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Defining Target Product Profiles (TPPs) for Aptamer-Based Diagnostics

Harleen Kaur, Bandhan Chaterjee, John G. Bruno, and Tarun Kumar Sharma

Contents

Abstract Defining target product profiles (TPPs) for aptamer-based diagnostics is crucial to the success or failure of aptamer businesses or products. A well-conceived TPP will place the aptamer in an assay for a target against which antibodies are ill-suited or have difficulty detecting the analyte, such as some highly related proteins or poorly immunogenic small molecule haptens. Strong TPPs can also take advantage of the unique nucleic acid nature of aptamers, to produce assays with

Harleen Kaur and Bandhan Chaterjee contributed equally to this work.

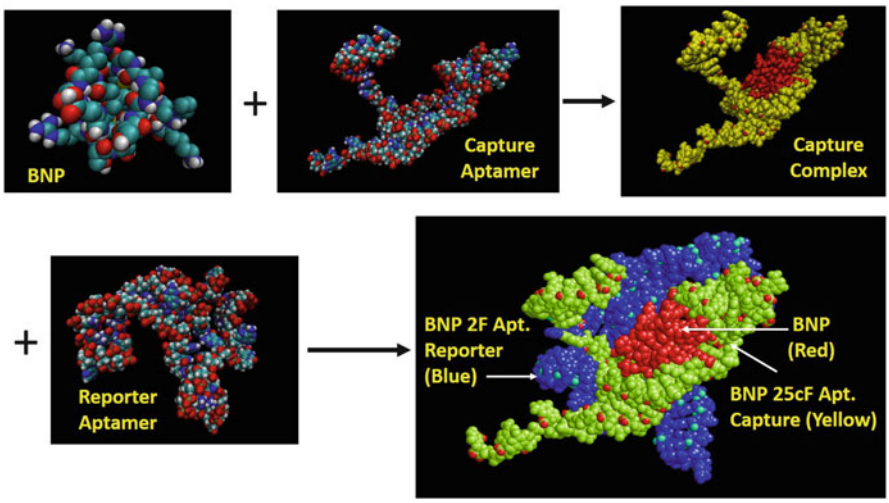
H. Kaur
Aurobindo Biologics, Hyderabad, India

B. Chaterjee and T. K. Sharma (✉)
Centre for Biodesign and Diagnostics, Translational Health Science and Technology Institute (THSTI), Faridabad, Haryana, India
e-mail: tarun@thsti.res.in

J. G. Bruno
Nanohmics Inc., Austin, TX, USA

longer shelf life or special chemical properties and ability to be modified versus protein-based antibodies. The following chapter reviews the essence of well-conceived TPPs especially with respect to aptamer targets for diagnostics and illustrates several examples of commercial aptamer diagnostic success.

Graphical Abstract



Keywords Aptamer, Diagnostics, Health, TPPs

1 Introduction

With two simultaneous seminal works by Ellington and Szostak [1] and Tuerk and Gold [2] in 1990, the genesis of a new era started. What was discovered was going to be a potential panacea to diagnostic and therapeutic challenges. They realized what was already known from studies on HIV and adenovirus in the 1980s, which established that viruses code structured RNAs that then bind proteins with high affinity and specificity [3], and that the capacity of single-stranded nucleic acids to form limited, but sophisticated, 3D structures, conferred their ability to bind ligands specifically. However, the moment of epiphany was that random single-stranded nucleic acid sequences can be screened for candidates that will be binding to a specific analyte with affinity and specificity at par with antibodies, hence the discovery of nucleic acid "antibodies." Both the groups independently devised an iterative selection process known as SELEX (Systematic Evolution of Ligands by EXponential enrichment) for screening of chemically synthesized random nucleic

acid sequences against a target, and the resultant high-affinity sequences were termed as "aptamers" (a sobriquet coalescing the Latin word *aptus* ("to fit") and the Greek word meros ("part")) [4, 5]. The development of aptamers includes designing a library of random nucleic acid sequences, typically 60–90 bp long, forming secondary structures (these structures may range from or include stem, loop, bugle, pseudoknot, G-quadruplex, and kissing hairpin) [6, 7] which in turn is functionally analogous to the binding site of antibodies (Fab fragment). Sequential cycles of repetitive selection against a target and PCR-assisted enrichment follow. The final product of SELEX is aptamers which can bind their cognate targets with high affinity and specificity.

