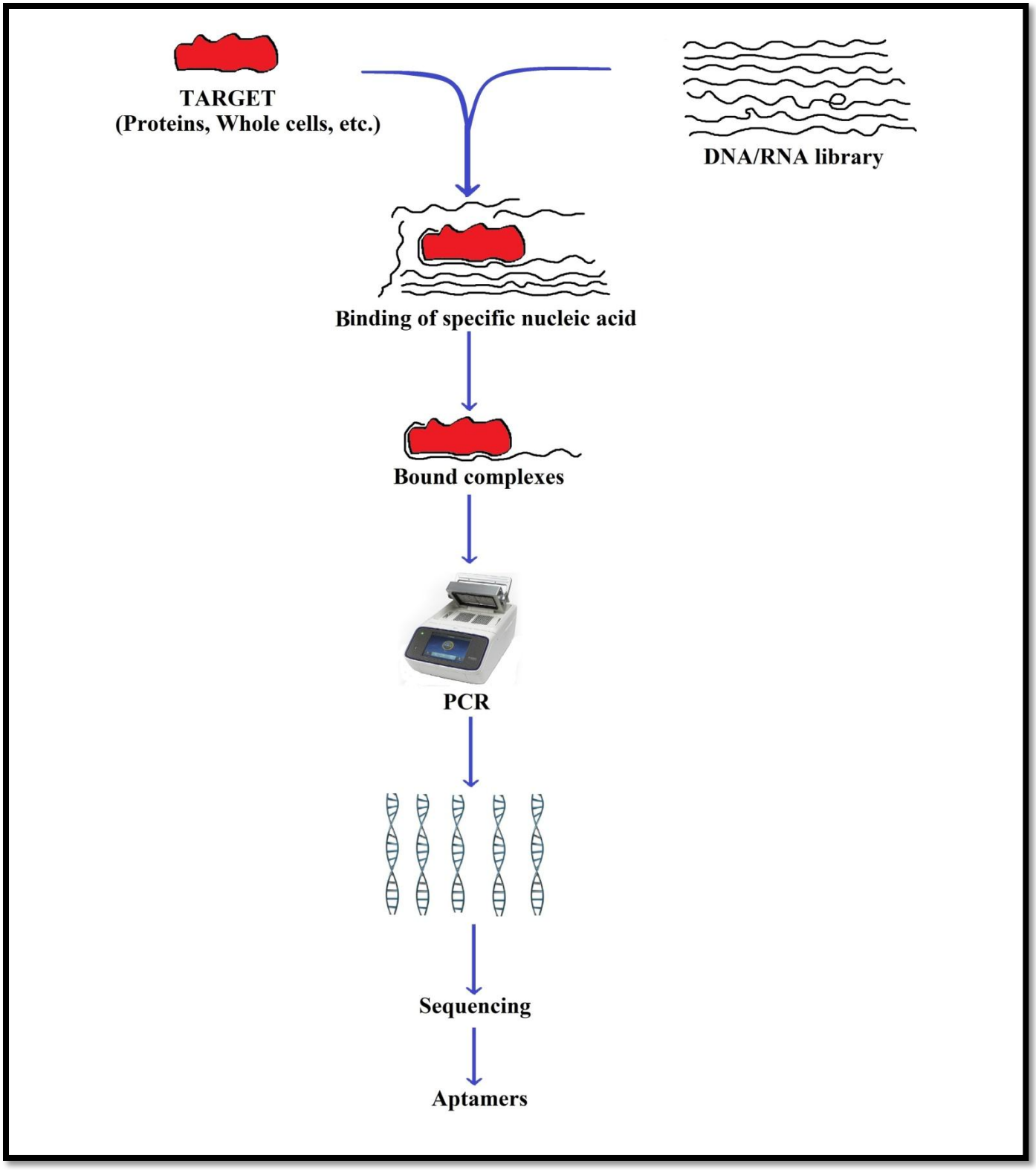

