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APPLICATION OF BACTRIAN CAMEL DERIVED NANOBODIES IN THE DETECTION OF FOODBORNE PATHOGENS

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ABSTRACT

Foodborne pathogens pose a significant threat to public health and the limitations of traditional detection methods have underscored the urgent need for rapid and sensitive novel technologies. Nanobodies (Nbs), owing to their unique structural features, exhibit substantial potential for the detection of foodborne pathogens. This review systematically examines the structural characteristics, biological properties, advantages as detection tools and preparation methods of Bactrian camel-derived Nbs. It elaborates on their applications in detecting various foodborne pathogens, including Salmonella, *Cronobacter sakazakii* (*C. sakazakii*) and *Vibrio parahaemolyticus* (*V. parahaemolyticus*). Nb-based technologies such as enzyme-linked immunosorbent assay (ELISA), immunochromatographic test strips and colourimetric sensors have demonstrated high sensitivity, specificity and rapid detection capabilities. Meanwhile, this paper analyses the current challenges, such as insufficient antibody affinity and non-specific binding in complex matrices and looks forward to future directions, including modification of antibodies and development of integrated detection platforms in combination with emerging technologies. This provides a reference for in-depth research and application of Nbs in foodborne pathogen detection.

Key words: Bactrian camel, food safety, foodborne pathogens, immunoassays, nanobodies

Illnesses caused by foodborne pathogens pose a serious threat to global public health, with a large number of people falling ill or even dying each year from consuming contaminated food. Foodborne pathogens are reported to cause approximately 600 million cases of illness and 420,000 deaths worldwide annually (Maguire Van Seventer and Hamer, 2017). Traditional methods for detecting foodborne pathogens, such as microbial culture, remain the gold standard but are time-consuming, low-throughput and inefficient (Hendrickson *et al,* 2019). Molecular biology methods, although accurate, specific and sensitive, require specially designed probes, cumbersome sample preparation and are prone to false positives. They are also limited by the need for expensive equipment and skilled operators (Mandal *et al,* 2011; Zhang *et al,* 2020). With the rapid pace of the food supply chain, there is an urgent need for rapid, sensitive and accurate detection technologies to enable early monitoring and control of foodborne pathogens.

Immunoassays based on the specific antigen-antibody reaction, characterised by simple operation, high accuracy and rapid detection, have been developing rapidly and playing an increasingly important role in the detection of foodborne pathogens. As an emerging immune biomolecular tool, Nbs have emerged in the field of foodborne pathogen detection in recent years. Their unique structure confers many excellent properties, providing a new approach to addressing the bottlenecks of traditional detection techniques. As an important source of Nbs, Bactrian camel, due to its special physiological adaptations and immune properties, produces Nbs with high diversity and unique functions. The application of Bactrian camel-derived Nbs in foodborne pathogen detection is of great significance for enhancing food safety and protecting public health.

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2. Overview of nanobodies

2.1. Structural features of nanobodies

Antibodies in camelids differ from those of other mammals in that they contain two naturally occurring structural antibodies, a conventional tetrameric antibody and a dimeric antibody composed solely of heavy chains, referred to as the heavy chain antibody (HCAb) (Hamers-Casterman *et al,* 1993). Although the HCAb lacks the light chain and the first constant region of the heavy chain (CH1), it remains functionally intact as an antibody. Upon cloning the variable domain of HCAb in camel, a single domain antibody called VHH or Nb was obtained. It has a diameter of 2.5 nm, a length of 4 nm and a molecular weight of only 15 kDa—merely one-tenth that of a conventional antibody. This makes it the smallest genetically engineered antibody with full antigen-binding functionality (Muyldermans, 2013; Hassanzadeh-Ghassabeh *et al,* 2013; Jin *et al,* 2023).

