Enzyme-linked immunosorbent assay (ELISA) was developed for the detection and quantification of proteins, peptides and a variety of complex target molecules. ELISA has an array of applications from use as a diagnostic tool in medicine to quality-control testing in industry. (Yolken et al., 1980, Aydin., 2015) An ELISA exploits the specificity of antibodies to detect and quantify an analyte.

The process of sensing a target molecule is carried out via detection agents such as fluorophores, enzyme probes or other recognition tags. These can be directly conjugated to a primary antibody, which binds directly to the target, or to a secondary antibody, which is able to identify the primary antibody. The detection molecules produce a visible signal such as a colour change, enabling not only detection, but also quantification. (Liddell, J., 2003)

Three formats of ELISA are most commonly used; direct detection, indirect detection and a capture detection sandwich method. (Figure 1) (Gan & Patel., 2013) All three formats require immobilization of either the target molecule or an antibody to surfaces such as a 96-well microtiter plate.

For a direct ELISA, the target of interest is immobilized to a surface and recognised by a labelled antibody that in turn produces a detectable signal to confirm the presence of a target. This is a rapid technique requiring only one labelled primary antibody. Disadvantages include the immunoreactivity of the antibody potentially being affected by the conjugation of a label, decreasing the target detection efficiency. (Vira et al., 2010) Labelling specific
antibodies can be very time consuming and expensive. Hence the most common ELISA format utilises the indirect detection mechanism. (Fig. 1b)

Indirect ELISA incorporates a labelled secondary antibody that recognises and detects a primary antibody bound to a target. (Yolken et al., 1980) Consequently, a variety of primary antibodies can be used in conjunction with a common labelled secondary antibody, while also permitting the immunoreactivity of the primary antibody to be at maximum capacity. However, indirect ELISA comes with its own disadvantages. Most prominently, cross-reactivity can occur between the two antibodies resulting in nonspecific signalling. (Holmseth et al., 2012)

A sandwich or capture ELISA can be employed to address some of the aforementioned shortcomings. The sandwich ELISA functions by immobilising a capture antibody specific to the target of interest. The captured target is then detected through a primary antibody, which can be labelled directly or recognised by a secondary antibody. (Fig. 1c) This method requires the target to contain two antigenic epitopes capable of being recognised by separate antibodies. One major advantage of this method is that the target does not need to be purified before use.

A rivalling technique—that not only reproduces the capabilities of an ELISA but has also been suggested to improve on the existing method of detection and quantification of target molecules—is the enzyme-linked oligonucleotide assay (ELONA). Systematic evolution of ligands by exponential enrichment (SELEX) is used to generate a nucleic acid aptamer that is highly specific to a selected target. (Gopinath., 2011) The aptamer is the main molecular recognition element in an ELONA. An ELONA is essentially an ELISA but with the antibody recognition molecule replaced by an aptamer. Therefore, any existing functions of the ELISA method can be carried out using ELONAs but with certain added benefits.

Figure 2 ELONA formats: (A) Direct Assay- Target of interest is immobilised on to the assay surface and consequently detected by the specific labelled aptamer (B) Aptamer Sandwich assay- A primary aptamer specific to the target of interest immobilized to the assay surface binds to the target, the target is then recognised by a labelled secondary aptamer.
One advantage of using aptamers in ELONA is that unlike antibodies, labelling of aptamers has a minimal effect on their sensitivity and specificity for their target. (Luzi et al., 2003) As such, a secondary aptamer is not required to detect a primary aptamer. (Fig. 2a)

The SELEX process of aptamer production can also be optimised to include aptamers tagged with specified labels during selection, ensuring labelled aptamers still bind tightly and specifically with the target of interest. Compared to antibodies, the labelling and production of aptamers can be more cost effective and efficient.

Similar to an ELISA, ELONAs can also be used for a target whose immobilisation may be challenging, exploiting a sandwich detection method. A capture aptamer that binds to the target can be immobilised to a surface, with a primary labelled aptamer then detecting the bound target. (Fig. 2b) The sandwich ELONA format increases the specificity of the assay because two aptamers that are highly specific to the target are required for detection. Two aptamers recognising the target of interest at different sites can easily be produced during the aptamer selection strategy by virtue of selection producing an easily refined polyclonal pool. A counter-selection step can be carried out where a target specific aptamer is introduced during aptamer selection, eliminating any potential aptamers that bind to the same site. (Hohmura et al., 2013) This ELONA format may be favourable when two antibodies cannot be raised for an indirect ELISA method, due to the lack of epitopes on the target.

These two methods have widely been advertised as in competition, understandable as they employ different methods of target detection. However, due to the versatility of aptamers, the two families of detection molecules can actually be used in conjunction. Aptamers can easily be incorporated into existing ELISA platforms and an aptamer-antibody based approach can be utilised. As aptamers can be produced for almost any molecule, they can be raised to target specific primary antibodies. The aptamer can then be employed to replace the secondary detection antibody. (Fig. 3a) Due to the simple chemical synthesis and labelling methods of oligonucleotides, aptamer-based methods of detecting primary antibodies makes for a cheaper and efficient option when compared to the production of labelled secondary antibodies. Aptamers can also be used to identify a target once it’s bound by a capture antibody or can act as capture molecules with detection carried out using a labelled primary antibody. (Fig. 3b &3c)
Aptamers have been raised and used in ELONAs successfully for the detection of various target molecules such as *Mycobacterium tuberculosis* secreted protein MPT64, West nile virus E protein and Hepatitis C virus Glycoprotein E2. (Zhu *et al.*, 2012, Bruno *et al.*, 2012, Chen *et al.*, 2009) The sensitivity and specificity shown in these studies demonstrate the versatility of aptamers and ELONA.

Along with the aforementioned advantage of chemical synthesis (reducing time and cost, whilst allowing an incredibly versatile labelling strategy), there are other benefits associated with aptamer use; low batch to batch variability, improved stability and completely *in vitro* selection. (Jayasena, 1999)

The ability of aptamers to recognise a diverse array of targets from small molecules to cells, makes ELONA a perfect assay for testing target molecules where antibodies have failed, such as molecules with low immunogenicity or small in size.