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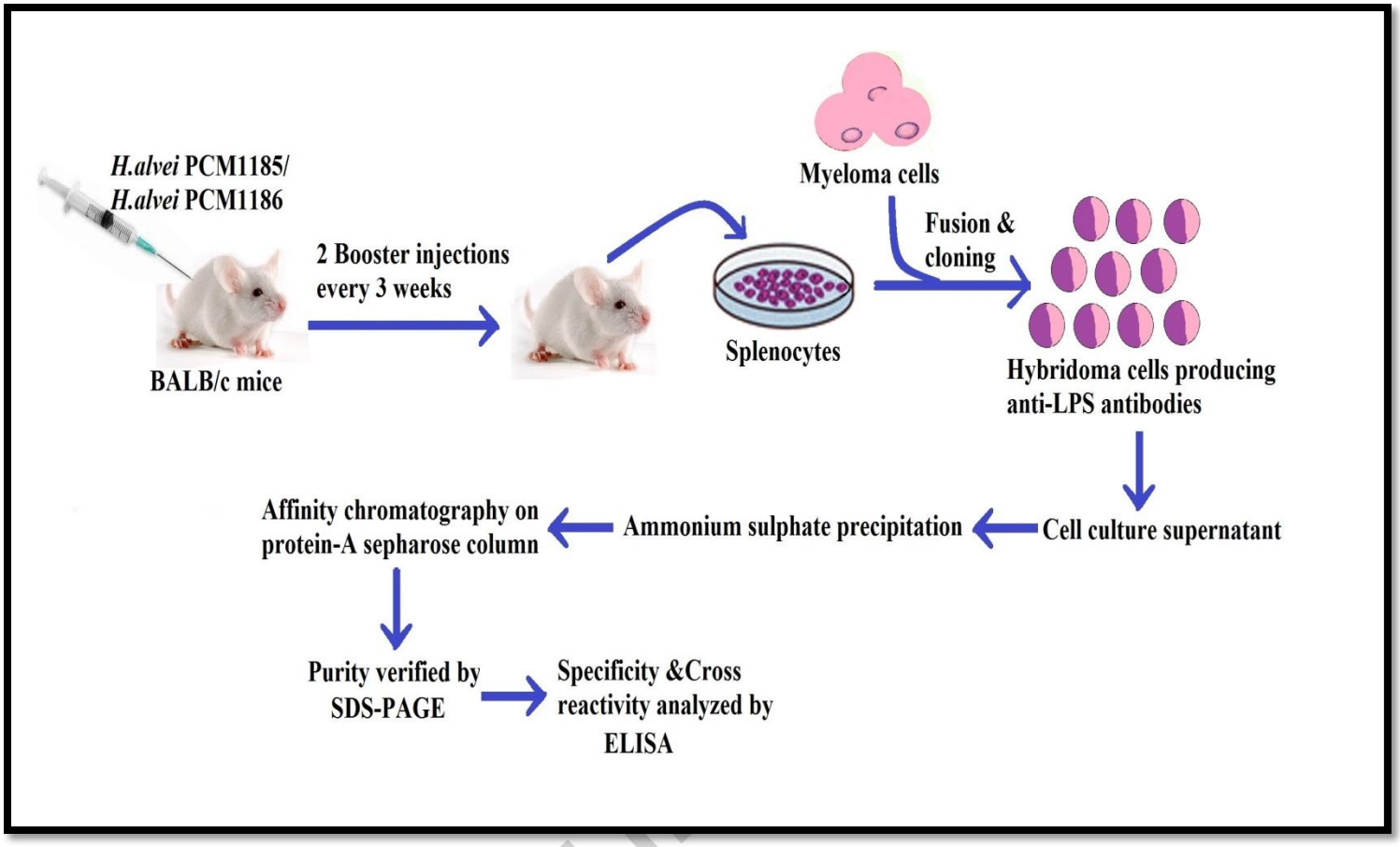