Nb consists of four framework regions (FRs) and three complementarity determining regions (CDRs). The variable region of the human heavy chain (VH) shares over 80% homology with VHH, yet exhibits significant differences (Arbabi Ghahroudi *et al,* 1997). VH contains six CDRs in total, whereas VHH possesses only three, with CDR1 and CDR3 being longer in VHH. The extended CDR1 and CDR3 in VHH compensate, to some extent, for the absence of light chains in antigen-binding capability. The convex loop structure formed by CDR3 facilitates antigen binding, enabling VHH to recognise cryptic antigenic epitopes more effectively (De Genst *et al,* 2006). The FR2 of VH harbours four highly conserved hydrophobic amino acids (V42, G49, L50, W52), which are substituted by four hydrophilic amino acids (F42, E49, R50, G52) in VHH. This substitution significantly enhances VHH hydrophilicity and improves its water solubility (Vu *et al,* 1997; Harmsen *et al,* 2000). In addition, both FR1 and FR3 contain a cysteine that forms a disulfide bond, conferring greater stability to Nb and enabling it to withstand extreme temperatures, pH ranges and chemical reagent treatments (Muyldermans *et al,* 1994). Furthermore, Nbs may form additional disulphide bonds at CDR1-CDR3, FR2-CDR3 and CDR3-CDR3, further enhancing their structural stability (Melarkode Vattekatte *et al,* 2020).

2.2. Biological properties of nanobodies

2.2.1. High stability and water solubility

Owing to the absence of light chains and CH1, Nbs exhibit more compact intramolecular interactions and multiple internal disulfide bonds, rendering their molecular structure relatively stable. This stability enables them to maintain activity in extremely harsh environments and at high temperatures (Li *et al,* 2018). Compared with conventional antibodies, Nbs show greater stability in complex detection environments, a feature that ensures the reliability of Nb-based detection reagents during storage and transport. Hydrophilic amino acids in the VHH increase the water solubility of the Nb when replacing hydrophobic amino acids exposed on the surface of the VH (De Genst *et al,* 2013). This high water solubility allows the Nbs to remain uniformly dispersed in complex solvents, avoiding the problem of activity loss due to hydrophobic aggregation in conventional antibodies.

2.2.2. High affinity and specificity

Nbs with long CDR3 and highly diverse sequences can form unique antigen-binding sites, enabling them to recognise antigenic epitopes that are inaccessible to conventional antibodies. This significantly enhances the accuracy and sensitivity of detection (De Genst *et al,* 2006). The loop structure of CDR3 improves antigen-antibody binding capacity, thereby enhancing the specificity and affinity of Nbs for antigens.

2.2.3. Low immunogenicity and simple humanisation

Nb has a simple structure and lacks a fragment crystallisable (Fc), which avoids the complement reaction caused by Fc contained in conventional antibodies. Moreover, they have high homology with human VH and can be easily cleared *in vivo* and can be humanised by simple modification of Nb (Liu *et al,* 2025). In detection systems, Nbs are less prone to trigger immune rejection or non-specific binding, effectively reducing background signals. This feature makes them particularly suitable for detecting foodborne pathogens in complex food matrices, as it minimises interference from food components and enhances detection reliability.

2.2.4. Small molecular weight and tissue penetration

With a molecular weight of only about 15 kDa, Nbs are able to rapidly penetrate the tissue interstitium and extracellular matrix (Arbabi-Ghahroudi, 2017). When detecting pathogenic bacteria that are wrapped in food matrix or hidden in the deeper layers of tissues, Nbs can rapidly reach the target location and bind to it, breaking through the spatial site-blocking limitation of conventional antibodies due to the larger molecules, which facilitates rapid detection.

2.2.5. Easily modified by genetic engineering

Nbs are encoded by a single gene, enabling genetic engineering to readily introduce tags, enzyme molecules, or other functional domains for constructing diverse assay systems. For instance, conjugating Nbs with nanoparticles (Zhang *et al,* 2022a) or magnetic beads (Bai *et al,* 2023) facilitates the development of detection technologies such as immunochromatography and immunomagnetic separation (IMS). Fusing Nbs with reporter enzymes (Gu *et al,* 2022) enables signal amplification in ELISA or biosensors. Additionally, directed evolution and structural modification (Wang *et al,* 2023a; Liao *et al,* 2024) can further optimise Nb properties, such as enhancing stability, affinity, or altering specificity.

