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comparable to, and in some cases even surpass those of monoclonal antibodies. For example, aptamers have been selected that have dissociation constants (Kd) in the nanomolar or picomolar range. Aptamers are discovered using an *in vitro* process called Systematic Evolution of Ligands by EXponential enrichment (SELEX), a procedure where target-binding oligonucleotides are selected from a random pool of sequences through iterative cycles of affinity separation and amplification (see Fig. 1) (Ellington & Szostak, 1990; Tuerk & Gold, 1990). The SELEX process begins with a large, random oligonucleotide library whose complexity and diversity can be tailored through its distribution and number of random nucleotide regions (Luo et al., 2010). These sequences are exposed to the molecule of interest and those with an affinity for the target are separated from non-binding sequences. The elution of the binding sequences from the target and the polymerase chain reaction (PCR) amplification of those binders yields an enriched pool for subsequent, more stringent, selection rounds. After several rounds, this pool is cloned, sequenced, and characterized to find aptamers with the desired properties.

![Fig. 1. Schematic overview of the SELEX procedure for the selection of aptamer sequences.](https://www.intechopen.com)

Targets for which aptamers can be developed are varied and range from small molecules (Huizenga & Szostak, 1995), to proteins and even whole cells. The *in vitro* nature of the selection process allows for the discovery of aptamers for even non-immunogenic or highly toxic substances. In addition to this advantage, aptamer technology offers several other benefits over antibodies. First, high-purity aptamers can be chemically synthesized at a low cost and can be easily modified with dyes, labels, and surface attachment groups without affecting their affinities. Second, aptamers are more chemically stable under most environmental conditions, have a longer shelf life, and can be reversibly denatured without loss of specificity. These properties make aptamers attractive in the development of low-cost, robust diagnostics and biosensors.

An examination of the development of aptamers and aptamer-based biosensors for food safety related targets can be found below. The chapter will be divided into two main parts: aptamers for small molecule food contaminants and aptamers for food safety-related pathogens.

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<table>
<thead>
<tr>
<th>Target Class</th>
<th>Target</th>
<th>DNA/RNA</th>
<th>$K_d$ (nM)</th>
<th>Biosensor Platform</th>
<th>LOD</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tetracyclines</strong></td>
<td>Chloramphenicol</td>
<td>RNA</td>
<td>2100</td>
<td></td>
<td>-</td>
<td>(Burke et al., 1997)</td>
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<tr>
<td></td>
<td>Tetracycline</td>
<td>RNA</td>
<td>1000</td>
<td></td>
<td>-</td>
<td>(Berens et al., 2001)</td>
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<tr>
<td></td>
<td></td>
<td>DNA</td>
<td>64</td>
<td>Electrochemical</td>
<td>10 nM</td>
<td>(Niazi, Lee Gu, 2008)</td>
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<tr>
<td></td>
<td></td>
<td>DNA</td>
<td>-</td>
<td>Electrochemical</td>
<td>1 ng/mL</td>
<td>(Zhang et al., 2010)</td>
</tr>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td>Oxytetracycline</td>
<td>DNA</td>
<td>10</td>
<td></td>
<td>-</td>
<td>(Niazi, Lee, Kim et al., 2008)</td>
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<td></td>
<td>Streptomycin</td>
<td>RNA</td>
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<td></td>
<td>-</td>
<td>(Wallace &amp; Schroeder, 1998)</td>
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<tr>
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<td>Tobramycin</td>
<td>RNA</td>
<td>30-100</td>
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<td>-</td>
<td>(Goertz, Colin Cox Ellington, 2004a)</td>
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<tr>
<td></td>
<td></td>
<td>RNA</td>
<td>20000</td>
<td></td>
<td>-</td>
<td>(Morse, 2007)</td>
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<td></td>
<td></td>
<td>RNA</td>
<td>9</td>
<td>2'-OMe-RNA</td>
<td>Electrochemical (Impedence) 0.7 µM</td>
<td>(González-Fernández et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Kanamycin</td>
<td>RNA</td>
<td>180</td>
<td></td>
<td>-</td>
<td>(Kwon et al., 2001)</td>
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<tr>
<td></td>
<td></td>
<td>RNA</td>
<td>10-30</td>
<td></td>
<td>-</td>
<td>(Goertz, Colin Cox Ellington, 2004b)</td>
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<tr>
<td></td>
<td>Neomycin</td>
<td>RNA</td>
<td>Low nM</td>
<td></td>
<td>-</td>
<td>(Goertz, Colin Cox Ellington, 2004b)</td>
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<tr>
<td></td>
<td></td>
<td>RNA</td>
<td>1800</td>
<td>2'-OMe-RNA</td>
<td>Electrochemical (Impedence) &quot;sub µM&quot;</td>
<td>(de-los-Santos-Alvarez et al., 2007)</td>
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<tr>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td><strong>Mycotoxins</strong></td>
<td>Ochratoxin A</td>
<td>DNA</td>
<td>200</td>
<td></td>
<td>-</td>
<td>(Cruz-Aguado &amp; Penner, 2008a)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fluorescence Polarization</td>
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<td>(Cruz-Aguado &amp; Penner, 2008b)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Electrochemical</td>
<td>30 pg/mL</td>
<td>(Kuang et al., 2010)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Electrochemiluminescent</td>
<td>0.007 ng/mL</td>
<td>(Z. Wang et al., 2010)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Colorimetric</td>
<td>20 nM</td>
<td>(Yang et al., 2010)</td>
</tr>
</tbody>
</table>
2.1.1 Antibiotics
In addition to their use in the treatment of bacterial diseases in humans and animals, antibiotics are important in animal husbandry because they significantly enhance growth when added to animal feed. However, the accumulation of antibiotics in food-producing animals is a potential cause of the increasing occurrence of antibiotic resistance. For this reason, European Union (EU) legislation has forbidden the practice of adding antibiotics to animal feed since 2006. As a result, fast, sensitive methodologies are being developed and used by food-safety control laboratories to ensure the control of antibiotic residues in live animals and animal products (Cháfer-Pericás et al., 2010).