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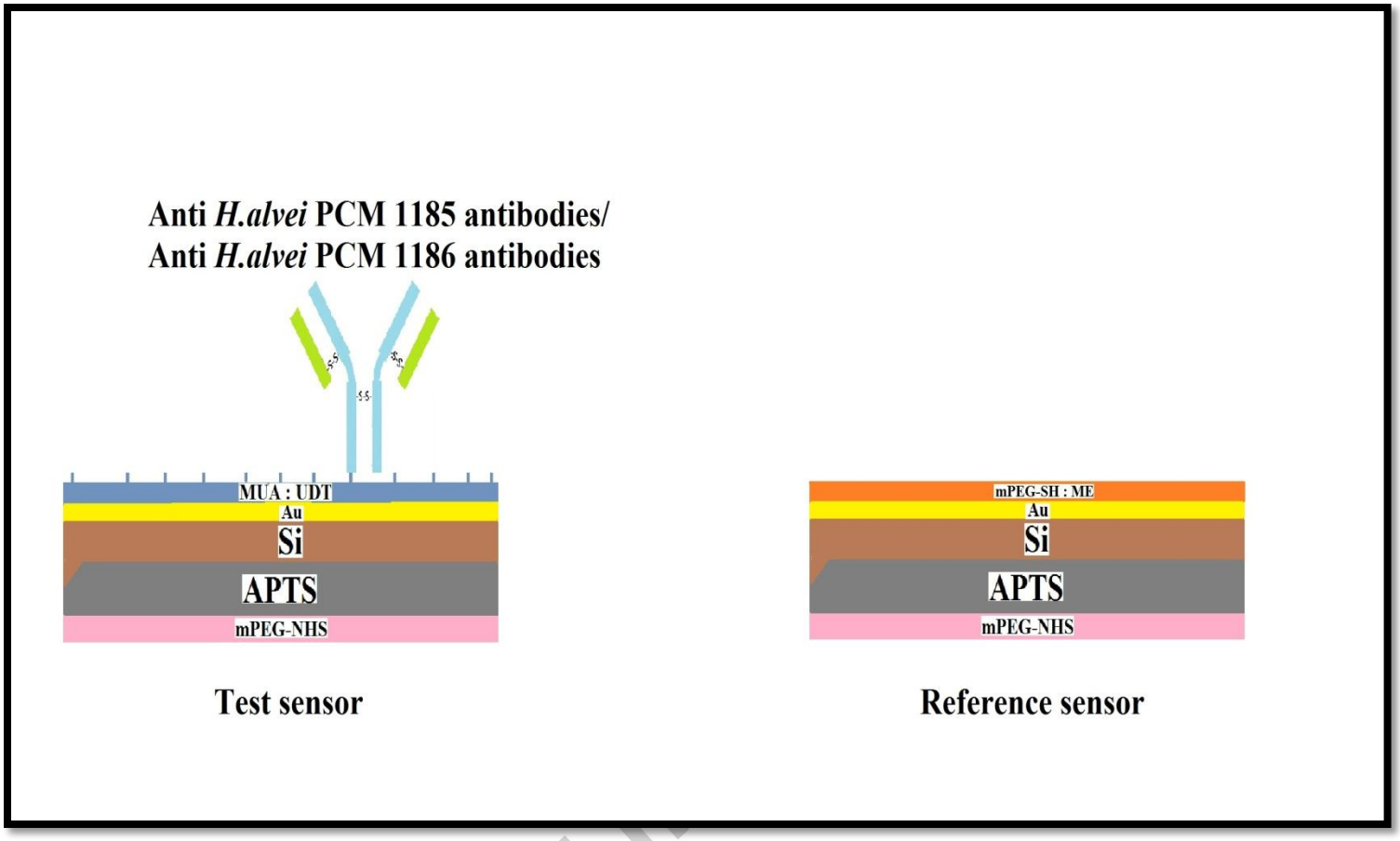

