

BARD1 PRESENTS EXO-NET® EXOSOME ISOLATION DATA AT ISEV2021 CONFERENCE

- **BARD1's US-based research team is presenting a poster demonstrating the superior performance of EXO-NET® at the ISEV Annual Meeting 18-21 May 2021**
- **Data indicates that EXO-NET® provides a novel, rapid and scalable exosome isolation solution**
- **Poster presentation supports BARD1's launch of its EXO-NET® product for research use only**

Melbourne, Australia, 19 May 2021: BARD1 Life Sciences Limited (ASX:BD1) (**BARD1** or the **Company**) is pleased to announce that it will be presenting a poster entitled "EXO-NET®: A Novel, Rapid, Scalable Exosome Isolation Technology" at the virtual International Society for Extracellular Vesicles (ISEV) Annual Meeting from 18-21 May 2021.

The [ISEV2021](#) conference showcases the best in extracellular vesicle (EV) science, covering all aspects of fundamental, translational and clinical research, disseminating cutting-edge developments in EV research. ISEV2021 brings together scientists from academia, clinics, and industry, who have a common goal of better understanding EVs and applying this knowledge for societal and economic benefit.

The results outlined in the poster clearly demonstrate that EXO-NET® is a novel approach for the rapid, pure and high yield capture of exosomes from complex samples, including body fluids such as plasma, urine and saliva. BARD1 researchers showed that EXO-NET® provides superior exosome-specific nucleic acid and protein yield and purity compared to market leading products and methods. Importantly, EXO-NET® captures exosomes in just 15 minutes, is easy to use and compatible with small volume, high-throughput sample analysis, allowing ready integration of EXO-NET® into multiple downstream research applications.

BARD1 Chief Scientific Officer, Dr Peter French, said: "Being able to showcase the superior performance, flexibility and ease of use of EXO-NET® on the global stage at ISEV2021 provides the Company with a great opportunity to embed EXO-NET® into a range of research projects that are isolating and characterising exosomes for potential diagnostic and therapeutic applications."

BARD1 CEO, Dr Leearne Hinch, said: "It is exciting to release the results of our exosome research demonstrating that our next-generation EXO-NET® product out-performed competitor products capturing exosomes rapidly, with high purity and yield from complex biofluids. EXO-NET® has the potential to become the exosome isolation product of choice for researchers globally."

A copy of the EXO-NET® poster accompanies this release.

Authorised by the Company Secretary, Tony Di Pietro.

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ABOUT BARD1 LIFE SCIENCES LTD

BARD1 Life Sciences Ltd (ASX:BD1) is a leading Australian diagnostics company with an innovative portfolio of diagnostic technologies and products. The Company is focused on developing and commercialising best-in-class diagnostic solutions based on its BARD1, SubB2M, and Molecular NETs platforms for healthcare professionals and patients. The cancer diagnostics portfolio includes the commercialised hTERT test used as an adjunct to urine cytology and development-stage tests for ovarian, breast, prostate and pancreatic cancers. The Company is also commercialising its Molecular NETs platform for sample preparation and has launched its first proprietary EXO-NET[®] exosome capture tool for use in research for exosome-based diagnostics and therapeutics. For more information on BARD1 and EXO-NET, visit www.bard1.com and www.exo-net.com.

FORWARD LOOKING STATEMENTS

This announcement contains certain 'forward-looking statements' within the meaning of the securities laws of applicable jurisdictions. Forward-looking statements can generally be identified by the use of forward-looking words such as 'may,' 'should,' 'expect,' 'anticipate,' 'estimate,' 'scheduled' or 'continue' or the negative version of them or comparable terminology. Any forecasts or other forward-looking statements contained in this announcement are subject to known and unknown risks and uncertainties and may involve significant elements of subjective judgment and assumptions as to future events which may or may not be correct. There are usually differences between forecast and actual results because events and actual circumstances frequently do not occur as forecast and these differences may be material. The Company does not give any representation, assurance or guarantee that the occurrence of the events expressed or implied in any forward-looking statements in this announcement will actually occur and you are cautioned not to place undue reliance on forward-looking statements

BACKGROUND

The EXO-NET® technology represents an innovation in exosome capture for researchers and routine pathology laboratories. It consists of a covalently linked, multilayered three-dimensional matrix comprising several exosome-specific antibodies and spacer and linker molecules that interact to confer a characteristic topology to maximize specific binding and capture of exosomes from complex biofluids, in a reproducible manner.