Consider the following points to understand how aptamers are at least an ideal surrogate, if not better, for antibodies:

- First of all, antibodies are generated in biological systems, typically in horses or sheep. This poses a limitation, as antibodies cannot be generated against toxins that cannot be tolerated by the animal system. Further, antibodies cannot be generated against non-immunogenic (targets that do not illicit immune response in the host systems) entities. Conversely, as aptamers are generated chemically and the selection is in vitro, the intended number of targets can be theoretically endless.
- The synthesis procedure of aptamers is rapid and cheap and suffers only minimally from batch to batch variations. Antibody synthesis is opposite to it on every account. Furthermore, the selection process for monoclonal antibodies is far more time-consuming and costly than aptamer selection.
- Both offer comparable range of affinity (low nanomolar to picomolar range, though aptamers have gone to zeptomolar (10^{-21} M) [8]) and selectiveness. Because aptamers are chemically synthesized and selected in vitro, aptamers offer greater room for modifications than antibodies. Features like the ability to be chemically modified as per requisite, selection of target epitope, even pharmacokinetic (PK) parameters can be tailored according to needs, and simply do not exist with antibodies.
- Aptamers have the ability to refold into their functionally active native state after high-temperature exposures and thus have less stringent storage conditions than antibodies which only remain functional when stored in refrigerated conditions.

Because of such technical superiority aptamers were destined for the diagnostics and therapeutics market. Despite being only discovered in 1990, the industry's worth estimate runs as high as $2.1bn by 2018 [9] and is poised to grow at unprecedented rate in the coming years. With such optimism and opportunities, a profound understanding of the requirements from end users and the aptamer's intended use becomes of paramount importance. Thoughtful consideration of facts, such as (1) what is the specific use of the espoused product and if it aligns properly with the needs of the end users or the target populations and (2) what features to be incorporated so that the product can compete with the contemporary gold standards, facts that concern the investors and the remaining stakeholders, is indispensable for a viable market product. Luckily such motley but germane questions can be conjoined in a document

that will be used as a tool to guide the entire process of the product development, known as target product profile (TPP). A TPP is nothing but an elaborate and well-deliberated plan, spanning all the relevant sections from product development to marketing [10]. A well-designed TPP ensures that the R&D is well directed to develop products that satisfy the context and need of the end users properly. Organizations like the *F*oundation for *I*nnovative *N*ew *D*iagnostics (FIND) further simplify the process by convening the various stakeholders like researchers, clinicians, and end users to gather their views on the product, to develop a better TPP.

While most target product profiles (TPP) for aptamers are similar to almost any other business or product development plan to sell a "widget" which targets a specific need, aptamers present some unique opportunities and challenges when compared to their antibody or immunodiagnostic competitors which have been quite commercially successful. Thus, planning of specific aptamer development for particular targets (TPPs) is extremely important to the eventual success or failure of aptamer-based diagnostics. We begin with a general discussion of TPPs with emphasis on aptamer diagnostic products below.

2 Designing of TPP, Benefits, and Its Key Features

A TPP document can be used as a strategy planning tool in the development of aptamers for diagnostic assays and also other biomolecules. The development of the TPP and its execution is a joint effort between various stakeholders, such as the team of scientists (or technical team), regulatory authorities, investors, supply chain, and senior management, and can often be used as a reference document for post development discussions.

2.1 Benefits of Designing a TPP

Defining TPP as per its intended use from the beginning of the execution of a project increases the chance of success many folds, as it allows the researchers to design their aptamer as per the requirement of regulatory bodies. The other main advantage of a TPP is that it can serve as an excellent tool for designing, communicating, and tracking the progress and modifications during the aptamer-based diagnostics development. In addition, as it is not limited to aptamer diagnostics, TPPs could also be employed for drug discovery research as well as drug development and have already been utilized for in vitro diagnostics (IVD)-based products for several companies [11]. Also, this kind of exercise could gather a lot of information and feedback from a wide variety of stakeholders from different disciplines, background, and geographies which helps to make well-informed decisions and provide value to the end users, investors, and sponsors.