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Table 1: List of common uropathogens corresponding with their toxins produced and sources

SL. No.	Uropathogen	Toxin produced	Source	References
1.	*Escherichia coli*	Endotoxin	Contaminated	Das et al., 2014

			food & water	
2.	*Proteus mirabilis*	Cytotoxin, agglutinin	Soil, water, human intestinal tract	Praveen & Mobley 2008
3.	*Klebsiella pneumonia*	Endotoxin	Soil	Cahill et al., 2015
4.	*Pseudomonas aeruginosa*	Exotoxin A, endotoxin	Soil, water, skin flora, normal environment	Liu., 1974
5.	*Salmonella paratyphi*	Endotoxin	Feces, contaminated food & water	Lin et al., 2001
6.	*Staphylococcus aureus*	Endotoxin, cytotoxin (hemolysin alpha, beta, gamma)	Skin flora, nasal secretions	Alexander., 2001; Dinges et al., 2000
7.	*Providencia stuartii*		Urine, human feces	Shima et al., 2012
8.	*Enterococcus faecalis*	Endotoxin; cytolysin	Root canal treated teeth	Rocas et al., 2004; Coburn et al., 2003
9.	*Proteus vulgaris*	Cytotoxin (hemolysin Hpm A)	Soil, water, fecal matter	Praveen et al., 2009
10.	*Staphylococcus epidermidis*	Polysaccharide intercellular adhesion (PIA) molecule	Skin flora, mucosal flora	Fey et al., 2010; Otto., 2009; Cogen et al., 2008
11.	*Morganella morgani*	Haemolysin	Normal intestinal flora of mammals and reptiles	Singla et al., 2010;
12.	*Citrobacter freundii*	Cytotoxin	Soil, contaminated food and water, intestinal tract of animals	Rath et al., 2014; Wang et al., 2000
13.	*Serratia marcesens*	hemolysin	Damp environments	Hertle 2000
14.	*Coagulase negative staphylococci*	hemolysin	Human skin flora	Tufariello & Lowy., 2015
15.	*Klebsiella oxyntoca*	Endotoxin	Soil, water and intestinal flora	Khalifa et al., 2012; Wojtusik et al., 2015
16.	*Vibrio cholera*	Endotoxin	Feces	Das et al., 2014
17.	*Brucella melitensis*	O-Polysaccharide	Unpasteurized dairy products, infected aerosols	Ortuno et al., 2006; Vigeant et al., 1995
18.	*Treponema pallidum*	Endotoxin	External genitals	Das et al., 2014
19.	*Corynebacterium*		Human skin flora	Mandar., 2013

	jekeium			
20.	*Vibrio parahemolyticus*	Thermostable direct haemolysin (tdh)	Raw or improperly cooked sea food (eg., oysters)	Noria et al., 2010
21.	*Acinetobacter baumanni*	Endotoxin	Contaminated environment	Rath et al., 2014; Mishra et al., 2013; Luke et al., 2010
22.	*Pseudomonas cepacia*	Hemolysin	Contaminated disinfectant solutions, moist environment	Thomson et al., 2012; Ryall et al., 2008
23.	*Proteus rettgeri*	Endotoxin	Improperly stored food, fecal contaminated food	Kushwaha et al., 2014; Tu et al., 2014
24.	*Actinobaculum schaali*	Hemolysin	Commensal flora of human genitals	Cattoir., 2012; Bank et al., 2010
25.	*Hafnia alvei*	Cytotoxin	Dairy and poultry products, meat, sewage, soil.	Rahman et al., 2014; Abbott et al., 2011

Table 2: Represents uropathogen detection method along with the sensitivity, incubation time and organism

