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REVIEW

Electrochemical Biosensing of Exosomes. From Separation Strategies to Clinical Diagnostics

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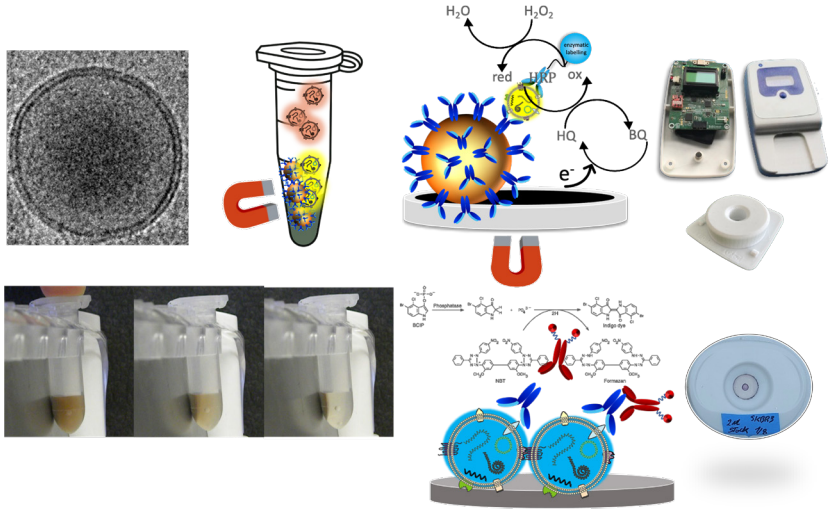

Exosomes are nano-sized extracellular vesicles secreted by cells and they have emerged as promising biomarkers for non-invasive clinical diagnostics. However, their small size (30–200 nm) and low abundance in biofluids pose significant challenges for isolation and detection. This review highlights recent advances in electrochemical biosensors for exosome detection, with particular emphasis on immunomagnetic separation (IMS) strategies and electrochemical platforms developed for clinical diagnostics. Within this context, this review discusses a coherent series of exosome biosensing platforms developed within our research group, contextualized with representative contributions from other teams in the field. The reviewed platforms rely on immunological, enzymatic, and nucleic acid–based recognition strategies, and integrate selective exosome capture, typically assisted by magnetic separation, with electrochemical or related readouts for sensitive detection in complex biological matrices. These approaches integrate selective exosome capture, typically assisted by magnetic separation with electrochemical transduction, enabling sensitive detection in complex biological matrices. Methodologically, the platforms exploit different exosomal features, including surface-associated proteins, intrinsic enzymatic activity, and nucleic acid cargo, illustrating the versatility of electrochemical biosensing for exosome analysis. Reported limits of detection range from approximately 10^5 down to 10^2 exosomes μL^{-1}, with demonstrated capability to discriminate clinically relevant samples (e.g., cancer versus healthy controls, Alzheimer's disease versus controls). The integration of magnetic separation with electrochemical readout in portable formats supports sensitive, specific, and rapid analysis, highlighting the potential of these approaches for POC applications and resource-limited settings. In this regard, this article pays tribute to Prof. Kubota's legacy in biosensing by reflecting his long-standing emphasis on transforming innovative analytical concepts into solutions suited for real diagnostic settings.

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INTRODUCTION

Exosomes are a subtype of extracellular vesicles (EVs) typically 30–200 nm in diameter that originate from the endosomal pathway as intraluminal vesicles inside multivesicular bodies.[1] Upon fusion of multivesicular bodies with the plasma membrane, these nano-sized vesicles are released as exosomes, carrying membrane and cargo molecules reflective of their cell of origin.[2] Exosomal membranes are enriched in tetraspanins (CD9, CD63, CD81), integrins, and other membrane proteins, while their lumen contains a rich cargo of biomolecules including proteins, mRNAs, microRNAs, DNA, and lipids.[3] Through this molecular cargo, exosomes mediate intercellular communication, transferring genetic information and signaling proteins between cells and even to distant tissues. Exosomes have been found in virtually all biofluids – blood, urine, saliva, breast milk, cerebrospinal fluid, ascites, etc.[4] – highlighting their ubiquity in physiological and pathological processes (Figure 1).

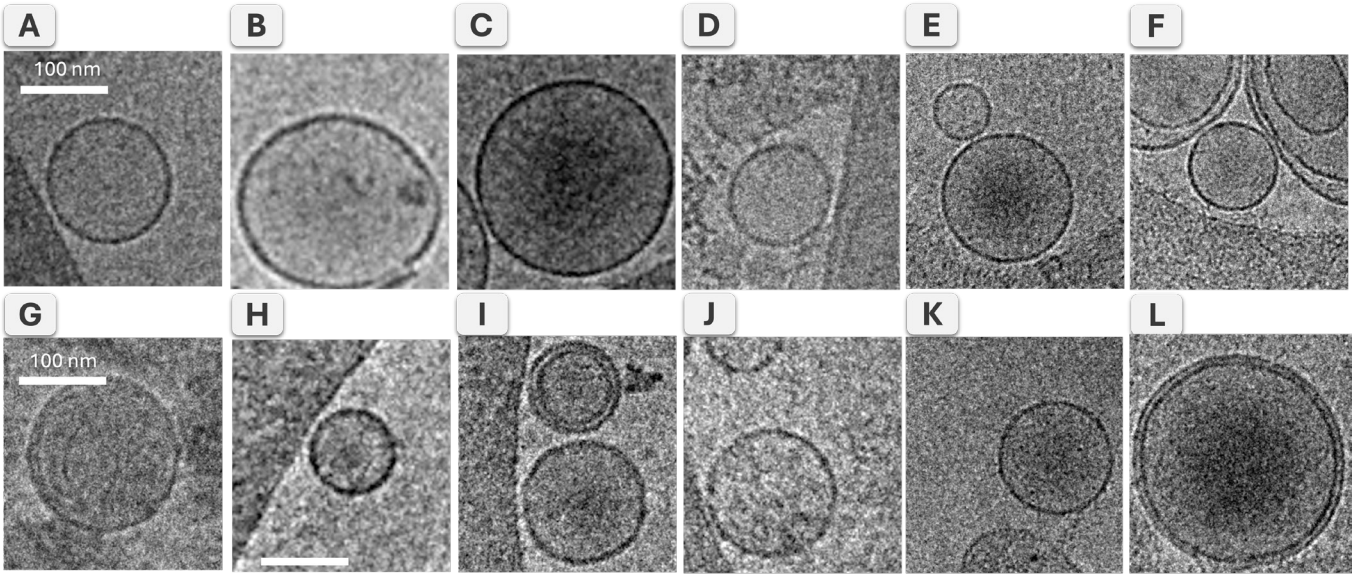

Figure 1. Cryo-TEM characterization of exosomes derived from breast cancer cells (upper panels) and brain-derived exosomes (lower panels), obtained from cell culture supernatants and biological fluids including human plasma and cerebrospinal fluid (CSF). (A–D) Exosomes isolated from cell culture supernatants of MRC-5 (human lung fibroblasts, healthy control), SK-BR-3, MDA-MB-231, and MCF-7 (human breast cancer cell lines). (E–F) Exosomes purified from human plasma of breast cancer patients (E) and healthy donors (F). (G) Exosomes obtained from the SH-SY5Y cell line (human neuroblastoma, neuronal model). (H–L) Exosomes isolated from cerebrospinal fluid (CSF) of patients with neurodegenerative disorders: DCL− (mild cognitive impairment, biomarker-negative), DCL+ (mild cognitive impairment, biomarker-positive), DEM (dementia), QSM− (quantitative susceptibility mapping-negative, without magnetic abnormalities), and healthy control (L). All samples exhibit vesicles with the typical cup-shaped morphology and size distribution of exosomes (≈30–200 nm). Images were acquired at the Servei de Microscòpia, Universitat Autònoma de Barcelona (UAB) using a JEOL JEM-2011 transmission electron microscope operated at 200 kV, under cryogenic low-dose conditions. Scale bars = 100 nm (shown in A and G).

Exosomes have attracted intense interest for their roles in human disease. They are implicated in facilitating tumor progression and neurodegeneration by shuttling oncogenic or pathogenic factors between cells.[4] At the same time, their presence and composition in bodily fluids make them promising minimally invasive biomarkers for diagnosis and prognosis. For example, circulating exosomal proteins and RNAs are being explored as biomarkers in breast cancer, where tumor-derived exosomes carry signatures of the tumor and are found at elevated levels in patients.[4] Genomic and proteomic profiling of exosomes has revealed distinct patterns in breast cancer patients versus healthy controls, highlighting their potential for early cancer

detection and monitoring of treatment response. In neurodegenerative diseases like Alzheimer's, brain-derived exosomes in the blood and CSF carry pathological peptides (e.g. Aβ, tau) and disease-specific RNAs, suggesting utility as diagnostic indicators.[5] Indeed, studies have identified exosomal biomarkers (such as NCAM-labeled exosomal Aβ and miR-384 from blood) that can distinguish Alzheimer's disease patients from controls.[6] These examples illustrate the broad clinical relevance of exosomes as disease biomarkers.

Beyond diagnostics, exosomes are also being investigated as therapeutic agents and drug delivery vehicles. Because they are naturally derived nanoparticles, exosomes tend to be less immunogenic and more biocompatible than synthetic liposomes or polymeric nanocarriers.[7] They possess an innate ability to protect and transport bioactive cargo, and can be engineered to deliver drugs, siRNAs, or gene editing molecules to target cells. For instance, loading exogenous therapeutics into exosomes (by electroporation, incubation, or genetic engineering of donor cells) has shown promise for cancer therapy and neurodegenerative disease treatment.[1] Early studies suggest that exosomes can cross biological barriers, including the blood–brain barrier, and exhibit preferential uptake by specific cell types, highlighting their potential as targeted drug delivery vehicles.[8,9] Overall, the unique biological properties of exosomes – stability in circulation, rich and customizable cargo capacity, and inherent cell targeting – position them as valuable tools in next-generation diagnostics and therapeutics.[3]

Exosome separation methods

The purification of exosomes is challenging due to their nanometric size, complex composition, and low abundance in biological fluids.[10] A variety of isolation methods have been developed, each with distinct principles, advantages, and limitations.[11]

Ultracentrifugation

Differential ultracentrifugation is the most widely used exosome isolation technique and is considered the gold standard.[12] The sample is subjected to sequential centrifugation at increasing speeds to pellet components by size: low speeds remove cells and debris, while ultrahigh speeds (~100,000×*g*) pellet small extracellular vesicles (exosomes).[13] Differential ultracentrifugation can process large volumes and yields a concentrated exosome pellet with relatively high recovery.[14] It requires little sample pretreatment and has low reagent costs. However, the method is time-consuming (often several hours to an overnight spin) and requires specialized ultracentrifuge equipment. Harsh forces can cause vesicle deformation or aggregation, and co-pelleting of other particles (protein aggregates, larger vesicles) can reduce purity.[15] To improve purity, exosomes can be banded at their buoyant density by density-gradient ultracentrifugation (e.g. sucrose or iodixanol gradients).[16] After ultracentrifugation (typically 14–18 h at 100,000–160,000×*g*), exosomes form a discrete band separated from proteins and other vesicles by density. This approach yields highly pure exosomes, removing most non-vesicular contaminants.[17,18] However, gradient ultracentrifugation is laborious and very time-intensive (often requiring overnight runs). It also demands ultracentrifuges and expertise in gradient preparation. Yield can be lower than simple pelleting, and prolonged high g-force or osmotic gradient exposure may cause loss of some exosome functionality.