The EXO-NET® matrix is coated onto magnetic beads for rapid and highly specific exosome isolation from any liquid biopsy sample. EXO-NET® has demonstrated compatibility with multiple downstream chemistries for analysis of lipid, protein and nucleic acids. The technology is highly scalable and is compatible with existing automated testing systems.

MATERIALS

SAMPLES

- Purified breast cancer cell line MCF-7 exosomes (SBI, Erivan Bio)
- Exosome-free FBS (SBI)
- Healthy and pancreatic cancer human plasma (BioIVT)
- Artificial human plasma (SeraCare)

ANTIBODIES

- Anti-CD9 antibody (exosome marker)
- Anti-CD63 antibody (exosome marker)
- Anti-calnexin antibody (Golgi marker)
- Anti-GPC1 antibody (Minomic Intl. Ltd.)

COMMERCIAL KITS

- miR21 RT-qPCR reagents (Thermo)
- RNA Isolation kits (Qiagen, Promega)
- ExoIP kit (Diagenode)
- Streptavidin-gold 700nm nanorods (Nanopartz)

METHODS & RESULTS

FIGURE 1. EXO-NET® BEAD STRUCTURE AND FUNCTION

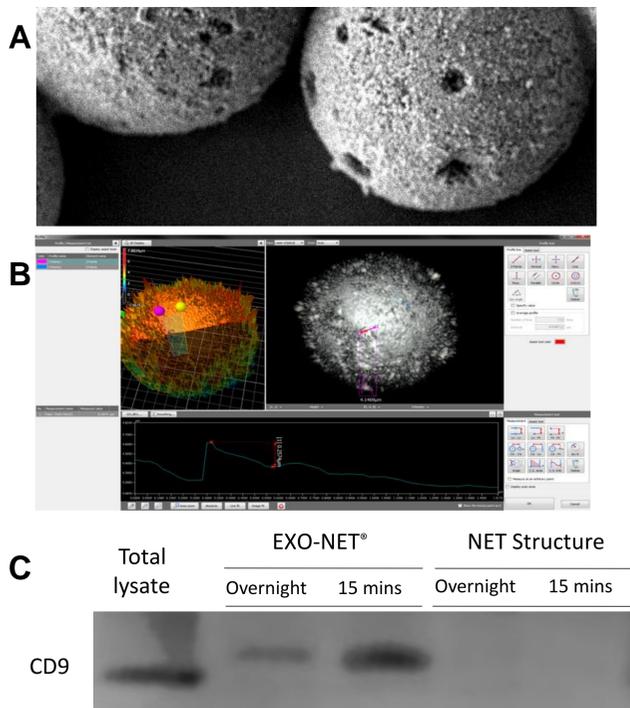


Figure 1. (A) Scanning electron micrograph showing ultra-structure of the EXO-NET™ matrix, including pores, coated on 2.5 um magnetic particles.

(B) 3D digital microscopic image (Keyence) of MCF-7-derived exosomes ranging in size from 40-250 nm bound to the surface of an EXO-NET® matrix-coated magnetic bead. Yellow and pink pseudo-colored spheres are bound exosomes, and their spatial separation and thickness were measured using Keyence software.

(C) Western blot showing the contribution of affinity vs. porosity in binding and recovering CD9 protein from human exosomes captured by EXO-NET® or by a NET matrix ("NET Structure") consisting of non-exosome specific antibodies but with similar pores. A 15 min incubation time is optimal for maximal exosome protein recovery. Total MCF-7 exosome lysate ("Total lysate") was used as the positive control.

Figure 2. (A) Western blot of proteins extracted from control ("blank") beads or EXO-NET®-coated beads incubated with human or artificial plasma samples for 30 minutes.

(B) Particle size analysis (Nanoparticle Tracking Analysis (NanoSight)) of exosome-free FBS spiked with MCF-7 exosomes (~9E¹⁰ - "Input"), exosome-free FBS ("Matrix") and the Input sample after incubation with EXO-NET® beads for 30 mins ("Depleted"). The data shows almost complete depletion of the spiked exosomes by EXO-NET®.

