AnteoBind™

Next-Generation Bioconjugation Technology

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Executive Summary

AnteoBind™ brings a paradigm shift in bioconjugation technology, offering a revolutionary alternative to conventional EDC/NHS chemistry through its proprietary metal-ion complex-based surface activation.

Developed by AnteoTech Ltd (ASX:ADO), this innovative technology addresses significant limitations in traditional bioconjugation methods and products.

Key value proposition components include:

- **Superior Performance:** Up to a 45-times improvement in assay performance compared to market dominant EDC/NHS chemistry[1]
- **Simplified Workflow:** One-step activation process versus multi-step traditional methods[2]
- **Cost Optimisation:** Up to 90% reduction in antibody requirements or consumption for equivalent sensitivity[3]
- **Extended Stability:** A single batch of activated AnteoBind™ particles can be used multiple times for up to 12 months post activation versus immediate use requirements for EDC[4]
- **Enhanced Compatibility and Specificity:** Enables higher specificity conjugation of challenging biomolecules that perform poorly with traditional methods.[1,5]
- **Reduced Wastage:** Through staged workflow, versus all-in-one processes with short 'use by' timeframes.

The global bioconjugation market, valued at US$5 billion[6] in 2024, is projected to reach US$11-16 billion[6] by 2029-2035, with consumables representing the largest part of the market. With its global reach **AnteoBind™** is well positioned to capture significant market share in this growing market as the product is commercialised and sales revenues grow.

AnteoBind™ is redefining bioconjugation technology to enable fast, reliable, robust, low-cost application development such as in field activities including vaccine development, diagnostic tests, immunoassays and research. Allowing our customers to achieve reproducible results and get to market faster.

Introduction

The Bioconjugation Challenge

Bioconjugation—the process of chemically linking biomolecules to surfaces or other molecules—is fundamental to modern diagnostics, therapeutics development, and medical research applications.

Traditional methods, primarily EDC/NHS (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxy succinimide) chemistry, have dominated the field for decades despite inherent limitations they have including:

- **Hydrolysis susceptibility:** EDC degrades rapidly in aqueous solutions.
- **Multi-step use complexity:** Requires precise pH control and multiple reagent additions.
- **Limited protein compatibility:** Poor performance with certain protein structures.
- **Storage instability:** Activated surfaces must be used immediately. Creating wastage.
- **High antibody consumption:** Inefficient conjugation leads to significant material waste.

The AnteoBind™ Innovation

AnteoBind™ technology represents a fundamental departure from current covalent bioconjugation approaches, utilising proprietary polymeric metal-ion complexes that facilitate biomolecule attachment through strong coordination bonding. This "molecular level binding" creates a nanometer-thin (2nm -15nm) activation layer that serves as an interface between synthetic surfaces and biomolecules.

[1] "Development and validation of a respiratory syncytial virus multiplex immunoassay", NMI Natural and Medical Sciences Institute. https://link.springer.com/article/10.1007/s15010-024-02180-6

[2] https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/385/793/almpn100-protocol.pdf

[3] Case Studies available upon request

[4] Company research and "A Simplified and Robust Activation Procedure of Glass Surfaces for Printing Proteins and Subcellular Micropatterning Experiments". https://pmc.ncbi.nlm.nih.gov/articles/PMC8946821/

[5] https://www.labroots.com/it/webinar/applying-anteobind-activated-estapor-europium-particles-development-quantitative-fluorescent-lateral-2

[6] Bioconjugation Market: Growth, Size, Share and Trends January 2025 https://www.marketsandmarkets.com/Market-Reports/bioconjugation-market-232696083.html

[7] https://www.labroots.com/it/webinar/applying-anteobind-activated-estapor- europium-particles-development-quantitative-fluorescent-lateral-2

Technology Overview

Mechanism of Action

AnteoBind™ operates through a unique metal-ion coordination mechanism that differs fundamentally from traditional covalent approaches.

Activation Phase:

- Polymeric metal-ion complexes form a nanometer-thin molecular layer on surfaces
- Multiple coordination sites are created for biomolecule attachment
- The activation process can be completed in 30-60 minutes at room temperature

Conjugation Phase:

- Biomolecules bind through multiple coordination interactions
- Resulting in strong overall attachment through avidity effects
- Maintaining native protein conformations and functionality

Stabilisation:

- Activated surfaces remain stable for up to 12 months
- No hydrolysis issues occur which are common with EDC chemistry
- **AnteoBind™** is compatible with standard storage and shipping conditions (Temperature etc)

When **AnteoBind™** binds to a surface, it forms a nanometre thin layer of the metal ion complex (of around 2nm – 15nm thickness depending on available binding groups, e.g. carboxyl) and primes the surface for biomolecule attachment (activation) functioning like a double-sided molecular tape or binder. The **AnteoBind™** activated surface is able to robustly bind with the electron-donating functional groups (e.g. carboxyl, hydroxyl, thiol) on other surfaces or biomolecules via strong coordinate bonds as represented in Figure 1 below:

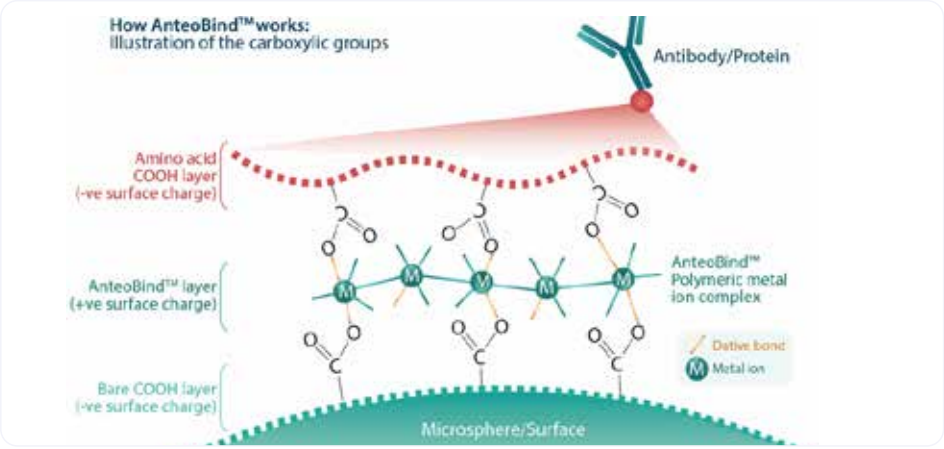

Figure 1. Schematic representation of **AnteoBind™** facilitating the conjugation of synthetic surfaces (illustrated by carboxylic acid) and biomolecules (antibody/protein) via coordinate bonds.

The majority of the purified antibodies used in developing life sciences applications contain a histidine rich cluster. This is found in the Fc (fragment crystallisable) region of antibodies that are derived from many common hosts, including mice, humans and rabbits[8]. This cluster in the Fc region allows the coordinate complex-mediated orientation of the Fab (fragment, antigen-binding) regions to orientate away from the **AnteoBind™** surface and free to bind to available antigens. This ideal functional orientation for the antibody, achievable through **AnteoBind™** use, facilitates increased sensitivity and helps reduce antibody usage by as much as 90%, increasing the number of functional binding sites available for interactions with the analytes or the antigen of interest. It creates a highly favourable orientation for attachment of molecules, a beacon!

Product Portfolio

AnteoBind™ technology is available in multiple formulations optimised for different applications:

AnteoBind™ NXT:

- Optimised for particles <5 µm diameter
- Enhanced performance in multiple immunodiagnostic applications including lateral flow and chemiluminescence assays
- Cost-competitive with traditional EDC chemistry

AnteoBind™ Biosensor:

- Designed for unconventional bioconjugation surfaces (COC plastics, carbon and gold-plated electrodes etc.)
- Optimised to work best with carboxylated surfaces
- Compatible with several readout formats (electrochemical[9] and optical[10] biosensing)

Activation kit Multiplex Microspheres:

- Designed for particles used in multiplexing platforms (such as e.g. Magplex® and Microplex® provided by Luminex)
- Equally Compatible with both antibodies[11] and antigens[12] with minimal optimisation required

[8] Hale JE, Beidler DE. Purification of humanized murine and murine monoclonal antibodies using immobilized metal-affinity chromatography. Anal Biochem. 1994 Oct;222(1):29-33. doi: 10.1006/abio.1994.1449. PMID: 7856866.).

[9] https://www.sciencedirect.com/science/article/pii/S2212017317300361

[10] https://www.mdpi.com/2227-9059/10/1/188

[11] https://www.nature.com/articles/s41586-023-06717-x

[12] https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2023.1190404/full

Comparative Performance Analysis

Superior Protein Binding Efficiency

Peer-reviewed studies have established the exceptional performance of **AnteoBind™** over traditional methods:

a) RSV Multiplex Immunoassay Study (NMI Institute):

- G protein subtype A: 7-45 fold improvement in signal intensity G protein subtype B: 1-5 fold improvement in signal intensity
- Enabled successful detection of previously challenging antigens

b) Lateral Flow Assay Applications:

- Upto 2× higher sensitivity compared to EDC/NHS methods
- Upto 50% reduction in antibody consumption
- Improved test reliability and batch-to-batch consistency

Workflow Comparison

Parameter	EDC/NHS Traditional	AnteoBind™ Technology
Activation Steps	2-3 steps	1 step
Total Time	3-5 hours	1-2 hours
pH Requirements	Strict pH 4.5-7	Tolerant of standard buffers
Storage Stability	Minutes-hours	Up to 12 months
Antibody Usage	Standard baseline	50% reduction typical
Buffer Compatibility	Limited	Broad compatibility
Protein Compatibility	Variable	Enhanced for difficult proteins

Versatility: Improved compatibility across a range of biomolecules and surfaces

AnteoBind™'s key competitive advantage is its compatibility with a broad range of particles sizes (40 nm-5.6µm), functional groups (-COOH/-OH/-silica), and materials (polystyrene, silica, polyvinyl alcohol, gold). **AnteoBind™** performs best overall when coupled with a carboxylated surface.