SL. No.	Method	Sensitivity	Incubation time	Organism	References
1.	Rabbit pyrogen test	0.5 EU/ml	>180 mins	-	Hoffman et al., 2005
2.	ELISA	10 pg/ml	5 h	-	Mohanan et al., 2011
3.	Colorimetric cell based assay	0.0084 µM	15 mins	*E.coli* 055:B5	Wang et al., 2014
4.	Human endothelial cell based assay	1 µg/ml	4 h	*E.coli* 0111:B4	Unger et al., 2014

4.	Dual signal amplification	5 fM	60 mins	-	Su et al., 2011
5.	Magnetoelastic sensor	0.0105 EU/ml	20-120 mins	*E.coli* 0111:B4	Ong et al., 2006
7.	TLR-4/MD2 complex based biosensors	0.0002 EU/ml	30 mins	*E.coli* 055:B5	Yeo et al., 2011
8.	Urinary lactoferrin based biosensor	145 pg/ml	120 mins	-	Y.Pan et al., 2010
9.	Solution based circuits	1 cfu/ µl	2-5 mins	*E.coli, S.aureus, P. aeruginosa*	Lam et al., 2013
10.	Electronic tongue for detection of LPS	10 pg/ml or 0.03 EU/ml	15 mins	*Salmonella enterica*	Heras et al., 2010
11.	Aptamer based impedance biosensor	1 pg/ml to 1ng/ml	15 mins	*E.coli 055:*B5	Su et al., 2012
12.	DNA biosensor	5 ng of DNA	10 mins	*Vibrio cholerae*	Chua et al., 2011
13.	Titanium based MALDI bacterial chips	10^3 cfu/ml	180 mins	*S.aureus, P.aeruginosa*	Gopal et al., 2013
14.	Bioluminescence using mutant luciferase enzyme	0.0005 EU/ml	15 mins	-	Noda et al., 2010
15.	End-point LAL assay	0.125 EU/ml	15 mins	-	Lindsay et al., 1989
16.	Kinetic chromogenic LAL assay	0.002 EU/ml	76 mins	-	Noda et al., 2010
17.	Kinetic turbidimetric method	0.0005 EU/ml	138 mins	-	Lindsay et al., 1989

Table 3: Depicts companies manufacturing toxin detection kits, basic principle and detection limits

SL. No.	Manufacturing Company	Kit	Principle	Detection limit (EU/ml)
1.	Pyrostar	Pyrostar™ ES-F/plate	Kinetic turbidimetric LAL	0.1
		Pyrostar™ ES-F	Kinetic turbidimetric LAL	0.001
		Limulus color KY series	Chromogenic LAL	0.0002
		Limulus PS	Adsorption on Pyrosep resin	0.001
2.	Associates of Cape Cod Corporated	Pyrochrome®	Chromogenic LAL	0.001
		Pyrosate®	Gel-clot LAL	0.03
		Pyrotell®	Gel-clot LAL	0.03
		Pyrotell®-T	Turbidimetric LAL	0.001
		Chromo-LAL	Kinetic chromogenic LAL	0.005
3.	Lonza BioSciences	QCL-1000	Endpoint chromogenic LAL	0.1
		Pyrogent 5000	Kinetic turbidimetric LAL	0.01
4.	Charles river	KTA	Kinetic turbidimetric & Gel-clot	0.015
		KTA2	Kinetic turbidimetric LAL	0.005
		Endochrome-K™	Kinetic chromogenic LAL	0.005
		Endosafe® gel-clot	Gel-clot LAL	0.015
5.	Sigma	E-toxate	Endpoint chromogenic LAL	0.05
6.	InvivoGen	HEK-Blue LPS detection kit	Interaction between TLR4 and LPS	0.03

Highlights:

1. This review highlights the recent advances in biosensor based uropathogen detection methodologies.
2. Representation of the common uropathogens in a tabular form.
3. Illustration of working mechanism behind each biosensor system.
4. Special attention has been given for LAL based assay and its successful fabrication into a biosensor.
5. A brief study on the commercially available diagnostic kits available in market.