2.2 Features of a TPP

In order to develop the business strategy for TPPs and quantify the potential market value, five key differentiating features must be put in place for product positioning, raising capital, and discussions with investors and key stakeholders. The key features are discussed in detail below:

1. *Statement of intended use*: This section of the TPP provides a brief description of the assay or product under development. The following questions need to be addressed by the stakeholders during this phase of product development:
   - What is the purpose of the assay? Is it for screening, monitoring, or diagnosis?
   - What is the target population? Is it limited to a particular geography or demographics?
   - Is it a quantitative or qualitative diagnostic assay? How can the end result(s) be expected, and how can it assist the physician to understand the entire clinical picture and take a well-informed decision related to the treatment?
   - What is the analyte or marker of interest? Is it well characterized or validated for the assay development?
   - What instruments or additional diagnostics tests are required to execute the assay?
   - What is the "gold standard" against which performance of your test will be compared?
2. *Brief explanation of test*: A concise summary about the diagnostic test along with the usefulness to the patients is helpful to complete this section of the TPP. Questions raised during this section could be as follows:
   - What is the intent of the test?
   - What kind of test will it be? Is it a point-of-care test, laboratory test, or use at home test?
   - What analytes can be detected from the test?
   - Does the test and target of interest need to be validated per the regulatory guidelines?
   - What could be accomplished from the test?
   - When should the test not be used for detection?
3. *Summary of test procedure*: A good understanding about the simplicity or complexity of the diagnostic test is useful to determine whether or not the target location is ideal for performing the test. For simple and easy-to-use diagnostic kits, such as glucose level monitoring or pregnancy detection, robustness and reliability would be important. On the other hand, complex laboratory tests, such as pathogen detection or tumor biopsy in a clinical laboratory, must be carried out under the supervision of trained and qualified laboratory personnel. The following questions need to be addressed in order to complete this section:

- What is the layout of the entire test starting from sample collection, sample analysis to final results?
- What equipment (or instruments), reagents, and assistance are required to perform the test?
- Is the diagnostic assay kit easily accessible in different geographies?
- In case of complex testing, what is the turnaround time from sample collection to availability of results?

4. *Interpretation of results*: The results of a clinical test could be interpreted by visual observation and manual calculation or could be determined through computer software. Regardless of how the results are obtained and reported, this key feature should include how the results are calculated and interpreted. In order to do so, the following questions must be answered and taken into consideration.

   - How will the results be calculated?
   - What are the acceptable criteria for a positive or negative/clinically insignificant test result?
   - What format should be used for reporting the result?
   - What factors can impact the test results? For example, improper handling of samples or reagents, calibration of equipment, and storage of samples or reagents.

5. *Performance characteristics*: The last key step to complete the TPP for a particular diagnostic assay would be to summarize the critical performance characteristics that would be used as a standard to build the road map of the studies required to generate data to test and validate the specifications. A checklist of performance characteristics for a diagnostic test is as follows.

   - Robustness: Robustness is a measure of a diagnostic test capacity to remain unaffected by small but deliberate variations in test parameters and, thus, provides an indication of its reliability during normal usage.
   - Specificity: Specificity is the ability of a diagnostic test to assess unequivocally the analyte in the presence of components which may be expected to be present such as impurities and degradants.
   - Sensitivity: Sensitivity is the ability of a diagnostic test to detect the minimum concentration of an analyte in a sample.
   - Reproducibility: The reproducibility of a diagnostic test is the ability of the diagnostic test to measure the sample and generate data within acceptable range under the same operating conditions.
   - Limit of quantification (LOQ) and limit of detection (LOD): The quantitation limit of a diagnostic test is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The detection limit of a diagnostic test is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

To understand TPPs better, consider this example. In 2017, the WHO outlined an overarching TPP for developing a test for predicting the advancement of tuberculosis

infection to active disease. For this, the WHO conducted two surveys, first in May 2016 and second in January 2017. The latter had more specific participation, engaging all stakeholders including academia, multilateral and international agencies, NGOs, civil society and community representatives, endemic countries, test developers, and members of the New Diagnostics Working Group (NDWG) to arrive at a consensus TPP. The consensus meeting report outlines TPPs for optimal features of the test and can be summarized as follows [12]:

Intended Use

- *Goal of test* – To predict and quantitatively correlate the progression of infection to active disease within the next 2 years, to reflect the effect of treatment
- *Type of specimen* – Invasive specimen like capillary whole blood (finger-prick sample) or noninvasive specimens like saliva, urine, stool, or breath
- *Target population* – Persons who highly likely came in contact with affected persons, individuals showing early symptoms marking the progression of the disease
- *Target user* – Health workers with minimal lab training
- *Setting* (*lowest level of implementation in healthcare system*) – Health post or tertiary level

Performance Characteristics

- *Sensitivity* – $\geq$90% sensitivity
- *Specificity* – $\geq$90% specificity
- *Reproducibility* – Inter-assay CV $\leq$10.0% at high and low extremes of the assay

Operational Characteristics

- *No. of steps* – $<$2
- *Sample preparation* – None or fully integrated
- *Volume measurement* – None
- *Data analysis* – Integrated
- *Time to results* – $<$24 h
- *Biosafety* – Universal precautions
- *Operating temperature* – 5°–50°C, 90% humidity
- *Controls* – Internal positive only and positive and negative both for external controls
- *Maintenance/calibration* – None
- *Power requirements* – Should not require any instrument for charging and should be equipped with rechargeable batteries with life up to 8 h
- *Result capturing, documentation, data display* – Reader should be able to save or print results without any special instrumentation
- *Data export (connectivity and interoperability)* – The user should be able to export the data in the format compatible with his/her computer
- *Electronics and software* – None
- *Training* – $<$1-day long training for health workers with minimal lab training

Pricing

- *Cost of equipment* – <500 USD
- *Cost of consumables (reagents/test strips)* – <5 USD/test

3 Applications of Defining TPPs

3.1 TPP for Tuberculosis (TB) Diagnostics

Tuberculosis (TB) is one of the leading causes of deaths worldwide. Ten million people were diagnosed with TB, and 1.6 million died from the disease in 2017 [13]. With one-third of cases not reported and patients not undergoing drug susceptibility tests (DST), the new and improved diagnostic kits for TB could help to detect more cases and reduce diagnostic delays and transmission of the disease. Considering the gravity of the disease worldwide, resistance for the multiple TB drugs in the market and inspired by the success of Xpert MTB/RIF diagnostics, particularly in South Africa, investors and diagnostic companies have shown significant interest in TB diagnostics [14–16]. In order to develop a promising TB diagnostic kit, it is important to have a detailed target product profile (TPP) to understand the end user needs, market competition, and specifications.

In order to address the unmet needs, the Bill and Melinda Gates Foundation and Grand Challenges Canada have supported several ongoing activities globally and also announced grants to support the development of the diagnostics in global health. Nine potential TPPs were determined by a panel of experts from ten different countries at the TB Modelling and Analysis Consortium (TB MAC) meeting, held in 2013, which were further used to set the ten criteria and priorities in terms of market potential of the test, impact of the test on TB transmission, mortality and morbidity, as well as implementation and scalability of the test [17]. Each criterion was rated as high, medium, or low priority. The highest score was for a rapid, sputum-based, molecular test for microscopy centers (with the option of add on DST cartridge), followed by a rapid biomarker-based, instrument-free test and for non-sputum samples (that also detects childhood and extrapulmonary TB). TPPs that were ranked lowest score could not directly diagnose TB and could be used as a rule-in or rule-out method for TB detection. Due to their relatively low market potential, they were ranked low on their ability to reduce TB mortality and morbidity cases. Based on the obtained results, efforts are underway to develop detailed TPPs for the rapid sputum-based molecular test and a biomarker-based assay, which have been ranked first and second in the abovementioned assessment.