2.3. Preparation of Nanobodies

2.3.1. Nanobody libraries

Nb libraries can be classified into naïve libraries, immune libraries and semi-synthetic/synthetic libraries. A naïve library is constructed by directly extracting mRNA from peripheral blood lymphocytes of unimmunised Bactrian camels and cloning variable domain genes without immune antigens. This library contains the full diversity of Nb genes naturally present *in vivo*. The method saves time and cost, enabling screening for toxic, weakly immunogenic, or hardly preparable antigens (Tu *et al,* 2016). However, screening is relatively challenging, requiring extensive efforts and ensuring the library has sufficient capacity and diversity to isolate Nbs with high specificity and affinity against foodborne pathogens.

In contrast, immune libraries are more targeted: immunising Bactrian camels with specific foodborne pathogens stimulates *in vivo* B cell proliferation to produce specific Nbs, thus avoiding *in vitro* affinity maturation (Lim *et al,* 2014). Immune libraries do not require large capacity to screen high-affinity and stable Nbs. However, each antigen necessitates re-immunisation and library construction, leading to a lengthy and costly cycle.

Library capacity and diversity are key factors affecting antibody screening. By modifying base sequences and constructing synthetic/semi-synthetic libraries completely or partially *in vitro*, the FRs and CDRs of Nbs can be designed and mutated as needed. Introducing diversified sequences enhances library diversity and capacity, also enabling customisation of Nb properties to meet special detection requirements—such as improving stability or altering specificity (Liu *et al*, 2018; Valdés-Tresanco *et al*, 2022; Liu *et al*, 2024).

2.3.2. Screening techniques for nanobodies

The screening of Nbs primarily involves phage display, ribosome display and yeast surface display techniques. Among these, phage display technology is the most widely used. Its principle lies in introducing exogenous genes into a phage vector, which are expressed as fusions with phage coat proteins and displayed on the phage surface (Sidhu, 2001). The collection of all phages displaying exogenous genes is termed a phage display library. High-affinity Nbs can be screened via biopanning, leveraging the specific binding of antigens to Nbs, making it commonly used for the initial screening of specific Nbs against foodborne pathogens.

Ribosome display technology is a cell-free screening method that does not depend on the transformation and expansion process of cells. It is based on the principle that transcription and translation are performed *in vitro* so that the VHH gene, mRNA and ribosomes form a stable complex, enabling the display of Nbs on the ribosome surface (Bencurova *et al,* 2015). This technique allows for rapid *in vitro* evolution and screening of Nbs and is particularly suitable for studies where affinity is optimised.

Yeast surface display uses yeast as a display vector, fusing specific VHH genes with yeast-secreted proteins to display Nbs on yeast cell surfaces (McMahon *et al,* 2018). Compared to phage display libraries, yeast display libraries allow for more complex post-translational modifications of expressed Nbs, aiding in forming the correct spatial conformation. This makes them suitable for Nb screening with high stability and activity requirements (Ryckaert *et al,* 2010).

With the development of sequencing technology, single-cell sequencing technology has been gradually applied to Nb screening. This technology is capable of directly sequencing individual B cells to obtain their antibody gene sequences. In the screening of Nbs against foodborne pathogens, individual B cells can be isolated from immunised Bactrian camel lymphocytes and their VHH gene sequences can be analysed by single-cell sequencing technology, so that potentially specific Nb genes can be screened out by bioinformatics analysis (Yi *et al,* 2024). Single-cell sequencing technology can avoid the bias during library construction and directly obtain the natural Nb sequences, which provides a new way to screen highly specific Nbs.