Size-Exclusion Chromatography (SEC)

SEC separates vesicles by size using porous polymer beads as the stationary phase. Larger particles (exosomes) elute first, while smaller proteins and molecules are retained.[19] SEC is a gentle, reagent-free method that preserves vesicle integrity and biological activity.[20] It provides high purity fractions making it excellent for downstream "-omics" analyses. Columns are inexpensive and can be reused after cleaning, and protocols can be completed in ~20–30 minutes for a single run. However, SEC generally requires moderate sample volumes (typically 0.5–2 mL per run). Resolution is limited by column pore size; very small exosomes or protein aggregates may co-elute in the same fractions, yielding a broader size distribution than ultracentrifugation or immunocapture methods. Elution can dilute the sample, so an extra concentration step may be needed.

Membrane filtration

Ultrafiltration devices or membranes with defined pore sizes (e.g. 0.1–0.22 μm) can physically separate exosomes from larger components.[21] Often, a series of filters is used or filtration is combined with centrifugation to improve yield. Filtration is simple and rapid, typically less time-consuming than ultracentrifugation. It can be done with relatively common lab equipment (syringe filters, centrifugal filter units) and yields comparable recovery to ultracentrifugation for many samples. However, membranes can clog due to sample debris or exosome aggregates, causing loss of vesicles and requiring force that may damage exosomes. Filters are typically single-use and can be expensive, especially for processing larger volumes. Moreover, deformable larger vesicles might squeeze through pores, and some exosomes can be retained non-specifically.[22] Thus, filtration is often combined with other methods to ensure purity. Sample volume is moderate; volumes above a few milliliters may exacerbate clogging issues unless tangential-flow filtration setups are used.

Polymer precipitation

This method uses volume-excluding polymers (most commonly polyethylene glycol, PEG) to aggregate and precipitate exosomes out of solution.[23] A precipitating reagent is added to the biofluid and incubated, then a low-speed centrifugation (on the order of 1,500–10,000×*g*) pellets the polymer-bound exosomes. Polymer-based precipitation is user-friendly and does not require special equipment beyond a standard centrifuge. It can isolate exosomes from relatively small sample volumes (tens to hundreds of microliters), which is advantageous for clinical samples where volume is limited. The procedure is fast (often <2 hours total) and gentle, preserving exosomal bioactivity. Several commercial kits are available, making the approach accessible and reproducible.[24] A major drawback is co-precipitation of contaminants — PEG precipitates other nanoparticles like lipoproteins, protein complexes, and polymeric debris along with exosomes. This lowers the purity of the exosome prep and can interfere with analyses (e.g. high protein background affects proteomic profiling). Indeed, precipitate from serum or plasma often contains abundant albumin and other proteins. Such contaminants can also lead to overestimation of particle counts by light-scattering methods (NTA), since non-vesicular particles are counted. Additionally, the polymer reagents themselves add cost (kits are relatively expensive) and must be removed or tolerated in downstream analyses. Despite these issues, polymer precipitation generally achieves the highest yield of small RNA and miRNA cargo compared to other methods, making it useful when nucleic acid analysis is the priority.

No single isolation method is ideal for all purposes. Table I compares the main exosome separation techniques on key parameters. In practice, combinations of methods are common (for instance, ultracentrifugation coupled with filtration or an immunocapture step).[24] The choice of method depends on whether the priority is yield, purity, speed, or maintaining bioactivity. The current landscape of exosome isolation is thus a compromise between these factors, and improving isolation efficiency and purity (especially from complex clinical samples) remains an active area of research and development.

Table I. Overview of exosome separation techniques: principles, advantages, and limitations

Method	Principle	Advantages	Limitations	Sample Volume	Equipment Required	Time
Differential ultracentrifugation	Serial centrifugation by increasing g-force to pellet particles by size (higher speeds pellet smaller vesicles).	- High yield of exosomes (concentrates them in a pellet). - Widely used; established protocols. - Low reagent cost (uses physical force only).	- Requires ultracentrifuge (high cost equipment). - Time-consuming (multiple spins, often ≥hours). - Co-pellets protein aggregates and other vesicles, reducing purity - High g-forces may damage exosomes (rupture or aggregation).	Typically large (several mL up to tens of mL) to obtain sufficient yield.	Ultracentrifuge (≥100,000×g rotor).	Long (several hours to overnight, depending on protocol).
Density-Gradient Ultracentrifugation	Ultracentrifugation in a density medium (e.g. sucrose or iodixanol gradient); exosomes band at their buoyant density, separating from lighter or heavier components6.	- Yields highly pure exosomes (separates out most non-vesicle proteins). - Preserves vesicle integrity and functionality. - Effective even for viscous samples (e.g. saliva) with optimized protocols.	- Very slow (often 14–18 h spin); labor-intensive setup. - Ultracentrifuge required; high operational cost. - Potential vesicle loss or damage from prolonged high g and osmotic stress. - Lower yield than direct pelleting (some exosomes discarded with gradient fractions).	Moderate to large (typically 1–5 mL per gradient tube; can process larger volumes by multiple tubes).	Ultracentrifuge + gradient-forming materials (gradient mixer or careful manual layering).	Very long (≥ overnight for ultracentrifugation + time to prepare/harvest gradient).
Size-Exclusion Chromatography (SEC)	Column chromatography using porous beads; particles above a certain size elute first, smaller molecules are retarded in pores.	- Gentle, no harsh conditions, maintains exosome structure and bioactivity. - Good purity: separates exosomes from soluble proteins. - Inexpensive and scalable; columns can be reused and run by gravity (no special equipment if using gravity flow).	- Dilution of sample; may need concentration post-elution. - Long column run for large volumes; (~20–30 min per run). - Resolution limits: smallest exosomes and protein complexes might co-elute, giving broader size distribution. - Column can clog with very particulate samples (pre-filtering recommended).	Small to moderate (~0.5–2 mL per run typically, larger volumes processed in batches).	Chromatography column (pre-packed SEC column or self-packed Sepharose). Pump or centrifuge optional (gravity flow often used).	Moderate (tens of minutes per sample run).
Filtration (Ultrafiltration)	Membrane filtration through size-specific pores (100 nm, 220 nm, etc.). Exosomes (30–150 nm) pass or are retained depending on setup; larger particles are retained or removed.	- Rapid and easy protocol; minimal equipment. - Can concentrate exosomes while isolating (if using a molecular weight cutoff ultrafilter to retain exosomes). - Yields comparable recovery to ultracentrifugation in many cases.	- Prone to filter clogging, which can trap exosomes and reduce yield. - Shear forces or pressure can damage vesicles. - Not highly specific: some non-exosomal particles of flexible shape/size may pass through. - Filters are single-use and add cost; not suitable for very large volumes without specialized systems.	Variable. Small volumes (≤1 mL) per spin filter;	Basic laboratory centrifuge (for spin filters) or vacuum/ pressure pump. No high-end equipment required.	Short (minutes to <1 hour, depending on volume and whether multiple filtration steps are used).
Polymer Precipitation (PEG)	Add a polymer solution (e.g. PEG) that binds water and forces exosomes out of solution (water-exclusion precipitation). Incubation followed by low-speed centrifugation collects the exosome-rich precipitate.	- Simple, mix-and-spin protocol; no advanced equipment needed. - Works with small sample volumes (100 µL–1 mL range) – ideal for limited clinical samples. - High yield recovery of exosomes and their RNA cargo; good for maximizing material for analysis.	- Low purity: co-precipitates abundant proteins (albumin, lipoproteins, etc.) with exosomes. - Downstream analyses (e.g. proteomics) can be confounded by contaminant proteins. - Requires removal of polymer and contaminants (wash steps or additional purification). - Reagents can be relatively costly (commercial kits).	Small to moderate (as low as ~100 µL; up to a few mL – larger volumes may need proportionally more reagent).	Standard microcentrifuge or tabletop centrifuge (only ~10,000×g or less needed).	Short (~1–2 hours total, including incubation).
Immunomagnetic Separation (Affinity Isolation)	Magnetic microbeads coated with antibodies (or other binders) specific to exosome surface markers (e.g. tetraspanins) are mixed with the sample. Target exosomes bind to beads, then are pulled out by a magnet.	- High specificity: enriches exosomes of chosen origin/antigen, reducing impurities. - Gentle, one-step capture preserves exosome structure. - Low equipment needs (only a magnet) and relatively low cost. - Can concentrate exosomes from dilute samples by capturing them onto a small solid phase.	- Only captures exosomes with the target marker; may miss other subpopulations. - No size selection – co-captures any vesicle (or even protein aggregates) bearing the target epitope. - Upfront sample clearing (low-speed spin) needed to remove bulk debris. - Potential interference from non-specific binding in complex fluids (e.g. serum proteins binding antibodies). - Additional step required if purified exosomes need to be released from beads for analysis.	Small (≤1 mL is typical; even <100 µL feasible for some kits) to moderate volumes. Bead volume/capacity can be scaled for larger samples if needed.	No major instruments. Requires magnetic beads and a strong magnet; plus basic lab mixer/rotator for incubation. (Centrifuge used only for initial clearing step.)	Short (~30 min – 2 h including incubation and washes).