The impact of the TPP criteria can be readily gauged by looking at the current trends of diagnostic research, with increasing emphasis being laid on the development of POC tests or devices, capable of swiftly yielding highly accurate results in a limited resource setting in sputum directly. Recently, Lavania et al. reported an

aptamer-based electrochemical sensor for the screening of pulmonary TB among presumptive TB subjects. The development of the sensor was guided by target product profiles (TPPs) demarcated by the WHO for community-based triage or referral testing (TPP #2), namely, swift, facile, and specific and can be performed at the primary healthcare center (microscopy center level). The test costed around $1–3/test and yields ~92.3% sensitivity (95% CI 64–100%) and ~91.2% specificity (95% CI 80.7–97%) in just 30 min [18].

3.2 TPP for Point-Of-Care (POC) Tests

In order to improve the management of POC testing, Pai et al. have highlighted the importance of defining the diversity of target product profiles, particularly in resource-limited settings [17]. Five TPP settings that are useful at different levels are TPP1 for homes, TPP2 for communities, TPP3 for clinics, TPP4 for peripheral laboratories, and TPP5 for hospitals (Fig. 1). Oral fluid-based HIV tests or lateral flow assay devices are a few examples that span the entire spectrum of TPPs that can be performed in home- to hospital-based settings. On the other hand, techniques such as ELISA, aptamer-linked immunosorbent assay (ALISA), and microscopy can only be performed by trained and qualified personnel, thus limiting the technology to laboratories and hospitals [19]. Each of the abovementioned settings has individual set of challenges for diagnosis monitoring, and treatment of a disease, and challenges can vary from country to country, within the country, and urban versus rural demographics. Furthermore, barriers could be economical and infrastructural, such as unaffordability of instruments at home or community level, frequent power outrage, and lack of adequate storage space or temperature control. It could also be due to supply chain deficiencies or lack of qualified and trained personnel and healthcare workers in low-resource countries.

With no TPP in place for aptamer research and promising potential of aptamers in diagnostics and therapeutics, Tarun and colleagues adopted the abovementioned TPP model to reduce the "bench-to-bedside" translation of aptamer technology [5, 20–22] (Fig. 2). Diagnostic assays such as aptamer-linked immunosorbent assay (ALISA), dot blot, and apta-PCR that are used for the detection of *Rickettsia* and food pathogens were positioned in laboratory and hospitals settings, as these assays required qualified and trained personnel along with the instrument to perform the diagnostics. On the other hand, assays equipped with handheld devices, such as fluorescence-based assays and vitamin D detection, were placed at community and clinical levels. The categorization of these diagnostic assays into different settings beforehand would not help to speed up the translation process but would also eliminate unnecessary steps and effort required for the development and validation of the assays, thereby bringing the product a step closer to the end user. Considering the wide success and affordability of the personal glucose meter (PGM) in improving and saving the lives of diabetic patients, Liu et al. developed a novel technology by conjugating functional DNA molecules to invertase enzyme for the detection of