Figure 3. (A) Supernatants containing MCF-7 exosomes were treated using the Qiagen ExoRNeasy exosome RNA isolation kit or incubated with an increasing number of EXO-NET® beads for 30 mins. Qiagen samples were eluted and PBS-washed EXO-NET® beads were re-suspended in the same volume prior to analysis by miR21 RT-qPCR. The average concentrations of miR21 recovered from MCF-7 exosomes using the different methods are shown.

(B) Western blot of samples recovered from EXO-NET® or an alternative (commercial) bead-based kit (ExoIP). Pooled human plasma (0.2mL) was incubated with EXO-NET® beads for 15 min at RT or with ExoIP for 24h at 4°C, per manufacturer's instructions.

Figure 4. (A) Exosomes were recovered from 0.2mL pancreatic cancer and healthy human plasma samples (K2EDTA) using EXO-NET® beads after a 15 minute incubation. Total RNA was extracted directly from exosome-bound EXO-NET™ using the Promega kit. RT and the miR21 qPCR assay was performed using a thermocycler (Applied Biosystems). Depicted is a heat map of miR21 Ct values obtained (GraphPad Software).

(B) EXO-NET® beads were incubated for 15 min with 0.020mL healthy or pancreatic cancer plasma samples, washed and probed for 15 min using anti-glypican-1 (GPC-1) biotinylated monoclonal antibody (Minomic). Detection antibody-exosome-EXO-NET® beads (EXO-NET® "immune complex") were then washed and incubated with 2uL of a 1:500 dilution of streptavidin-gold nanorods for 5 min prior to washing and spotting 1uL on microscope slides. Spots were dried and were visualized by 3D digital microscopy (Keyence). Shown are 2 representative results obtained from healthy and pancreatic samples.

CONCLUSIONS

1. EXO-NET® is a novel approach for rapid, pure and high yield capture of exosomes from complex samples.
2. Recovered exosome content is not contaminated with Golgi (Fig 2A), extraneous serum proteins, or free lipids (data not shown).
3. EXO-NET® provides superior exosome-specific nucleic acid and protein marker results compared to market leading products and methods.
4. EXO-NET® is compatible with small volume, high-throughput sample analysis using multi-omics, imaging or targeted approaches (MS/MS data not shown).
5. EXO-NET® is a cost-effective tool for rapid isolation of highly pure exosomes for downstream analysis.

FIGURE 2. PLASMA AND CELL CULTURE-DERIVED EXOSOMES

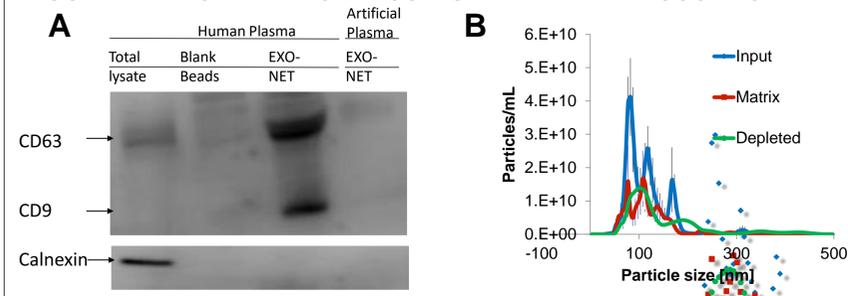


FIGURE 3. SUPERIOR YIELD vs COMPETITORS

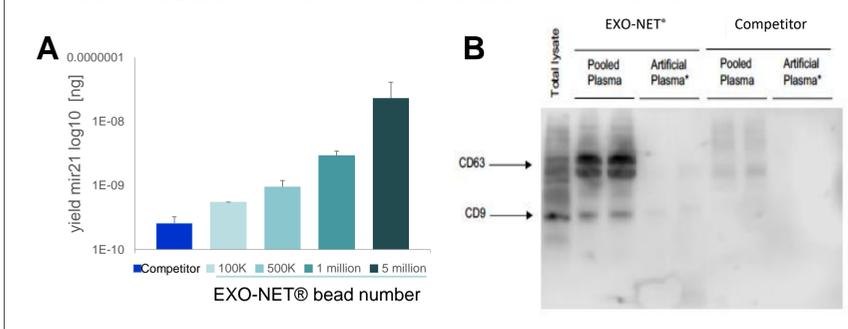


FIGURE 4. COMPATIBLE WITH DOWNSTREAM USES