2.3.3. Preparation process of nanobodies

The conventional procedure for preparing Nbs involves isolating peripheral blood lymphocytes from Bactrian camels immunised or unimmunised against specific foodborne pathogens, extracting total RNA, reverse-transcribing it into cDNA, amplifying the VHH gene fragment with specific primers, cloning it into phage vectors, transforming it into *Escherichia coli* (*E. coli*) cells and constructing a phage-displayed Nb (phage-Nb) library with the aid of helper phages. Phage-Nbs that specifically bind to the target antigen are screened through 3–4 rounds of adsorption, washing, elution and amplification using solid-phase biopanning. Finally, the Nbs are obtained by prokaryotic induction expression, affinity chromatography purification and activity determination, meeting the requirements for foodborne pathogen detection (Gavira-O'Neill *et al,* 2020; Zhang *et al,* 2021).

3. Application of nanobodies in the detection of foodborne pathogens

3.1. Enzyme-linked immunosorbent assay based on nanobodies

ELISA is one of the most widely used immunoassays and foodborne pathogens are typically detected by sandwich ELISA. Pathogens are first captured by a capture antibody and then reacted by horseradish peroxidase (HRP)-conjugated antibody as a detecting antibody with 3,3′,5,5′-tetramethylbiphenyl (TMB) to produce a measurable colour change (Chunglok *et al,* 2011). In the field of foodborne pathogen detection, ELISA based on Bactrian camel Nbs has emerged as a premier research direction due to its technical maturity and application potential (Table 1).

He *et al* (2020) established a sandwich ELISA for the detection of *Salmonella enteritidis* (*S. enteritidis*) by using a polyclonal antibody (pAb) as the capture antibody and Nb13 as the detection antibody, with a limit of detection (LOD) was 1.4×10^5 CFU/mL. The method was suitable for milk samples and 6 CFU/mL of *S. enteritidis* could be detected after 10 h of enrichment. However, in the development of sandwich ELISA, Nb is mostly used as detection antibody and pAb or monoclonal antibody (mAb) as capture antibody. This is due to the difficulty of pairing dual Nb in a limited number of Nb. In addition, the small size of Nb may hinder the fixation of binding sites on polystyrene microtitre plates. Some studies have reported that the affinity constants of Nb for antigens are typically 10^2–10^3 times lower than those of intact antibodies from immunised animals.

Notably, Gu *et al* (2022) established a sandwich ELISA for the detection of *S. enteritidis* in milk by genetic manipulation using SE-Nb9 as the capture antibody and SE-Nb1-vHRP as the probe. The method reduces the use of commercially available secondary antibodies, shortens the time of the assay and reduces the cost and can detect *S. enteritidis* as low as 5×10^4 CFU/mL. In order to efficiently avoid the interference of food matrices, to shorten the enrichment period and to improve the detection sensitivity. Phage display technology is a powerful and commonly used tool to amplify the signal and improve the sensitivity of the assay by using phage-Nb as a detection antibody to compensate for the lack of Nbs in affinity. Zhang *et al* (2022b) established phage-mediated dual Nb sandwich chemiluminescent enzyme immunoassay. The chemiluminescence reaction was used to replace the traditional colour development reaction and a highly sensitive detection of *Salmonella typhimurium* (*S. typhimurium*) was achieved with a LOD of 3.63×10^3 CFU/mL, which was 100-fold higher than that of the traditional Nb-ELISA.

Multivalent modification of Nbs has been investigated to further improve sensitivity, aiming to balance stability with enhanced affinity by increasing Nb size. Liao *et al* (2024) established a sandwich ELISA based on bivalent Nb (BNb-ELISA), with a LOD of 2.364×10^3 CFU/mL against *S. enteritidis*, which was a 7.5-fold improvement over the monovalent Nb ELISA. In addition, Bai *et al* (2023) developed a Nb-based sandwich ELISA combined with an IMS (IMS-ELISA) for the rapid enrichment and detection of *S. enteritidis* in food products. The IMS-ELISA achieved an LOD of 3.2×10^3 CFU/mL and reduced pre-enrichment time by 2 h in real sample analysis, effectively avoiding matrix interference and demonstrating potential for food pathogen monitoring. In summary, Bactrian camel Nb-based ELISA has continuously enhanced sensitivity and specificity in foodborne pathogen detection, progressing from laboratory research to rapid, precise food safety monitoring through antibody optimisation, signal amplification and pretreatment innovation.