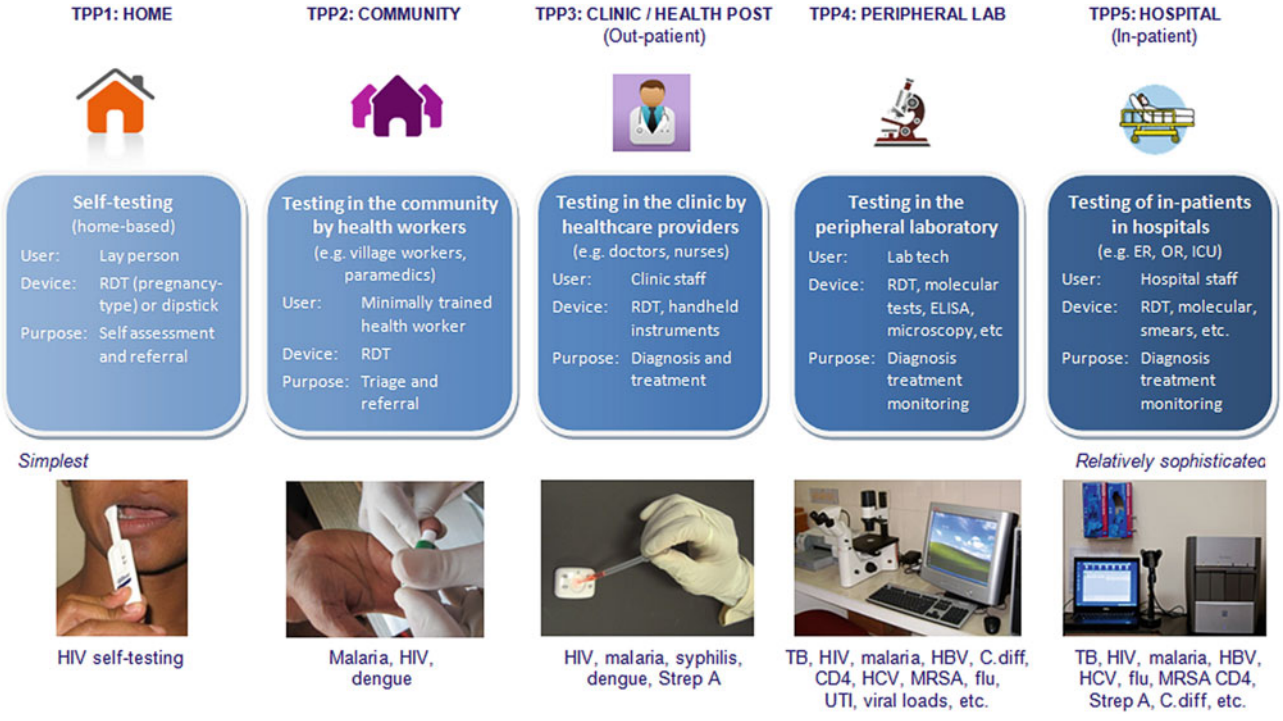


Fig. 1 Schematic representation of TPPs in different settings [17]

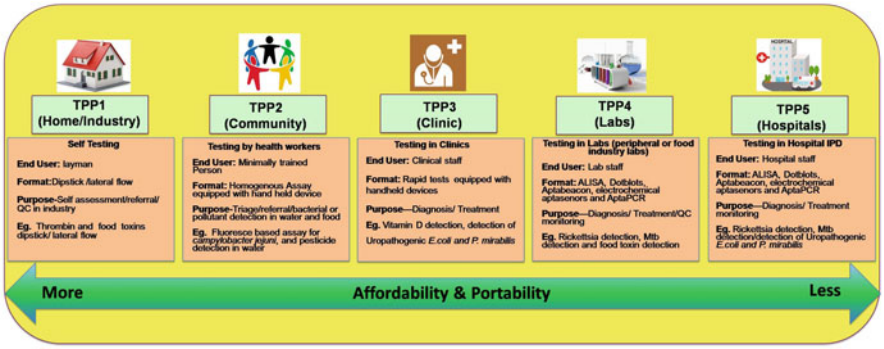


Fig. 2 Schematic representation of TPPs for aptamer diagnostics [22]

non-glucose targets, such as interferon gamma of tuberculosis, toxic metal ions, and food toxins [23]. The functional DNA molecule could either be an aptamer, aptazymes, or DNAzymes. In this study, the interaction between invertase-conjugated functional DNA and magnetic beads is disturbed in the presence of the target, resulting in the release of DNA-invertase conjugate that catalyzes the conversion of sucrose to glucose which can be read by the PGM. The amount of detected glucose is directly proportional to the amount of target present. The aforementioned work has a lot of potential in aptamer-based diagnostics, and further research and implementation of TTP strategies could be valuable to change the landscape of the medical diagnostics and environmental monitoring at home and in the field.

3.3 Diagnostic Detection of Bacterial Infection

Kapasi and colleagues [24] have employed TPP-based treatment in their work, in order to improve the patient outcome and side effects and make the treatment more economical, particularly in resource-limited settings. This work on improving the patient outcome was driven by the previous study that involved review of the host biomarkers to differentiate between bacterial and non-bacterial infections in acute febrile patients. In order to reduce bias, the follow-up work was conducted in collaboration with experts working in different geographies in the field of infectious disease, laboratory medicine, microbiology, global health, health economists, and diagnostic test development [24].