While several antibiotic families are used in veterinary medicine and are tested in foods, only a handful of aptamers have been developed that recognize them. As seen in Table 1, RNA aptamers have been developed against chloramphenicol and several aminoglycosides such as streptomycin, kanamycin, tobramycin and neomycin. In particular, several aptamers have been developed for members of the tetracycline family. An RNA and DNA aptamer exist that recognize tetracycline as well as a DNA aptamer for oxytetracycline. Several of these have been integrated into electrochemical based testing systems. Overall, these antibiotic aptamers have a wide range of affinities for their targets, having dissociation constants from micromolar to low nanomolar. However, the detection systems that have been developed that use these aptamers all display detection limits in the nanomolar range.

2.1.2 Mycotoxins
Toxic fungal metabolites known as mycotoxins can contaminate a wide range of agricultural commodities and are high priority targets for the development of new molecular recognition probes and biosensors. It is estimated that at least 25% of the grain produced worldwide is contaminated with mycotoxins. Problematically, even small concentrations of mycotoxins can induce significant health problems including vomiting, kidney disease, liver disease, cancer and death (Cheli et al., 2008). The first mycotoxin aptamer was developed for Ochratoxin A (OTA). This toxin is produced by Aspergillus ochraceus and Penicillium verrucosum and is one of the most abundant food-contaminating mycotoxins in the world (De Girolamo et al., 2011). OTA is a nephrotoxic toxin, with strong carcinogenic effects on rodents, as well as documented teratogenic and immunotoxic effects in humans (Cruz-Aguado & Penner, 2008a). Since its development in 2008, this aptamer has been integrated into several biosensor detection systems including electrochemical, electrochemiluminescent, colorimetric and fluorescent platforms as well as chromatographic and fluorescent test strips. More recently, an aptamer for fumonisin B1 (FB1) has recently been developed. Fumonisin mycotoxins are produced by Fusarium verticillioides and F. proliferatum species, fungi that are ubiquitous in corn (maize). Insect damage and some other environmental conditions result in the accumulation of fumonisins in corn-based products worldwide. FB1 is a nephrotoxin in all species tested, a carcinogen in rodents and a reproductive toxicant in rodents and likely in humans (Bolger et al., 2001). Both the FB1 and the OTA DNA aptamers bind to their target with nanomolar dissociation constants.