3.2. Other immunoassays based on nanobodies

Driven by detection technology advancements and market demands, the field of high-throughput, on-site rapid detection of foodborne pathogens has garnered significant attention (Table 2).

Immunochromatographic technology integrates thin-layer chromatography with immune recognition, offering advantages of speed, simplicity, low cost and requiring no specialised personnel or sophisticated equipment (Gondhalekar *et al*, 2020). However, traditional immunochromatographic test strips are facing significant challenges, such as insufficient sensitivity and the inability to conduct real-time quantitative detection.

Zhang *et al* (2022a) developed KMO@Au and Nb9-assisted colourimetric photothermal dual-mode immunochromatographic test strips for rapid, sensitive and quantitative detection of *S. typhimurium* in food. The small size of Nb9 enabled efficient conjugation with flower-like KMO@Au photothermal agents, synergistically enhancing detection sensitivity and specificity. This biosensor achieved a LOD of 10^4 CFU/mL in colourimetric mode and 10^3 CFU/mL in photothermal mode, with successful application to real sample analysis demonstrating high precision. Preparing nanoparticle-Nb conjugates with high stability and affinity remains a key challenge in lateral flow immunoassay (LFIA) development. Wang *et al* (2025) enhanced the bioactivity of gold-Nb nanoprobes by directionally conjugating biotinylated Nb9 with streptavidin-coated gold nanoparticles (AuNPs). The resulting Au/SA@Bio-Nb probe exhibited exceptional stability and affinity, enabling

Table 1. Enzyme-linked immunosorbent assay based on nanobodies for the detection of foodborne pathogens

Immunoassay	Foodborne pathogen	Capture antibody+Detection antibody	LOD (CFU/mL)	Reference
Traditional sandwich ELISA	*Listeria monocytogenes* (*L. monocytogenes*)	mAb+Nb	1×10^4	Tu *et al* (2016)
Traditional sandwich ELISA	*S. enteritidis*	pAb+Nb	1.4×10^5	He *et al* (2020)
Traditional sandwich ELISA	*E. coli* O157:H7	pAb+Nb	8.7×10^3	He *et al* (2025)
Sandwich ELISA without additional secondary antibodies	*S. enteritidis*	Nb+HRP-Nb	5×10^4	Gu *et al* (2022)
Divalent modified sandwich ELISA	*Salmonella*	Divalent Nb+Phage-Nb	2.364×10^3–1.501×10^4	Liao *et al* (2024)
Streptavidin-bridged sandwich ELISA	*Salmonella*	Biotinylated Nb+ Phage-Nb	4.23×10^3–9.15×10^3	Ren *et al* (2022)
Bispecific modified sandwich ELISA	*S. enteritidis V. parahaemolyticus*	Bispecific Nb+Phage-Nb	3.33×10^3-6.35×10^4	Wang *et al* (2023a)
Chemiluminescent sandwich ELISA	*S. typhimurium*	Nb+Phage-Nb	3.63×10^3	Zhang *et al* (2022b)
Chemiluminescent sandwich ELISA	*C. sakazakii*	Nb+Phage-Nb	1.04×10^4	Zhang *et al* (2023)
Immunomagnetic sandwich ELISA	*S. enteritidis*	Nb+Phage-Nb	3.2×10^3	Bai *et al* (2023)

Table 2. Other immunoassays based on nanobodies for the detection of foodborne pathogens