IMMUNOMAGNETIC SEPARATION AND DOWNSTREAM APPLICATIONS

Immunomagnetic separation (IMS) constitutes a solid-phase preconcentration strategy that enables the selective isolation of exosomes from complex biological matrices through antibody-mediated affinity capture on magnetic particles (MPs).[25] The technique exploits the ease of magnetic actuation to rapidly separate target vesicles from interfering matrix components, addressing key limitations of conventional bulk isolation methods such as ultracentrifugation and polymer precipitation, which are labor-intensive, poorly selective, and often incompatible with point-of-care workflows.[26]

Magnetic particles used for IMS are typically superparamagnetic micro- or nanoparticles that can be readily functionalized with biorecognition elements. Among the different surface chemistries available, tosyl-activated magnetic microparticles have been extensively employed due to their robustness and versatility. These particles consist of an iron oxide core encapsulated in a polymeric shell bearing p-toluenesulfonyl ester groups, which act as efficient leaving groups for nucleophilic substitution reactions. Covalent immobilization of antibodies occurs via the reaction of amine groups, primarily from lysine residues, under mild conditions, yielding stable antibody-modified MPs with high coupling efficiencies and minimal leaching,[27] as schematically shown in Figure 2.

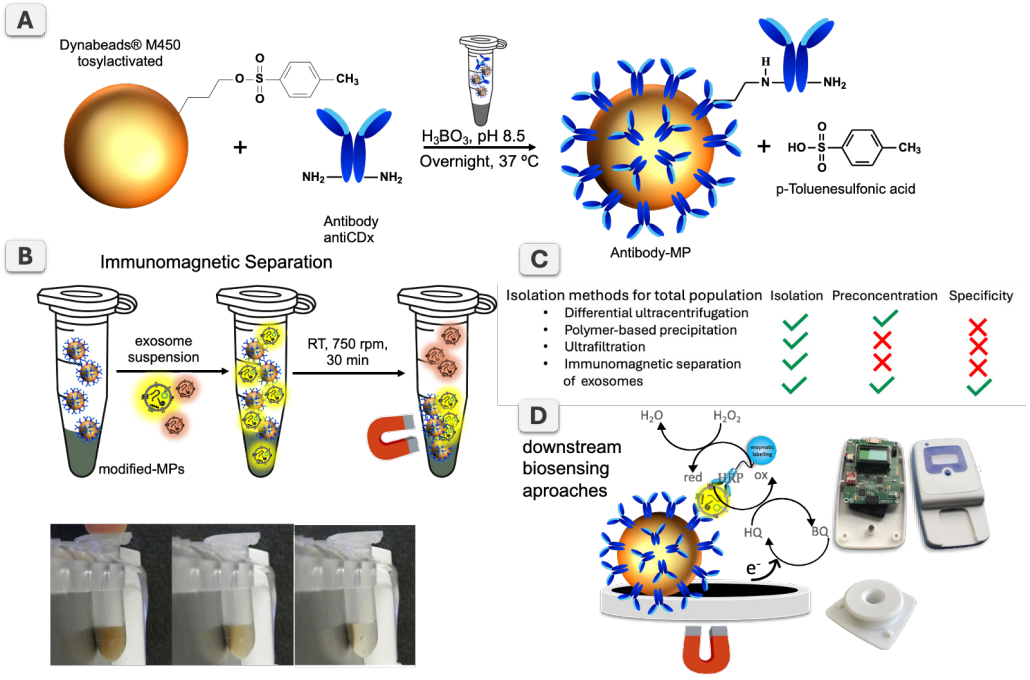

Figure 2. Immunomagnetic separation of exosomes using antibody-functionalized magnetic particles and integration with downstream biosensing platforms. (A) Covalent immobilization of antibodies onto tosyl-activated magnetic microparticles (Dynabeads® M450). Antibodies are coupled through nucleophilic substitution of tosyl groups by primary amines under mildly alkaline conditions (borate buffer, pH 8.5), yielding stable antibody-functionalized magnetic particles. (B) Immunomagnetic separation workflow. Antibody-modified MPs are incubated with an exosome-containing suspension, allowing selective binding of target exosomes in solution. Magnetic actuation enables rapid separation and washing, resulting in preconcentration of the captured vesicles. Representative photographs illustrate the magnetic separation process. (C) Comparison of exosome isolation strategies in terms of isolation of total vesicle populations, preconcentration capability, and molecular specificity. While conventional methods (differential ultracentrifugation, polymer-based precipitation, ultrafiltration) primarily isolate total vesicle populations, immunomagnetic separation uniquely combines preconcentration with molecular specificity. (D) Integration of immunomagnetically captured exosomes with downstream biosensing approaches. Exosomes immobilized on MPs can be directly interfaced with electrochemical biosensors, enabling enzymatic signal generation and portable readout formats suitable for point-of-care applications.

The specificity of IMS is largely determined by the choice of capture antibody. General exosomal markers such as the tetraspanins CD9, CD63, and CD81 are widely used to isolate total exosome populations, while additional surface receptors including CD24, CD44, CD54, CD171, CD326 (EpCAM), and CD340 (HER2) enable the selective enrichment of exosome subpopulations associated with specific cellular origins or disease states.[2] This molecular selectivity represents a major advantage over size or density-based separation methods, which isolate heterogeneous vesicle populations and limit the ability to distinguish biologically relevant EV subpopulations. Operationally, IMS is performed in suspension, allowing immunorecognition to occur under well-controlled conditions and improving binding kinetics relative to surface-immobilized formats. Following incubation, magnetic actuation enables rapid separation and washing steps, effectively reducing matrix effects and concentrating the captured vesicles into small volumes suitable for downstream analysis. The procedure can be implemented in microtubes or microplate formats and requires only basic laboratory equipment, making it compatible with automation and miniaturization.

Beyond isolation, IMS offers seamless integration with downstream analytical techniques.[27,28] Exosomes immobilized on MPs can be directly analyzed by flow cytometry, confocal microscopy, magneto-immunoassays, qRT-PCR, or electrochemical biosensors, often without the need for elution steps. This solid-phase format enhances analytical sensitivity while simplifying assay workflows, particularly in applications targeting low-abundance biomarkers in complex clinical samples.[27,28] Our research group has extensively applied and optimized IMS strategies for exosome isolation from cell culture supernatants and human biofluids, demonstrating their robustness, selectivity, and compatibility with multiple biosensing readouts. These studies position IMS as a key enabling technology that bridges exosome separation and electrochemical detection, forming the methodological foundation for the biosensing platforms discussed in the following sections.

ELECTROCHEMICAL BIOSENSING PLATFORMS FOR EXOSOME DETECTION

The combination of immunomagnetic separation with biosensing platforms enables the selective capture, preconcentration, and direct interrogation of exosomes in complex biological matrices. By immobilizing target vesicles onto a solid magnetic support, matrix interferences are minimized and the captured exosomes can be seamlessly interfaced with diverse transduction schemes (Figure 3). In this section, different biosensing strategies integrated with immunomagnetic separation are discussed, highlighting electrochemical and optical readout approaches that exploit distinct exosomal features, including surface proteins, enzymatic activity, and nucleic acid cargo.

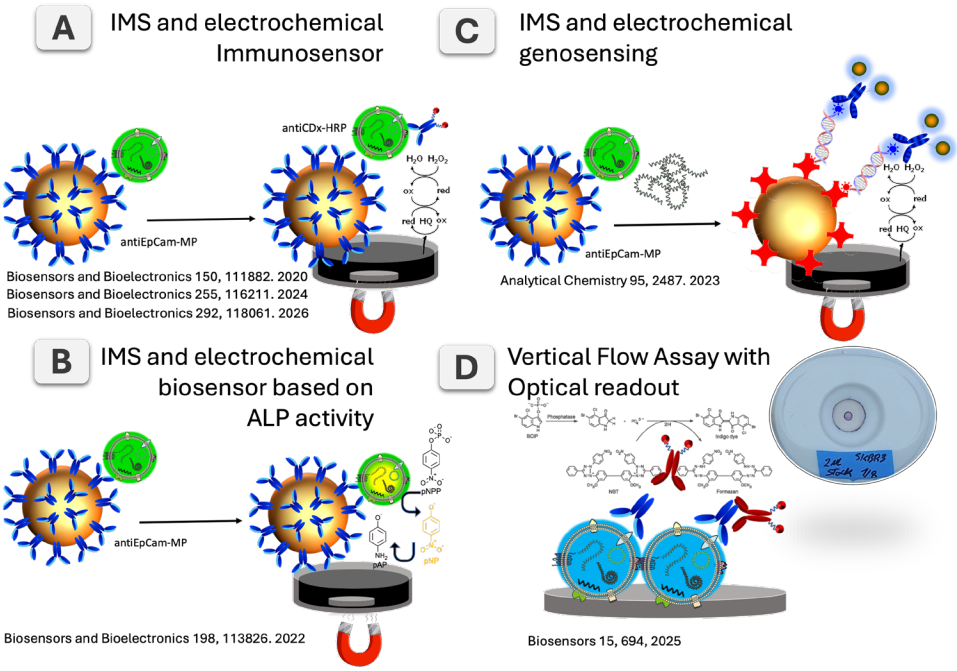

Figure 3. Integration of immunomagnetic separation with biosensing platforms for exosome analysis. Schematic overview of four representative platforms combining antibody-based immunomagnetic capture of exosomes with different signal transduction strategies. (A) Electrochemical immunosensor based on enzyme-labeled secondary antibodies following IMS. (B) Electrochemical biosensor exploiting the intrinsic alkaline phosphatase (ALP) activity associated with captured exosomes. (C) Electrochemical genosensor targeting exosomal nucleic acids after IMS and amplification. (D) Vertical flow assay coupled to optical readout for rapid exosome detection. Representative publications corresponding to each platform are also indicated.