3.4 Other Aptamer-Specific TPPs and Diagnostic Applications

As antibody-based diagnostics continue to dominate the diagnostics market, it will not be prudent to compete against them, unless aptamer-based diagnostics offer very convincing competitive advantages over antibody assays. Some advantages of nucleic acid aptamers and their in vitro generation that can be used for TPP development are:

- *Specificity*: Aptamers can be developed to discriminate nearly identical targets whether it be small molecules or macromolecules. The classic example of greater aptamer specificity for a small molecule target comes from the work of Jenison et al. who demonstrated a greater than 11,000-fold difference in dissociation constants for an RNA aptamer that could distinguish the bronchodilator theophylline from caffeine which differ by only one methyl group in structure. Ingesting a simple cup of coffee may interfere with a theophylline immunoassay result, but the Jenison aptamer enabled improved analyte discrimination [25]. Likewise, Cruz-Aguado and Penner developed aptamers capable of distinguishing ochratoxin A and B for wheat testing and incorporated these aptamers into successfully marketed diagnostic field tests for NeoVentures Biotechnology in Canada [26]. Bruno et al. developed aptamers capable of discriminating natural from recombinant human growth hormone (hGH) for the World Anti-Doping Agency (WADA) [27]. Recombinant hGH does exhibit amino acid modifications in about 2% of the recombinant proteins. More recently, Bruno's group has worked on chemically modified aptamers in an attempt to discriminate a variant of prostate-specific antigen (PSA) which is impossible to detect via antibodies but is indicative of aggressive cancer and demonstrated some degree of success [28]. An improved aptamer-based PSA or other cancer biomarker diagnostic test would be a fine example of a worthy aptamer TPP. This ability (specificity) to discriminate between two very similar targets can be incorporated in the TPP list.
- *Scope of the Diagnostics*: Aptamers can be developed against poorly immunogenic molecules, while it is difficult to generate antibodies against such targets. Aptamer development in vitro typically will work against molecules which are either too small (haptens), too short (peptides less than ~10 amino acids), or too repetitive to be immunogenic in animals. Numerous examples of high-affinity aptamers developed against the monomers of otherwise non-immunogenic polymers exist in the literature [8].
- Development of aptamers to bind and detect highly lethal toxins that might easily kill a host animal if injected. Numerous examples of this TPP exist for bacterial and marine toxins, as well as snake, insect, scorpion, spider venoms, etc. [29–32]. This feature of aptamers allows to broaden the scope of the developed test to places where antibodies have clear limitations.

- Assays which take advantage of specific chemical properties of nucleic acids (aptamers) including DNA "combing" (plastic adhesion at neutral pH) or facile modification of nucleic acids with attachment or reporter chemical groups and modified bases or exotic nucleotides to enhance binding [8]. Bruno utilized the ability of DNA (aptamers) to adhere to polystyrene to concentrate and isolate or purify aptamer-bound pathogenic bacteria on the inside of plastic cuvettes as the basis for a novel sensitive assay format called plastic-adherent sandwich assay or "PASA" [33, 34].
- *Storage and transport*: Aptamers are highly stable at a wide range of temperatures (15–90°C), a feature which enables higher shelf life and cost-effective storing conditions. Bruno demonstrated that his group's C-telopeptide aptamer beacon assay was unimpaired in its low ng/ml detection of this bone loss peptide even after 5.5 years of storage at ambient temperature in a lyophilized state [35]. While many dried antibody-based lateral flow test strips also have long shelf lives, few are validated beyond more than a few years in sealed or vacuum-packed envelopes.

4 Conclusions

Aptamers are a unique class of nucleic acids able to recognize their targets with high affinity and high specificity, similar to the popular antibodies. To date, aptamers have proven to be a versatile tool for healthcare and biomedical research, particularly in the diagnostics arena. In order to unleash their full potential in diagnosis and treatment of various diseases, it is important to develop, design, and understand the indispensable significance of the target product profile (TPP). The presence of this strategic document will be useful for the researchers and biological experts to review and assess the product development process, desired features, likely course of research and development, and all other scientific and technical information required to reach the desired product outcome. In addition, TPP is a valuable tool to reduce the bench-to-bedside translation times of aptamer molecules, thus making the process faster, more cost-effective, and accessible, particularly in resource-limited settings.

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