Immunoassay	Foodborne pathogen	Capture antibody+Detection antibody/Detection antibody	LOD (CFU/mL)	Reference
Colourimetric and photothermal dual-mode immunochromatography biosensor	*S. typhimurium*	mAb+Nb	Colourimetric: 10^4 Photothermal: 10^3	Zhang *et al* (2022a)
Colourimetric immunosensor	*V. parahaemolyticus*	Phage-Nb	Visual: 10^4 Quantitative: 10^3	Wang *et al* (2023b)
Chromogenic immunosensor	*C. sakazakii*	Nb	Visual: 10^3 Quantitative: 13^6	Chen *et al* (2024)
Streptavidin-biotin immobilised lateral flow immunoassay	*S. typhimurium*	pAb+Biotinylated Nb	Visual: 10^3	Wang *et al* (2025)

an improved LFIA for rapid, sensitive *S. typhimurium* detection. The Au/SA@Bio-Nb-LFIA achieved a visual LOD of 10^3 CFU/mL and a linear detection range of 10^3–10^7 CFU/mL.

Notably, the above methods rely on paired antibodies, limiting their application in foodborne pathogen detection. Thus, establishing immunoassays based on single recognition elements is crucial for real-time pathogen monitoring. Wang *et al* (2023b) developed a simple, sensitive colourimetric immunosensor for *V. parahaemolyticus* by leveraging thiolation of phage-Nb (phage-Nb-SH) on pVIII shell proteins to induce AuNP aggregation. Specific interactions between nanomolecules and bacteria prevented aggregation, altering surface plasmon resonance and triggering a visible colour change. The assay completed within 100 min, with a visual LOD of 10^4 CFU/mL and quantitative LOD of 10^3 CFU/mL, showing no cross-reactivity with other bacteria. Innovations from traditional immunochromatography to novel colourimetric methods, combined with Nbs and advanced materials, are driving foodborne pathogen detection toward high-throughput, high-sensitivity and on-site capabilities. These advancements break free from antibody-dependency limitations, providing diversified solutions for enhancing rapid food safety detection systems.

4. Conclusion and Outlook

Nbs, with their unique structural and performance advantages, have made significant progress in foodborne pathogen detection, offering a series of efficient, rapid and sensitive methods for food safety analysis. In practical applications, Nb-based detection technologies effectively address the limitations of traditional methods, playing a critical role in ensuring food safety by enabling rapid screening and accurate quantification of foodborne pathogens throughout food processing and distribution.

However, the application of Nbs in the detection of foodborne pathogens still faces some challenges. On the one hand, the large-scale production process of Nbs needs to be further optimised to reduce the cost and increase the yield to meet the increasing demand for detection. Currently, although a variety of expression systems have been used for Nb production, there is still room for improvement in yield and cost control. On the other hand, traditional phage display library screening requires multiple rounds of biopanning, with a long cycle and high antigen purity requirements. Complex pathogenic bacterial antigens (e.g. surface polysaccharides, protein complexes) may lead to insufficient affinity or specificity crossover of the screened Nbs. In addition, some Bactrian camel Nb-based detection techniques have long detection times, require supporting instrumentation and have limited on-site rapid detection capabilities; while certain methods are portable but have low sensitivity, making it difficult to meet trace contamination detection needs. Finally, although Nbs have good specificity, a certain degree of non-specific binding may still exist in complex food matrices, leading to elevated background signals or false-positive results affecting detection accuracy. Although Nbs have significant advantages in the detection of foodborne pathogens, they still face many challenges and need to make further breakthroughs in technological innovation and system optimisation.

With the continuous development of biotechnology, Nbs in foodborne pathogens detection will usher in a broader development prospect. In terms of technological innovation, an integrated and intelligent detection platform can be developed by combining emerging nanotechnology and microfluidic technology. For example, combining Nbs with nanosensors and microfluidic chips to build a portable, high-throughput rapid detection system for foodborne pathogens, which can achieve simultaneous, rapid and accurate detection of multiple pathogenic bacteria. Meanwhile, we will further expand the application of Nbs in the detection of new foodborne pathogens and in the monitoring of the whole food supply chain, so as to provide more comprehensive and reliable technical support for ensuring food safety.

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