Electrochemical immunosensing of exosomes

Electrochemical immunosensing combined with immunomagnetic separation (IMS) has been applied to the detection of tumor-associated exosomes in complex matrices, with particular emphasis on breast cancer liquid biopsy. Moura et al. reported a magneto-actuated electrochemical immunosensor in which exosomes are first immunocaptured on antibody-functionalized magnetic particles (MPs), followed by enzyme-amplified amperometric readout at disposable screen-printed electrodes.[28] A comparative evaluation of alternative assay configurations indicated that the most robust discrimination was obtained when the immunomagnetic step was used to enrich a marker-defined exosome subpopulation (e.g., CD24- or CD340-positive vesicles), followed by electrochemical readout using a broadly expressed exosomal reporter (CD63). This strategy benefits from the higher surface abundance and ubiquity of the general tetraspanin label, which improves signal-to-background while preserving the biological specificity at the capture step. In practice, combining selective capture with a general exosome reporting label provided better analytical performance than capturing a broad exosome population and subsequently probing for low-abundance tumor markers, even in magneto-ELISA format.[29] Importantly, the platform was validated using real clinical samples, comparing plasma from breast cancer patients and healthy donors (n = 10 per group; the study reports stage IV patients). Both formats yield low background currents in the absence of exosomes (MPs control) and enable discrimination between healthy and cancer cohorts for selected markers. In particular, the cancer group displays higher signals for tumor-associated surface markers (notably CD24 and CD340/HER2 in this dataset), whereas the healthy group shows lower and more compact distributions, consistent with the enrichment of marker-positive exosome subpopulations in patient plasma. The use of IMS is central to performance in plasma, as it provides both preconcentration and effective matrix cleanup prior to electrochemical transduction. The

authors report limits of detection on the order of 10^5 exosomes μL^{-1}, within a range relevant for circulating exosome concentrations, and demonstrate that electrochemical readout supports sensitive measurements in minimally processed samples. Overall, this study illustrates how IMS–electrochemical immunosensing can move beyond proof-of-concept in spiked buffers and deliver clinically discriminative readouts in real biofluids, supporting its suitability for liquid biopsy workflows.

Peptide ligands selected by phage display can replace antibodies as affinity elements in immunomagnetic/electrochemical exosome workflow. Alves et al. (2024) reported a biotinylated peptide ligand (biotin-C3) that selectively recognizes breast cancer–derived exosomes and can be implemented within magneto-actuated assay formats by immobilization on streptavidin-coated magnetic supports.[30] In this configuration, the peptide is used as the specific recognition component in the selective labelling step, while enzymatic amplification (e.g., HRP-based) enables quantitative readout. Overall, this approach illustrates that antibody-free affinity reagents can be engineered to target disease-associated exosome subpopulations, potentially improving assay robustness and simplifying reagent handling compared with antibodies. Notably, the peptide-based sensor achieved limits of detection on the order of 10^2 exosomes μL^{-1} for the cancer exosomes, with reported LODs of 175 exosomes μL^{-1} for MCF-7 exosomes (and below 500 exosomes μL^{-1} for two other cancer lines). These LODs reflect roughly a three-fold improvement over analogous antibody-based assays in the same study. The authors attribute this enhancement to the small size of peptides allowing denser surface packing and closer proximity to the sensor transducer, as well as potentially higher affinity of the selected peptide compared to some antibodies. Another approach addresses the need for a blood-based assay for early Alzheimer's disease (AD) diagnosis. Rossi et al. (2026) developed an electrochemical immunosensor that isolates brain-derived exosomes (BDEs) from plasma and measures an AD-specific protein on these vesicles.[31] In this strategy, magnetic particles were functionalized with an antibody against neuroligin-3 (NLGN3), a neuronal membrane protein found enriched on exosomes originating from neurons.[32] This allowed selective capture of BDEs from plasma, separating them from the bulk of exosomes released by other tissues. After immunomagnetic isolation, the captured exosomes were interrogated for the presence of β-site amyloid precursor protein cleaving enzyme 1 (BACE-1), a membrane enzyme (β-secretase) involved in AD pathology. BACE-1 on the exosome surface was detected by a biotinylated anti-BACE-1 antibody, followed by a streptavidin–HRP label for electrochemical readout. The combination of NLGN3 as a capture ligand (to enrich brain-derived vesicles) and BACE-1 as a detection target is unique; this dual-marker approach effectively creates a sandwich immunoassay on intact exosomes, where one antibody pulls out a specific exosome subpopulation and a second antibody probes it for a disease marker. The assay was evaluated on three different readout platforms (optical ELISA, chemiluminescence, and electrochemical) for sensitivity comparison. The electrochemical detection (using a portable reader operated by batteries) achieved a limit of detection of ~1.5×10^4 exosomes μL^{-1}. Moreover, the biosensor could distinguish plasma samples from patients at different stages of cognitive impairment: AD patients showed higher exosomal BACE-1 signals than mild cognitive impairment (MCI) patients, who in turn were higher than healthy controls. Although the differences had a moderate statistical significance ($p < 0.1$ in initial tests), the trend suggests potential for stratifying patient risk and detecting AD in prodromal stages. The entire assay was completed on a small volume of plasma, and the use of magnetic beads and a hand-held electrochemical reader highlights its point-of-care potential. This immunosensor exemplifies how targeting origin-specific exosomes (brain-derived) can enhance the specificity of liquid biopsy for a neurological disease, and how coupling immunocapture with electrochemical transduction yields a sensitive, portable diagnostic tool. Future clinical studies are expected to further validate the NLGN3/BACE-1 exosomal biomarker combination and potentially expand the panel to improve AD diagnostic accuracy.

Electrochemical biosensing exploiting enzymatic activity in exosomes

Another biosensing strategy is based on the intrinsic enzyme present on exosomes as the signal generator, thereby simplifying the assay design by avoiding external labels. Essentially, the exosome's own enzymatic activity serves as the biorecognition element and signal transducer in one, eliminating the need for a secondary

antibody or labeling step. Moura et al. (2022) reported that exosomes derived from certain cell types exhibit alkaline phosphatase (ALP) activity on their surface, and they used this property for cancer diagnostics.[33] ALP is a membrane-bound enzyme (a metalloenzyme) involved in dephosphorylation reactions, commonly used as a clinical biomarker for liver, bone, and other diseases. In this study, tumor-derived exosomes, especially from cancers with bone involvement, were found to carry elevated ALP activity. In this format, epithelial-derived exosomes are first selectively enriched from serum via immunomagnetic capture using CD326 (EpCAM) as the isolation marker. The bead-bound exosomes are then incubated with para-nitrophenyl phosphate (pNPP) under alkaline conditions; ALP catalyzes pNPP hydrolysis to p-nitrophenol, which is subsequently converted electrochemically to p-aminophenol and quantified by square-wave voltammetry using boron-doped diamond electrodes. This enzyme-based biosensor was shown to detect exosomes from as little as 1 mL of human serum. It yielded a limit of detection of 4.39 mU L^{-1} in terms of ALP activity, which corresponded to approximately 1×10^5 exosomes μL^{-1}. In pooled serum-derived exosomes from breast cancer patients versus healthy donors (n = 10 per group), ALP activity measured in CD326-positive exosomes was significantly higher in the patient cohort (mean 4.5-fold; $p < 0.05$), enabling discrimination without a secondary labeling antibody. Going forward, exploring other endogenous exosomal enzymes (or combining multiple enzyme activities) might further enhance specificity for certain diseases. Additionally, integrating this sensor into a fully automated platform could lead to a robust point-of-care device for cancer monitoring.

Electrochemical genosensing exploiting transcript cargo in exosomes

Beyond surface proteins and enzymatic activities, the nucleic-acid cargo of exosomes constitutes an additional and highly informative biomarker layer. In particular, tumor-derived exosomes can carry mRNAs and microRNAs that mirror the transcriptional signature of the parental cells. In this context, Pallarès-Rusiñol et al. (2023) reported an electrochemical genosensing strategy to quantify an exosomal mRNA target in breast-cancer samples as an indirect readout of tumor-associated exosome burden.[34] The selected transcript was glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, used as a model target to demonstrate the feasibility of exosome genosensing within a clinically oriented workflow. Methodologically, the assay couples selective immunomagnetic enrichment with nucleic-acid amplification and electrochemical detection. Exosomes are first isolated from serum by immunomagnetic separation using anti-EpCAM–functionalized magnetic particles, thereby enriching epithelial-derived vesicles within a complex protein background. A lysis step releases the intravesicular RNA, followed by reverse-transcription PCR (RT-PCR) to amplify GAPDH-derived sequences. To enable electrochemical readout, the amplification employs a double-tagging design, generating amplicons bearing (i) a capture tag compatible with immobilization/hybridization on the electrode interface and (ii) a reporter tag enabling enzymatic or affinity-based signal generation.[35] In the reported configuration, poly(dT)-modified magnetic particles are used to anchor cDNA-related species, and a biotin tag supports downstream electrochemical quantification through streptavidin–enzyme coupling (e.g., HRP) or equivalent labeling chemistries. This integrated IMS + RT-PCR + electrochemical readout achieved a limit of detection of 4×10^2 exosomes μL^{-1}. When applied to clinical specimens, the platform discriminated breast cancer samples from healthy controls, yielding an average 3.3-fold higher GAPDH-related electrochemical signal in the cancer cohort ($p < 0.05$). The workflow was implemented using 1.0 mL of serum without ultracentrifugation, highlighting an analytically sensitive approach for liquid-biopsy settings. While thermocycling increases instrumentation demands, the workflow remains compatible with compact, field-deployable thermocyclers (e.g., miniPCR-type platforms), which can support PCR/RT-PCR under portable or near-point-of-care conditions.

Paper-based vertical flow assay for rapid exosome biomarker profiling

This platform describes a paper-based vertical flow assay (VFA) for rapid profiling of exosomal surface proteins in breast cancer models, providing a complementary approach to electrochemical biosensing. The method follows an ELISA-like format implemented on a nitrocellulose membrane integrated into a commercial

vertical-flow cassette, enabling fast assay times and minimal instrumentation. Exosomes derived from metastatic breast cancer cell lines (SKBR3 and MDA-MB-231) are directly applied to the membrane and probed with primary antibodies targeting canonical exosomal markers (CD9, CD63, CD81) as well as the tumor-associated receptor EGFR1.[36] Signal generation relies on an alkaline phosphatase (ALP)–conjugated secondary antibody and chromogenic NBT/BCIP substrate, producing an insoluble precipitate that can be read visually or quantified by smartphone-based image analysis, as schematically shown in Figure 4.