2.1.3 Heavy metals
Inorganic metals contaminate foodstuffs including fish and fish products, meat and meat products, milk and milk products, eggs, fats and oils as well as animal feeds. Arsenic is a toxic carcinogen found in many parts of the world. It can exist in four oxidation states (-3, 0,+3, and+5); however, arsenate [As(V)] and arsenite [As(III)] are the most abundant.
This strip relied on the competitive reaction between the DNA probe 1 (test line) and the target OTA. In the presence of OTA, the aptamer-GNP could not hybridize to the DNA probe 1 in the test line, thus causing the red color intensity to become weaker. Regardless of the concentration of OTA, the aptamer–GNP probe could hybridize with DNA probe 2 in the control line to ensure the validity of the detection test (see Fig. 4).

Fig. 4. Schematic of the detection principle of the strip. Reproduced with permission from Elsevier.

When using this strip for qualitative purposes, it was found to have a visual limit of detection (LOD) of 1 ng/mL. However, it is possible to use this strip for semi-quantitative purposes. Using a scanning reader, a quantitative calibration curve was constructed. Based on this, the quantitative LOD was 0.18 ng/mL which is better than the antibody-based strip method and comparable to the ELISA methods used for detection. Specificity of this strip assay was also tested using the mycotoxins deoxynivalenol, fumonisin B₁, zearalenone and microcystin-LR and was found to be specific for OTA. These fabricated strips were stable after 30 days. In addition, OTA analysis was performed on spiked wine samples. The recoveries were from 96% to 110% which met the detection requirements. Overall, this semi-quantitative assay proved to be rapid (less than 10 minutes), inexpensive and reliable in the detection of OTA.

2.2.3 Tetracyline determination in milk using an aptamer-modified electrode

Tetracycline is a naturally occurring, broad-spectrum polyketide antibiotic produced by species of *Streptomyces*. Tetracycline has been a popular and economically valuable drug for the last six decades as it can be easily isolated by fermentation. It is used against many bacterial infections due to its ability to inhibit protein synthesis. For these reasons, tetracycline has been widely used in human therapy, aquaculture and veterinary medicine. It is also extensively used as an animal growth promoter (Berens et al., 2001). As a result, tetracycline has been detected in food products, such as meat, milk, eggs and chicken.
Fluorescence emission from the quantum dot conjugated aptamer was measured and the detection limit in binding buffer was found to be 2.5 cfu/mL for both live and heat-killed *C. jejuni* bacteria (Fig. 8). Bacterial detection was also attempted in diluted food matrices such as 2% milk, chicken juice and ground beef wash. The detection limit for both live and heat-killed *C. jejuni* bacteria was determined to be in the range of 10-250 cfu/mL. In this case, a portable fluorometer could be effectively used as the fluorescence detector allowing this biosensor to serve as a practical field-based detection kit.

This aptasensor was also previously found to have very low cross-reactivity with other bacteria such as *E. coli* O157:H7, *L. monocytogenes* and *S. typhimurium*. In the present study, the aptasensor was further tested against different species of *Campylobacter* such as *C. coli* and *C. lari* in addition to other bacterial genera. The resulting cross-reactivity was observed to be very low for species outside the *Campylobacter* family but was fairly high for those within the genus, indicating potential use for detecting these other two pathogens.

![Graphs showing detection of bacteria in different food matrices](image)

**Fig. 8.** Detection of *C. jejuni* bacteria in various food matrices using spectrofluorometry. Tenfold serial dilutions (using binding buffer as diluent) from $2.5 \times 10^6$ cfu/mL to 0 bacteria, indicated by the direction of the arrows, were measured. Reproduced with permission from Springer.

### 2.4.3 Aptamer-based biochips for label-free detection of plant virus coat proteins by SPR imaging

Apart from human health as a factor that is accounted for in food biosensors, food quality and integrity are also of importance. The appearance of food can influence the consumer's
Advances in Aptamer-based Biosensors for Food Safety


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Environmental Biosensors


This book is a collection of contributions from leading specialists on the topic of biosensors for health, environment and biosecurity. It is divided into three sections with headings of current trends and developments; materials design and developments; and detection and monitoring. In the section on current trends and developments, topics such as biosensor applications for environmental and water monitoring, agro-industry applications, and trends in the detection of nerve agents and pesticides are discussed. The section on materials design and developments deals with topics on new materials for biosensor construction, polymer-based Microsystems, silicon and silicon-related surfaces for biosensor applications, including hybrid film biosensor systems. Finally, in the detection and monitoring section, the specific topics covered deal with enzyme-based biosensors for phenol detection, ultra-sensitive fluorescence sensors, the determination of biochemical oxygen demand, and sensors for pharmaceutical and environmental analysis.

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