AnteoBind™ has also been used to activate a selection of other non- particle-based surfaces including glass, biosensor chips, cyclic olefin copolymer (COC) and polystyrene 96- and 384- well plates.

EDC/NHS conjugation is dependent on the presence of lysine residues on proteins as they contain a primary amine group (ε-amino group) that readily reacts with the NHS ester, to form a stable amide bond. However, the quantity and distribution of lysine groups varies across different antibodies. This variability typically results in inconsistent performance and greater variability between batches of conjugates. Unlike EDC/NHS, **AnteoBind™** bonds with a wide range of functional groups on the surface of biomolecules by forming stable ligand-metal ion complexes.

Reproducibility

The core of the **AnteoBind™** technology lies in its affinity for "electron-donating" ligands (biomolecules) in order to form coordinate bonds. The oligomeric metal ions in **AnteoBind™** confer a **positive charge to the activated surface** enabling the tracking of particle size/contact angle and surface charge at each stage of the conjugation process.

Furthermore, **AnteoBind™** enables a simple, comprehensive in-process quality check throughout the conjugation process whereas for conventional conjugations this can only occur at the end of the process. This feature has the potential to save time and resources (improve workflows) during the conjugation process, and it can ensure greater batch-to-batch reproducibility, features that are important for decision making in the move to large scale production.

This reproducibility has been demonstrated using multiple assay development platforms including screen printed carbon electrodes used to develop electrochemical biosensors.

"The relative standard deviation (RSD) values obtained (n=8) were 6.1% and 6.8% for the assays performed on the same day in absence and in presence of 1 µg/mL APN, respectively, whereas RSD (n=8) values were 7.8% and 8.5%, respectively, for the measurements made on different days."[13]

Stability

While the **AnteoBind™** reagents are themselves stable for up to two years at room temperature, the surfaces treated with **AnteoBind™** have been shown to maintain the ability to reproducibly attach biomolecules for a longer period than traditional chemistries or products. **AnteoBind™** NXT (the newest **AnteoBind™** product from AnteoTech) activated particles can be used for conjugation for nine consecutive weeks.

This significant benefit is not available with EDC/NHS where best practice is to complete the bioconjugation within hours of starting the chemical reaction. This flexibility to perform bioconjugations at any time after the initial activation allows for improved workflow, but also the ability to bulk activate particles for subsequent uses improving reproducibility and reducing associated costs.

[13] https://www.sciencedirect.com/science/article/pii/S0956566315301718

Applications and Markets

Primary Market Segments

Point-of-Care Diagnostics:

- Lateral flow assays for infectious disease detection
- Cardiac markers and troponin tests
- Pregnancy and fertility testing
- COVID-19 and respiratory pathogen detection

In-Vitro Diagnostics:

- Multiplexed immunoassays
- Protein and biomarker detection panels
- Clinical chemistry applications
- Quality control standards

Pharmaceuticals/ Biomanufacturing:

- Conjugate production
- Adjuvant attachment
- Immunogenicity enhancement
- Process development and quality control

Research Applications:

- Protein immobilisation for biosensors
- Microcontact printing and surface patterning
- Fluorescence-based assays
- Electrochemical biosensors

Regulatory and Quality Considerations

Manufacturing Standards

AnteoTech maintains comprehensive quality systems aligned with industry standards, including:

- **ISO 9001:** Quality management system certification
- **Batch Documentation:** Comprehensive traceability and analytical testing
- **Stability Studies:** Extensive shelf-life and performance validation

Regulatory Compliance

- Compliance with global diagnostic regulations
- Support for customer regulatory submissions
- Previously used in EUA (FDA)[14] and CE-marked[15] point-of-care devices

Scientific Validation

Peer-Reviewed Publications

AnteoBind™ technology has been validated through multiple peer-reviewed studies:

Microsphere Applications:

- "Development and validation of a respiratory syncytial virus multiplex immunoassay" demonstrated superior G protein conjugation performance[8]
- "Autoantibodies against type I IFNs in humans with alternative NF-κB pathway deficiency"[12]

Electrochemical Biosensors:

- "Peptide-based direct electrochemical detection" validated **AnteoBind™** in SARS-CoV-2 detection applications[16]
- Electrochemical Immunosensor for Sensitive Determination of TGF β1 in Urine[10]

Clinical Validation:

- Multiple studies confirming performance in real-world diagnostic applications [14,15]
- Demonstrated batch-to-batch reproducibility and stability

[14] https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-antigen-test-first-over-counter-fully-home-diagnostic

[15] https://wcsecure.weblink.com.au/pdf/ADO/02362428.pdf

[16] https://www.sciencedirect.com/science/article/pii/S0925400522016951

Want to know more?

For a detailed white paper and further information on the comparative studies which have been completed that evaluate AnteoBind™ against EDC/NHS please visit our website at www.anteotech.com or contact us at support@anteotech.com.