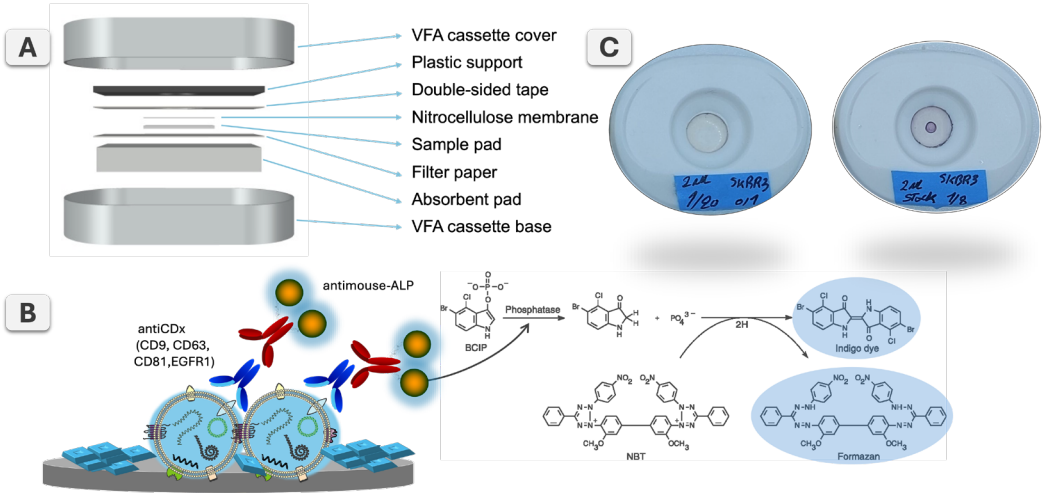

Figure 4. Paper-based vertical flow assay (VFA) for optical exosome biomarker profiling. (A) Schematic representation of the VFA cassette architecture, showing the assembly of the plastic support, double-sided adhesive layer, nitrocellulose membrane, sample pad, filter paper, and absorbent pad between the cassette base and cover. (B) Assay principle: exosomes immobilized on the nitrocellulose membrane are recognized by primary antibodies against exosomal surface markers (CD9, CD63, CD81, EGFR1), followed by an alkaline phosphatase (ALP)–conjugated secondary antibody. Enzymatic conversion of the NBT/BCIP substrate generates an insoluble colored precipitate, enabling visual detection and image-based quantification. (C) Photographs of representative VFA cassettes after assay completion, illustrating the absence of signal in control samples (left) and the localized colorimetric response obtained for breast cancer–derived exosomes (right).

The assay was optimized in terms of membrane pore size, blocking conditions, and enzymatic reaction time, resulting in low background and good reproducibility. Under the selected conditions, the VFA achieved limits of detection on the order of 6×10^7 exosomes μL^{-1} for both SKBR3 and MDA-MB-231 exosomes, with a total assay time below 20 minutes. Although the sensitivity is lower than that of electrochemical platforms, the VFA enables rapid, parallel profiling of multiple biomarkers without the need for complex instrumentation. Importantly, the expression patterns obtained with the VFA correlated well with those measured by bead-based flow cytometry, validating its utility as a semi-quantitative tool for exosome characterization. Control experiments confirmed that intrinsic ALP activity associated with exosomes did not significantly contribute to the signal under the selected assay conditions. Overall, this paper-based platform illustrates a simple and robust strategy for fast exosome biomarker profiling, well suited for screening applications and decentralized testing, and complementary to high-sensitivity electrochemical biosensors.

Comparison of biosensing platforms for exosomes

The sample type used for validation is a key parameter when assessing the analytical reliability and translational potential of electrochemical exosome biosensors. Matrix components may contribute to nonspecific adsorption, reduced capture efficiency, signal interference or decreased reproducibility.

In complex biological matrices, analytical performance may be affected by several concurrent mechanisms. Abundant proteins and other components can compete for the sensor surface or promote nonspecific adsorption, whereas differences in ionic strength, viscosity, pH, and redox-active endogenous compounds may alter mass transport, binding kinetics, electrode interfacial properties, and the background signal. Matrix effect may therefore influence both sensitivity and selectivity, particularly when calibration is performed in buffer rather than in matrix-matched standards. Reliable evaluation should consequently include appropriate negative and interference controls, recovery studies, assessment of nonspecific binding, and, whenever possible, calibration and validation in the same type of biological matrix intended for clinical use.[37] For this reason, Tables II and III specifies whether each platform was evaluated in buffer or artificial media or in real biological samples. Platforms validated in serum, plasma or other clinically relevant matrices provide stronger evidence of robustness and practical applicability than those demonstrated only under idealized laboratory conditions.

In the studies discussed here from our group, the purification and preconcentration procedures were kept consistent, and LOD values were referred to the original sample rather than only to the final detection suspension. This allows a more meaningful comparison of the influence of different bioreceptors, assay configurations and transduction platforms on analytical sensitivity. In this context, the platforms discussed here illustrate one specific analytical route: the use of magnetic separation as a preconcentration and matrix-cleanup step coupled to electrochemical or related readouts for targeted exosome detection. Each of these biosensors was designed to address specific challenges in exosome analysis. The immunosensor based on dual antibodies achieves both cellular-origin specificity (brain-derived exosomes) and disease specificity (AD marker), which is particularly valuable for a complex disorder such as Alzheimer's disease.[31] Its LOD (~10^4 exosomes μL^{-1}) was sufficient for clinical plasma samples and highlights the benefit of a well-chosen biomarker combination.

A useful comparison can be made with analogous immunomagnetic assays using optical, chemiluminescent or ELISA-like readouts. In the Alzheimer's disease platform, the same NLGN3/BACE-1 immunochemical scheme was evaluated using optical, chemiluminescent and electrochemical detection, yielding LODs of $1.91 \times 10^{4.5}$, 1.64×10^5 and 1.51×10^4 exosomes μL^{-1}, respectively, with the electrochemical format providing the lowest LOD and enabling portable analysis.[31] Similarly, in breast cancer exosome detection, the electrochemical immunosensor achieved LODs below 100 exosomes μL^{-1} for purified exosomes and approximately 10^5 exosomes μL^{-1} directly in human serum,[28] while the related magneto-ELISA format reported 218 exosomes μL^{-1} in PBS and 10^5 exosomes μL^{-1} in human serum.[29] These comparisons indicate that electrochemical readout can provide comparable or improved analytical sensitivity while offering clear advantages for miniaturization and point-of-need implementation.

The ALP-based sensor takes a different approach by utilizing an intrinsic enzymatic signal; while its LOD was slightly higher (~10^5 exosomes μL^{-1}), it simplifies the assay steps and reagent requirements.[33] A similar format can be integrated into paper-based platforms, such as vertical flow assays for exosome profiling with visual readout.[36] This makes it highly attractive for low-resource settings or rapid testing, where minimizing reagents and instrumentation is crucial. The genosensor achieved the highest sensitivity (~10^2 exosomes μL^{-1}) by amplifying a genetic marker from within the exosomes.[34] This approach is invaluable when sample volumes are limited or when early-stage disease might only shed very few exosomes. However, it does rely on nucleic acid amplification, which could be a limiting factor for point-of-care deployment unless portable PCR thermocyclers are implemented. Lastly, the peptide-based sensor represents an important innovation in capture technology. By replacing antibodies with peptides, it reduces costs and improves stability, and in this case even enhanced the detection limit.[30] Its successful application to detecting cancer exosomes suggests that with further development, peptide/aptamer-based systems could rival antibody-based ones in both performance and ease of use. Notably, all platforms integrate magnetic separation (IMS) as a preconcentration step, underlining the versatility of magnetic beads in exosome biosensing. IMS effectively addresses the low target concentration issue by enriching exosomes from a large volume into a small volume, which is reflected in the low detection limits achieved across these methods.

Table II. Summary of electrochemical biosensing platforms for exosome analysis, highlighting the biorecognition strategy, detected exosome biomarkers, sample matrices, limits of detection (LOD), and key methodological strengths

Ref.	Platform / approach	Target application	Biorecognition element(s)	Detected exosomal marker(s)	Sample / matrix	LOD	Key strengths
28	Magneto-actuated electrochemical immunosensor	Breast cancer exosomes	Immunomagnetic capture with anti-CD24 or anti-CD340/HER2; HRP-labelled anti-CD63 for detection	CD24, CD340/HER2; CD63 as general exosome reporter	Breast cancer cell line-derived exosomes; human plasma from breast cancer patients and healthy donors	<100 exosomes μL^{-1} in purified exosome samples ca. 10^5 exosomes μL^{-1} directly in serum/plasma	Selective enrichment of tumor-associated exosome subpopulations combined with general exosome reporting; validated in breast cancer plasma samples.
33	Electrochemical biosensor based on intrinsic ALP activity	Breast cancer-related exosomes	Capture with anti-EpCAM or anti-CD81; detection through endogenous ALP activity	ALP, EpCAM, CD81	hFOB-derived exosomes; human serum-derived exosomes from healthy donors and breast cancer patients	ca. 10^5 exosomes μL^{-1}; 4.39 mU L^{-1} ALP	Simplifies sandwich immunosensing by replacing the labelled secondary antibody with intrinsic enzymatic activity.
30	Electrochemical peptide-based biosensor	Breast cancer-derived exosomes	Phage-display-derived biotinylated peptide; anti-CD63 magnetic capture; streptavidin-polyHRP readout	Peptide-recognized membrane proteins; CD63 for capture	MCF-7, MDA-MB-231 and SKBR3-derived exosomes; depleted human serum	175 exosomes μL^{-1} for MCF-7	Replaces a specific detection antibody with a synthetic peptide ligand, improving stability and analytical sensitivity.
31	Portable electrochemical immunosensor	Alzheimer's disease / brain-derived exosomes	anti-NLGN3 capture; anti-BACE-1-HRP detection	NLGN3 and BACE-1	SH-SY5Y-derived exosomes; human plasma from controls, MCI and AD patients	1.5×10^4 exosomes μL^{-1}	Extends magneto-electrochemical exosome sensing to brain-derived exosomes and patient stratification; validated in AD plasma samples.
34	Electrochemical magneto-genosensor with double-tagging RT-PCR	Breast cancer exosomal RNA	anti-EpCAM immunomagnetic capture; poly(dT)-MPs; double-tagging RT-PCR; electrochemical genosensing	GAPDH mRNA	MCF-7-derived exosomes; 1 mL undiluted human serum from breast cancer patients and controls	4×10^2 exosomes μL^{-1} directly in serum/plasma	Strong sensitivity gain through RT-PCR amplification; accesses intravesicular RNA biomarkers. Specificity depends on the captured exosome subpopulation.
38	Disposable screen-printed electrochemical immunosensor	HER2-positive breast cancer exosomes	anti-CD9 capture; anti-HER2 detection	CD9 and HER2	BT-474 and MDA-MB-231-derived exosomes; HER2+ exosomes spiked in serum	4.7×10^5 exosomes μL^{-1}	Simple disposable SPE format; detects HER2+ exosomes within total exosome populations.
39	Integrated magneto-electrochemical exosome sensor, iMEX	Ovarian cancer EV/exosome profiling	anti-CD63 magnetic capture; HRP-labelled detection antibodies	CD63; EpCAM, CD24, CA125 and other markers	Undiluted plasma from ovarian cancer patients and controls	3×10^4 exosomes	Integrated, portable and multiplexed magnetic/ electrochemical platform.
40	Microfabricated electrochemical chip with metal nanoparticle labels	Prostate cancer exosomes and microsomes	anti-EpCAM aptamer capture; AgNP–anti-EpCAM and CuNP–anti-PSMA probes	EpCAM and PSMA	VCaP culture supernatant; serum from prostate cancer patients and controls	50 exosomes/sensor	Dual-marker electrochemical detection. Requires microfabrication and metal nanoparticle probes.

(continued on next page)

Table II. Summary of electrochemical biosensing platforms for exosome analysis, highlighting the biorecognition strategy, detected exosome biomarkers, sample matrices, limits of detection (LOD), and key methodological strengths (continued)

Ref.	Platform / approach	Target application	Biorecognition element(s)	Detected exosomal marker(s)	Sample / matrix	LOD	Key strengths
41	Aptamer-based electrochemical microfluidic biosensor	Total CD63-positive exosome detection	CD63 aptamer immobilized on gold electrodes; methylene-blue-labelled antisense probe	CD63	HepG2-derived exosomes in HEPES and DMEM + 10% FBS	1×10^6 particles mL^{-1}	Simple displacement aptasensor without antibody labelling or washing; detects total CD63+ exosomes. Not validated with clinical samples.
42	Magneto-mediated electrochemical sensor	Breast cancer exosomal protein profiling	CD63 aptamer-MBs; SiO_2 probes with MUC1, HER2, EpCAM and CEA aptamers	CD63, MUC1, HER2, EpCAM, CEA	Breast cancer cell line-derived exosomes; patient serum	Not explicitly reported; linear range 1.2×10^3 – 1.2×10^7 particles μL^{-1}	Multiplexed breast cancer exosome profiling.
43	Electrochemical aptasensor with cucurbit[7]uril–ferrocene host–guest recognition	Total exosome detection	Ferrocene-linked CD63 aptamer immobilized through cucurbit[7]uril on AuNP-electrodes	CD63	MCF-7-derived exosomes in PBS; exosomes spiked into exosome-free plasma	482 particles μL^{-1}	Combines electrochemical detection with mild exosome release for downstream analysis. Not validated with clinical samples.
44	Electrochemical aptasensor with multidirectional hybridization chain reaction (mHCR) amplification	EpCAM-positive tumorous exosome detection	EpCAM aptamer on Au electrode for exosome capture; cholesterol-modified H-shaped DNA anchor inserted into the exosomal membrane; biotin-labelled mHCR products recruiting SA-HRP for enzymatic signal amplification	EpCAM	MCF-7-derived exosomes; breast cancer serum exosomes	285 exosomes μL^{-1}	Multidirectional hybridization chain reaction (mHCR) improves sensitivity.
45	Electrochemical immunosensor with HRCA-responsive MOF amplification	PD-L1-positive breast cancer exosomes	CD63 for exosome capture; PD-L1 for breast cancer exosome identification	CD63 and PD-L1	MDA-MB-231-derived PD-L1+ exosomes; MCF-7- and L02-derived exosomes; undiluted serum from healthy controls, non-metastatic breast cancer patients and metastatic breast cancer patients	334 particles mL^{-1}	PD-L1+ exosome detection; relies on DNA amplification and MOF-based signal release.
46	Electrochemical micro-aptasensor with HCR amplification	EpCAM-positive exosome; lung cancer serum	CD63 aptamer-modified microelectrodes for exosome capture; anti-EpCAM aptamer-embedded HCR products carrying biotin/avidin-HRP for enzymatic signal amplification	CD63 and EpCAM	MCF-7-derived exosomes; lung cancer serum samples	5×10^2 exosomes mL^{-1}	CD63-based capture with EpCAM-specific recognition and HCR/HRP signal amplification; validated with serum from lung cancer patients.

Table III. Summary of paper-based platforms for exosome analysis, highlighting the biorecognition strategy, detected exosomal biomarkers, sample matrices, limits of detection (LOD), and key methodological strengths

Ref.	Platform / approach	Target application	Biorecognition element(s)	Detected exosomal marker(s)	Sample / matrix	LOD / sensitivity	Main comparative point
36	Paper-based vertical flow assay, ELISA-like format	Breast cancer exosome profiling	Immobilized antibodies on nitrocellulose; anti-mouse-ALP; BCIP/NBT colorimetric readout	CD9, CD63, CD81 and EGFR1	SKBR3 and MDA-MB-231-derived exosomes	ca. 6 × 10^7 exosomes μL^{-1}	Rapid visual/smartphone-compatible exosome profiling in <20 min; lower sensitivity than amplified methods but simpler operationally.
47	Lateral flow immunoassay for enriched exosomes	General exosome detection	Capture: anti-CD9 + anti-CD81; detection: anti-CD63-AuNPs	CD9, CD81, CD63	Ma-Mel-86c melanoma exosomes; human plasma and urine exosomes	8.54 × 10^5 exosomes mL^{-1}	Early LFIA for exosome detection; rapid 15 min assay but requires prior exosome enrichment.
48	Multiple-target LFIA / POC EV detection	General EV detection from plasma	Capture: anti-CD81 and anti-CD9; detection: anti-CD63-AuNPs; label comparison with AuNPs, carbon black and MNPs	CD9, CD81, CD63	EVs isolated from human plasma	3.4 × 10^6 EVs mL^{-1}	Optimizes LFIA sensitivity through multiple capture lines and label selection; total analysis time around 2 h.
49	Magnetic LFIA coupled to inductive sensor	Colorectal cancer EV biomarker detection	Capture: anti-CD9; detection: MNP-labelled anti-CD63 or anti-CD147	CD63 for total EVs; CD147 for CRC-associated EVs	EVs isolated from plasma of CRC patients	Total EVs: 3 × 10^7 EVs μL^{-1} by optical readout and 1 × 10^7 EVs μL^{-1} by magnetic readout; no independent CD147-specific LOD reported	Adds quantitative magnetic detection to LFIA and explores CD147+ EVs as CRC-associated biomarkers.
50	Lateral flow aptamer assay strip	NSCLC-derived exosome identification	CD63 aptamer-functionalized AuNPs; complementary aptamer strand on test line	CD63	A549 lung carcinoma cell-derived exosomes	No formal LOD reported; proof-of-concept dilution series from 6.4 × 10^9 particles mL^{-1}	Antibody-free lateral flow format based on aptamers; mainly qualitative/semiquantitative proof-of-concept.
51	Fluorescent nanosphere-based LFA with membrane biotinylation	General EV quantification	DSPE-PEG-biotin membrane labelling; streptavidin fluorescent nanospheres	EV phospholipid membrane; EpCAM characterized in Cal 27 EVs	Cal 27-derived EVs; saliva samples	2.0 × 10^3 particles μL^{-1}	Universal membrane-labelling approach independent of a specific EV protein; rapid and sensitive, but not disease-subpopulation specific.
52	Integrated SERS-vertical flow biosensor, iREX	Breast cancer diagnosis and molecular subtyping	Capture aptamers for MUC1, HER2 and CEA; CD63-conjugated SERS probes	MUC1, HER2, CEA; CD63 as common exosomal marker	Breast cancer cell-derived exosomes; serum exosomes from healthy donors and breast cancer patients	1.0–2.9 × 10^7 particles mL^{-1}	multiplexed quantitative profiling and molecular subtyping, but requires SERS probes and Raman instrumentation.

CONCLUSIONS

Electrochemical biosensors are increasingly positioned as analytical tools for exosome-based diagnostics, because they combine high analytical sensitivity with compact instrumentation and short assay times. From an analytical perspective, the performance of exosome biosensing platforms should not be evaluated solely on the basis of the reported LOD. The reviewed strategies differ in their recognition elements, capture or purification procedures, transduction formats, amplification strategies and sample matrices. Electrochemical biosensors provide clear advantages in terms of sensitivity, miniaturization, low reagent consumption, compatibility with portable instrumentation, and potential integration into point-of-care workflows. Nevertheless, important limitations remain, including the heterogeneity of EV isolation protocols. A further challenge is the absence of a universally accepted reference method for exosome quantification. This directly affects calibration, LOD calculation and the comparison of analytical performance across platforms. Therefore, the comparative data should be interpreted as a critical overview of the analytical landscape rather than as a direct ranking of methods. Platforms validated in real biological samples provide stronger evidence of translational relevance, while the original references should be consulted for detailed experimental conditions and additional performance characteristics.

The studies discussed here from our group show that coupling selective exosome enrichment, most effectively through immunomagnetic separation, with enzyme-amplified electrochemical readout or nucleic-acid amplification enables clinically relevant discrimination in minimally invasive samples, including the differentiation of cancer vs. healthy cohorts and AD vs. non-AD profiles.

Exosome heterogeneity remains one of the main biological and analytical limitations in this field.[3,17] Exosome populations differ in size, cellular origin, surface protein composition, biogenesis pathway, and molecular cargo.[2] Consequently, the signal generated by a biosensor depends not only on the total number of vesicles, but also on the abundance and accessibility of the selected marker and on the proportion of vesicles expressing it. This is particularly relevant for affinity-based and immunomagnetic assays, in which the capture bioreceptor determines the vesicle subpopulation that is ultimately isolated and analyzed. Capture through broadly expressed tetraspanins may provide wider EV enrichment but limited disease specificity, whereas capture through tissue- or disease-associated markers can improve selectivity while excluding vesicles that do not express the selected marker.[28,29] As emphasized in the MISEV2023 guidelines,[37] no single molecular marker can unequivocally define a specific EV subtype, and commonly used markers may be shared among different EV populations. Affinity-based biosensors should therefore be interpreted as measuring a marker-selected EV subpopulation rather than the entire exosome population. Differences in marker abundance, co-expression, epitope accessibility, and vesicle origin may affect capture efficiency, analytical sensitivity, and comparability across studies.

These limitations also highlight the need for greater standardization across the entire analytical workflow. Standardization should cover not only EV isolation, but also sample collection and storage, recovery controls, calibration procedures, reporting units, LOD calculation, and validation in clinically representative matrices. Shared reference materials and harmonized reporting guidelines will be important to improve reproducibility between laboratories, allow meaningful comparison between platforms, and support the clinical translation of exosome biosensors.

Although several electrochemical exosome biosensors show promising analytical performance, most reported platforms remain at the proof-of-concept or preliminary validation stage, and their clinical maturity is still limited by the lack of fully integrated sample-to-answer formats. Translation toward routine clinical and point-of-need implementation will depend on four priorities. First, tighter integration of sample preparation, magnetic capture, and detection into closed cartridges is needed to deliver true sample-to-answer workflows and reduce operator-dependent variability. Second, robustness can be reinforced by simplifying reagent panels and, where appropriate, substituting biologically derived bioreceptors with stable synthetic alternatives, together with scalable disposables such as screen-printed electrodes. Third, broader clinical validation remains essential to address exosome heterogeneity and to define decision thresholds. Finally, reducing the footprint of nucleic-acid amplification will be critical for translating genosensing approaches:

compatibility with compact thermocyclers or isothermal amplification modules can bring RT-PCR-class sensitivity closer to decentralized settings, enabling integrated workflows that link magnetic enrichment to on-cartridge amplification and electrochemical detection.

Clinical implementation will also require validation under standardized operating procedures, reproducible manufacturing of sensors and multicentre studies in sufficiently large and representative patient cohorts. From a regulatory perspective, these platforms will need to demonstrate consistent analytical performance, and compatibility with routine laboratory workflows. In parallel, battery-powered or smartphone-interfaced electrochemical readers could further facilitate deployment in settings that lack large analytical instruments.

Such a vision aligns closely with the career of Prof. Lauro T. Kubota, in whose honor this article is written, reflecting a commitment to innovation, practical application, and the training of future scientists in biosensor research. Continued interdisciplinary efforts, bringing together chemistry, materials science, and biomedicine, will accelerate the journey of these exosome biosensors from the benchtop to the point-of-need.

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Conflicts of interest

The author declares no conflicts of interest.

Use of artificial intelligence tools

The authors declare that no artificial intelligence tools were used in the preparation of this article.

REFERENCES

(1) Kim, H. I.; Park, J.; Zhu, Y.; Wang, X.; Han, Y.; Zhang, D. Recent advances in extracellular vesicles for therapeutic cargo delivery. *Exp. Mol. Med*. **2024**, *56*, 836–849. https://doi.org/10.1038/s12276-024-01201-6

(2) van Niel, G.; D'Angelo, G.; Raposo, G. Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol*. **2018**, *19* (4), 213–228. https://doi.org/10.1038/nrm.2017.125

(3) Sun, M.; Chen, Z. Unveiling the complex role of exosomes in Alzheimer's disease. *J. Inflamm. Res*. **2024**, *17*, 3921–3948. https://doi.org/10.2147/JIR.S466821

(4) Lee, Y.; Ni, J.; Beretov, J.; Wasinger, V. C.; Graham, P.; Li, Y. Recent advances of small extracellular vesicle biomarkers in breast cancer diagnosis and prognosis. *Mol. Cancer* **2023**, *22*, 33. https://doi.org/10.1186/s12943-023-01741-x

(5) Gomes, A.; Tzouanou, F.; Skolariki, K.; Vamvaka-Iakovou, A.; Noguera-Ortiz, C.; Tsirtsaki, K.; Waites, C. L.; Vlamos, P.; Sousa, N.; Costa-Silva, B.; Kapogiannis, D.; Sotiropoulos, I. Extracellular vesicles and Alzheimer's disease in the novel era of Precision Medicine: Implications for disease progression, diagnosis and treatment. *Exp. Neurol*. **2022**, *358*, 114183. https://doi.org/10.1016/j.expneurol.2022.114183

(6) Yang, T. T.; Liu, C. G.; Gao, S. C.; Zhang, Y.; Wang, P. C. The serum exosome derived microRNA-135a, -193b, and -384 were potential Alzheimer's disease biomarkers. *Biomed. Environ. Sci*. **2018**, *31* (2), 87–96. https://doi.org/10.3967/bes2018.011
(7) Chen, H.; Wang, L.; Zeng, X.; Schwarz, H.; Nanda, H. S.; Peng, X.; Zhou, Y. Exosomes, a new star for targeted delivery. *Front. Cell Dev. Biol.* **2021**, *9*, 751079. https://doi.org/10.3389/fcell.2021.751079
(8) Banks, W. A.; Sharma, P.; Bullock, K. M.; Hansen, K. M.; Ludwig, N.; Whiteside, T. L. Transport of Extracellular Vesicles across the Blood-Brain Barrier: Brain Pharmacokinetics and Effects of Inflammation. *Int. J. Mol. Sci.* **2020**, *21*, 4407. https://doi.org/10.3390/ijms21124407
(9) Cano, A.; Esteban-de-Antonio, E.; Bernuz, M.; Puerta, R.; García-González, P.; de Rojas, I.; Olivé, C.; Pérez-Cordón, A.; Montrreal, L.; Núñez-Llaves, R.; et al. Plasma extracellular vesicles reveal early molecular differences in amyloid positive patients with early-onset mild cognitive impairment. *J. Nanobiotechnol.* **2023**, *21*, 54. https://doi.org/10.1186/s12951-023-01793-7
(10) Johnstone, R. M.; Adam, M.; Hammond, J. R.; Orr, L.; Turbide, C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J. Biol. Chem.* **1987**, *262* (19), 9412–9420. https://doi.org/10.1016/S0021-9258(18)48095-7
(11) Pallares-Rusiñol, A.; Bernuz, M.; Moura, S. L.; Fernández-Senac, C.; Rossi, R.; Martí, M.; Pividori, M. I. Advances in exosome analysis. In: Makowski, G. S. (Ed.). *Advances in Clinical Chemistry*. Elsevier: Amsterdam, 2023, Vol. 112, Chapter Two, pp 69–117. https://doi.org/10.1016/bs.acc.2022.09.002
(12) Théry, C.; Amigorena, S.; Raposo, G.; Clayton, A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr. Protoc. Cell Biol.* **2006**, *30* (1), 3.22.1–3.22.29. https://doi.org/10.1002/0471143030.cb0322s30
(13) Swatler, J.; Dudka, W.; Piwocka, K. Isolation and characterization of extracellular vesicles from cell culture conditioned medium for immunological studies. *Curr. Protoc. Immunol.* **2020**, *129* (1), e101. https://doi.org/10.1002/cpim.96
(14) Livshits, M. A.; Khomyakova, E.; Evtushenko, E. G.; Lazarev, V. N.; Kulemin, N. A.; Semina, S. E.; Generozov, E. V.; Govorun, V. M. Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. *Sci. Rep.* **2015**, *5*, 17319. https://doi.org/10.1038/srep17319
(15) Lobb, R. J.; Becker, M.; Wen, S. W.; Wong, C. S.; Wiegmans, A. P.; Leimgruber, A.; Möller, A. Optimized exosome isolation protocol for cell culture supernatant and human plasma. *J. Extracell. Vesicles* **2015**, *4*, 27031. https://doi.org/10.3402/jev.v4.27031
(16) Witwer, K. W.; Buzás, E. I.; Bemis, L. T.; Bora, A.; Lässer, C.; Lötvall, J.; 't Hoen, E. N. N.; Piper, M. G.; Sivaraman, S.; Skog, J.; Théry, C.; Wauben, M. H.; Hochberg, F. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J. Extracell. Vesicles* **2013**, *2* (1), 20360. https://doi.org/10.3402/jev.v2i0.20360
(17) Kowal, J.; Arras, G.; Colombo, M.; Jouve, M.; Morath, J. P.; Primdal-Bengtson, B.; Dingli, F.; Loew, D.; Tkach, M.; Théry, C. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proc. Natl. Acad. Sci. U.S.A.* **2016**, *113* (8), E968–E977. https://doi.org/10.1073/pnas.1521230113
(18) Tauro, B. J.; Greening, D. W.; Mathias, R. A.; Ji, H.; Mathivanan, S.; Scott, A. M.; Simpson, R. J. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. *Methods* **2012**, *56* (2), 293–304. https://doi.org/10.1016/j.ymeth.2012.01.002
(19) Böing, A. N.; van der Pol, E.; Grootemaat, A. E.; Coumans, F. A.; Sturk, A.; Nieuwland, R. Single-step isolation of extracellular vesicles by size-exclusion chromatography. *J. Extracell. Vesicles* **2014**, *3*, 23430. https://doi.org/10.3402/jev.v3.23430
(20) Gámez-Valero, A.; Monguió-Tortajada, M.; Carreras-Planella, L.; Franquesa, M. L.; Beyer, K.; Borràs, F. E. Size-exclusion chromatography-based isolation minimally alters extracellular vesicles characteristics compared to precipitating agents. *Sci. Rep*. **2016**, *6*, 33641. https://doi.org/10.1038/srep33641

(21) Cheruvanky, A.; Zhou, H.; Pisitkun, T.; Kopp, J. B.; Knepper, M. A.; Yuen, P. S.; Star, R. A. Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. *Am. J. Physiol. Renal Physiol.* **2007**, *292* (5), F1657–F1661. https://doi.org/10.1152/ajprenal.00434.2006
(22) Yakubovich, E. I.; Polischouk, A. G.; Evtushenko, V. I. Principles and problems of exosome isolation from biological fluids. *Biochem. (Mosc.) Suppl. Ser. A Membr. Cell Biol.* **2022**, *16* (2), 115–126. https://doi.org/10.1134/S1990747822030096
(23) Niu, Z.; Pang, R. T. K.; Liu, W.; Li, Q.; Cheng, R.; Yeung, W. S. B. Polymer-based precipitation preserves biological activities of extracellular vesicles from an endometrial cell line. *PLoS One* **2017**, *12* (10), e0186534. https://doi.org/10.1371/journal.pone.0186534
(24) Zarovni, N.; Corrado, A.; Guazzi, P.; Zocco, D.; Lari, E.; Radano, G.; Muhhina, J.; Fondelli, C.; Gavrilova, J.; Chiesi, A. Integrated isolation and quantitative analysis of exosome shuttled proteins and nucleic acids using immunocapture approaches. *Methods* **2015**, *87*, 46–58. https://doi.org/10.1016/j.ymeth.2015.05.028
(25) Rembaum, A.; Yen, R. C. K.; Kempner, D. H.; Ugelstad, J. Cell Labeling and Magnetic Separation by Means of Immunoreagents Based on Polyacrolein Microspheres. *J. Immunol. Methods* **1982**, *52*, 341–351. https://doi.org/10.1016/0022-1759(82)90006-0
(26) Reddy, L. H.; Arias, J. L.; Nicolas, J.; Couvreur, P. Magnetic Nanoparticles: Design and Characterization, Toxicity and Biocompatibility, Pharmaceutical and Biomedical Applications. *Chem. Rev*. **2012**, *112*, 5818–5878. https://doi.org/10.1021/cr300068p
(27) Bernuz, M.; Pallarès-Rusiñol, A.; Rossi, R.; Fernández-Senac, C.; Martí, M.; Pividori, M. I. Magnetic Separation of Cell-Secreted Vesicles with Tailored Magnetic Particles and Downstream Applications. In: Vainio, S. (Ed.). *Cell-Secreted Vesicles. Methods in Molecular Biology*. Springer: New York, NY, 2023, vol. 2668, pp 257–276. https://doi.org/10.1007/978-1-0716-3203-1_18
(28) Moura, S. L.; Martín, C. G.; Martí, M.; Pividori, M. I. Electrochemical Immunosensing of Nanovesicles as Biomarkers for Breast Cancer. *Biosens. Bioelectron*. **2020**, *150*, 111882. https://doi.org/10.1016/j.bios.2019.111882
(29) Moura, S. L.; Martín, C. G.; Martí, M.; Pividori, M. I. Multiplex detection and characterization of breast cancer exosomes by magneto-actuated immunoassay. *Talanta* **2020**, *211*, 120657. https://doi.org/10.1016/j.talanta.2019.120657
(30) Alves, R. F.; Pallarès-Rusiñol, A.; Rossi, R.; Martí, M.; Vaz, E. R.; de Araújo, T. G.; Sotomayor, M. D. P. T.; Pividori, M. I. Peptide-Based Biosensing Approaches for Targeting Breast Cancer-Derived Exosomes. *Biosens. Bioelectron.* **2024**, *255*, 116211. https://doi.org/10.1016/j.bios.2024.116211
(31) Rossi, R.; Cano, A.; Pallarès-Rusiñol, A.; Ruiz, A.; Martí, M.; Pividori, M. I. Electrochemical biosensor for early Alzheimer's detection and patient risk stratification using plasma exosomes. *Biosens. Bioelectron.* **2026**, *292*, 118061. https://doi.org/10.1016/j.bios.2025.118061
(32) Pividori, M. I.; Rossi, R.; Martí, M. BACE-1 in Brain-Derived Extracellular Vesicles as a Biomarker for Alzheimer's Disease. European Patent EP25382155.7, priority date February 20, 2025.
(33) Moura, S. L.; Pallarès-Rusiñol, A.; Sappia, L.; Martí, M.; Pividori, M. I. The activity of alkaline phosphatase in breast cancer exosomes simplifies the biosensing design. *Biosens. Bioelectron.* **2022**, *198*, 113826. https://doi.org/10.1016/j.bios.2021.113826
(34) Pallares-Rusiñol, A.; Moura, S. L.; Martí, M.; Pividori, M. I. Electrochemical genosensing of overexpressed GAPDH transcripts in breast cancer exosomes. *Anal. Chem.* **2023**, *95* (6), 2487–2495. https://doi.org/10.1021/acs.analchem.2c04773
(35) Lermo, A.; Campoy, S.; Barbé, J.; Hernández, S.; Alegret, S.; Pividori, M. I. In Situ DNA Amplification with Magnetic Primers for the Electrochemical Detection of Food Pathogens. *Biosens. Bioelectron.* **2007**, *22* (9–10), 2010–2017. https://doi.org/10.1016/j.bios.2006.08.048
(36) Pallarès-Rusiñol, A.; Marfà, J.; Rossi, R.; Martí, M.; Pividori, M. I. Exosome biomarker profiling using a paper-based vertical flow assay. *Biosensors* **2025**, *15*, 694. https://doi.org/10.3390/bios15100694

(37) Welsh, J. A.; Goberdhan, D. C. I.; O'Driscoll, L.; Buzas, E. I.; Blenkiron, C.; Bussolati, B.; Cai, H.; Di Vizio, D.; Driedonks, T. A. P.; Erdbrügger, U.; et al. Minimal Information for Studies of Extracellular Vesicles 2023 (MISEV2023): From basic to advanced approaches. *J. Extracell. Vesicles* **2024**, *13*, e12404. https://doi.org/10.1002/jev2.12404

(38) Yadav, S.; Boriachek, K.; Islam, M. N.; Lobb, R.; Möller, A.; Hill, M. M.; Al Hossain, M. S.; Nguyen, N.-T.; Shiddiky, M. J. A. An electrochemical method for the detection of disease-specific exosomes. *ChemElectroChem* **2017**, *4*, 967–971. https://doi.org/10.1002/celc.201600391

(39) Jeong, S.; Park, J.; Pathania, D.; Castro, C. M.; Weissleder, R.; Lee, H. Integrated magneto-electrochemical sensor for exosome analysis. *ACS Nano* **2016**, *10*, 1802–1809. https://doi.org/10.1021/acsnano.5b07584

(40) Zhou, Y. G.; Mohamadi, R. M.; Poudineh, M.; Kermanshah, L.; Ahmed, S.; Safaei, T. S.; Stojcic, J.; Nam, R. K.; Sargent, E. H.; Kelley, S. O. Interrogating circulating microsomes and exosomes using metal nanoparticles. *Small* **2016**, *12*, 727–732. https://doi.org/10.1002/smll.201502365

(41) Zhou, Q.; Rahimian, A.; Son, K.; Shin, D. S.; Patel, T.; Revzin, A. Development of an aptasensor for electrochemical detection of exosomes. *Methods* **2016**, *97*, 88–93. https://doi.org/10.1016/j.ymeth.2015.10.012

(42) An, Y.; Li, R.; Zhang, F.; He, P. Magneto-mediated electrochemical sensor for simultaneous analysis of breast cancer exosomal proteins. *Anal. Chem.* **2020**, *92*, 5404–5410. https://doi.org/10.1021/acs.analchem.0c00106

(43) Liu, Q.; Yue, X.; Li, Y.; Wu, F.; Meng, M.; Yin, Y.; Xi, R. A novel electrochemical aptasensor for exosomes determination and release based on specific host–guest interactions between cucurbit[7]uril and ferrocene. *Talanta* **2021**, *232*, 122451. https://doi.org/10.1016/j.talanta.2021.122451

(44) Wang, L.; Zeng, L.; Wang, Y.; Chen, T.; Chen, W.; Chen, G.; Li, C.; Chen, J. Electrochemical aptasensor based on multidirectional hybridization chain reaction for detection of tumorous exosomes. *Sens. Actuators, B* **2021**, *332*, 129471. https://doi.org/10.1016/j.snb.2021.129471

(45) Cao, Y.; Wang, Y.; Yu, X.; Jiang, X.; Li, G.; Zhao, J. Identification of programmed death ligand-1 positive exosomes in breast cancer based on DNA amplification-responsive metal–organic frameworks. *Biosens. Bioelectron.* **2020**, *166*, 112452. https://doi.org/10.1016/j.bios.2020.112452

(46) Zhang, W.; Tian, Z.; Yang, S.; Rich, J.; Zhao, S.; Klingeborn, M.; Huang, P. H.; Li, Z.; Stout, A.; Murphy, Q.; Patz, E.; Zhang, S.; Liu, G.; Huang, T. J. Electrochemical micro-aptasensors for exosome detection based on hybridization chain reaction amplification. *Microsyst. Nanoeng.* **2021**, *7*, 63. https://doi.org/10.1038/s41378-021-00293-8

(47) Oliveira-Rodríguez, M.; López-Cobo, S.; Reyburn, H. T.; Costa-García, A.; López-Martín, S.; Yáñez-Mó, M.; Cernuda-Morollón, E.; Paschen, A.; Valés-Gómez, M.; Blanco-López, M. C. Development of a rapid lateral flow immunoassay test for detection of exosomes previously enriched from cell culture medium and body fluids. *J. Extracell. Vesicles* **2016**, *5*, 31803. https://doi.org/10.3402/jev.v5.31803

(48) Oliveira-Rodríguez, M.; Serrano-Pertierra, E.; García, A. C.; Martín, S. L.; Mo, M. Y.; Cernuda-Morollón, E.; Blanco-López, M. C. Point-of-care detection of extracellular vesicles: sensitivity optimization and multiple-target detection. *Biosens. Bioelectron.* **2017**, *87*, 38–45. https://doi.org/10.1016/j.bios.2016.08.001

(49) Moyano, A.; Serrano-Pertierra, E.; Duque, J. M.; Ramos, V.; Teruel-Barandiarán, E.; Fernández-Sánchez, M. T.; Salvador, M.; Martínez-García, J. C.; Sánchez, L.; García-Flórez, L.; et al. Magnetic lateral flow immunoassay for small extracellular vesicles quantification: application to colorectal cancer biomarker detection. *Sensors* **2021**, *21*, 3756. https://doi.org/10.3390/s21113756

(50) Yu, Q.; Zhao, Q.; Wang, S.; Zhao, S.; Zhang, S.; Yin, Y.; Dong, Y. Development of a lateral flow aptamer assay strip for facile identification of theranostic exosomes isolated from human lung carcinoma cells. *Analytical Biochemistry* **2020**, *594*, 113591. https://doi.org/10.1016/j.ab.2020.113591

(51) Dong, D.; Zhu, L.; Hu, J.; Pang, D. W.; Zhang, Z. L. Simple and rapid extracellular vesicles quantification via membrane biotinylation strategy coupled with fluorescent nanospheres-based lateral flow assay. *Talanta* **2019**, *200*, 408–414. https://doi.org/10.1016/j.talanta.2019.03.069

(52) Su, X.; Liu, X.; Xie, Y.; Chen, M.; Zheng, C.; Zhong, H.; Li, M. Integrated SERS-vertical flow biosensor enabling multiplexed quantitative profiling of serological exosomal proteins in patients for accurate breast cancer subtyping. *ACS Nano* **2023**, *17*, 4077–4088. https://doi.org/10.1021/acsnano.3c00449