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## **Blood Card and Vesicle-based Medical Tests**

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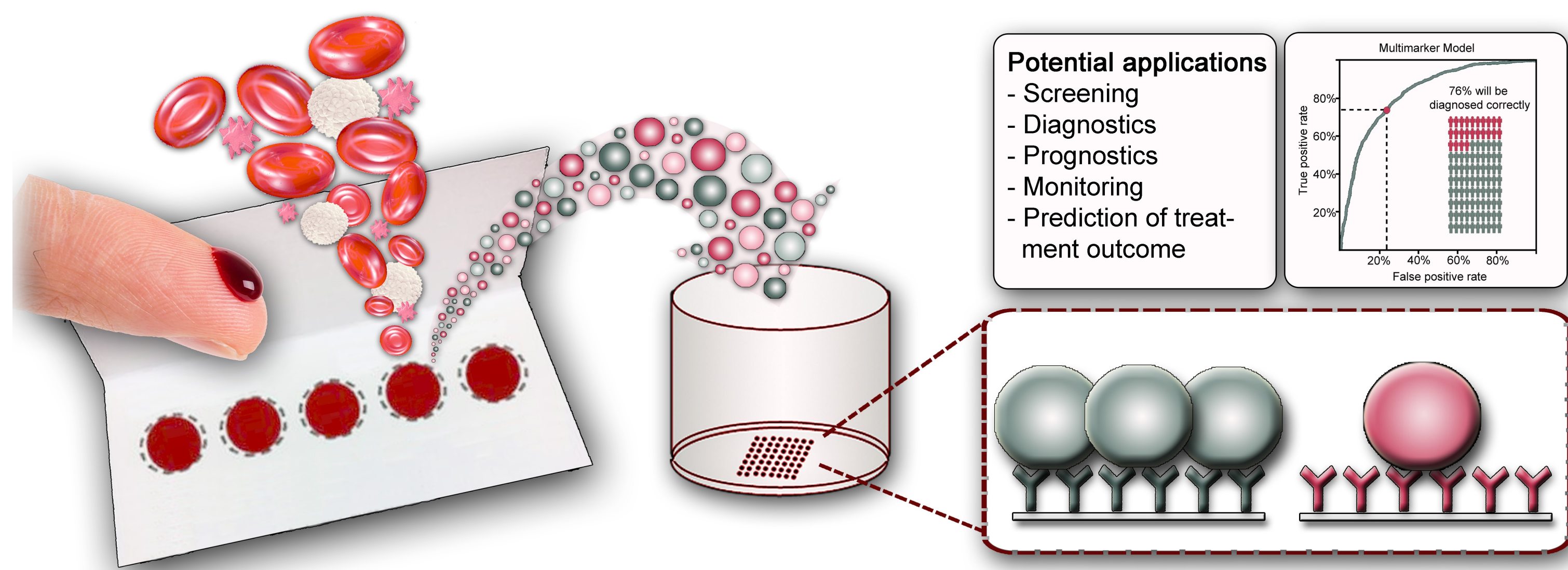
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# Blood Card and Vesicle-based Medical Tests

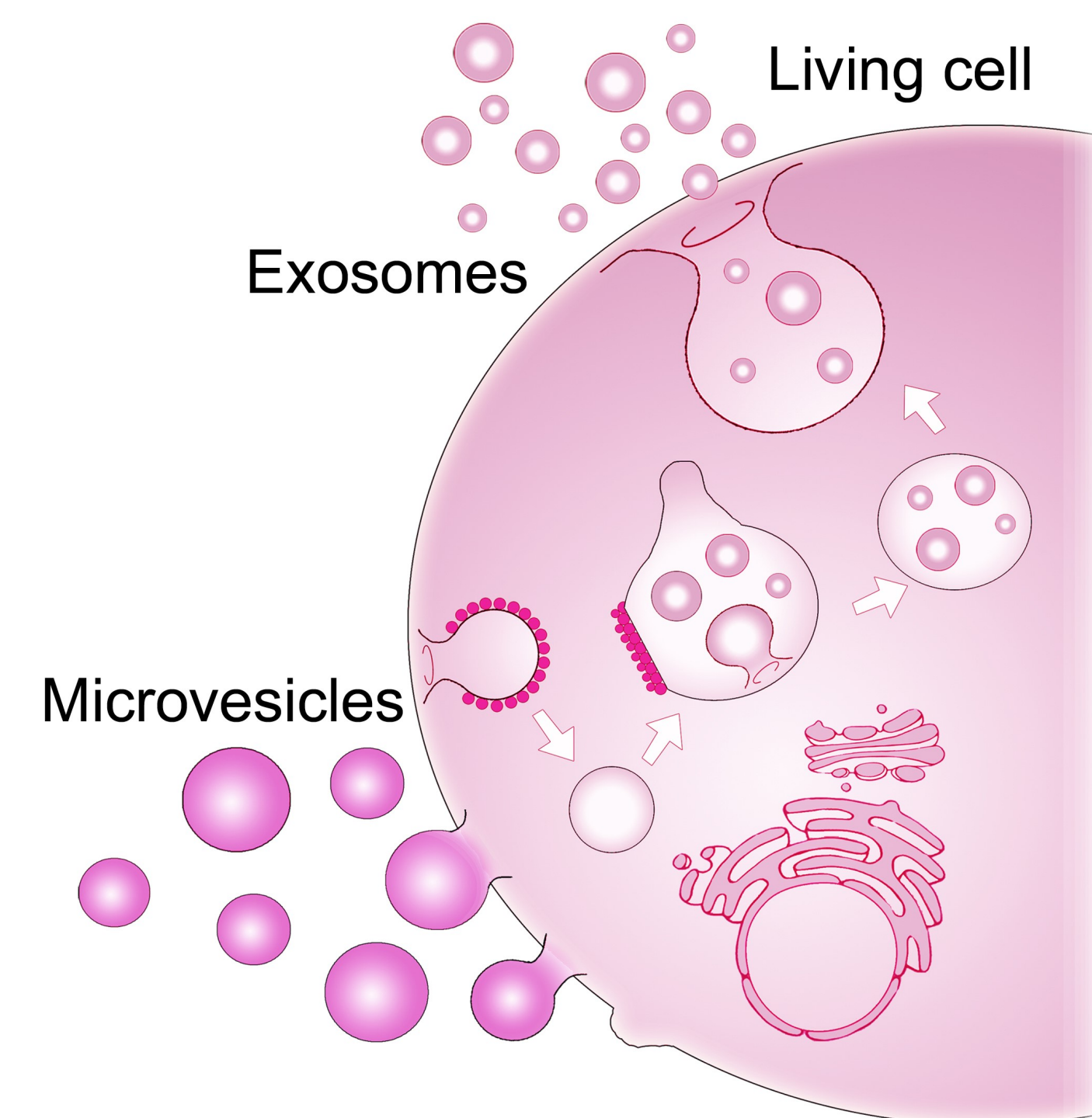


Venous blood is a **convenient source** of circulating extracellular vesicles (EVs). However, blood sampling requires authorized personnel and immediate purification of the vesicles. The present study demonstrate that intact EVs can in fact **be obtained from dried blood card samples** (or dried blood spots; DBS), which can be prepared by **unauthorized personnel**, or even at home by the user and shipped by regular mail. Intact EVs can be detected in extracts from dried blood spot samples even after prolonged storage. Being able to isolate and characterize small EVs from DBS **opens up a number of new opportunities** such as monitoring of disease, e.g. progression of cancer and response to treatment, only with involvement of a lab technician and responsible clinician.

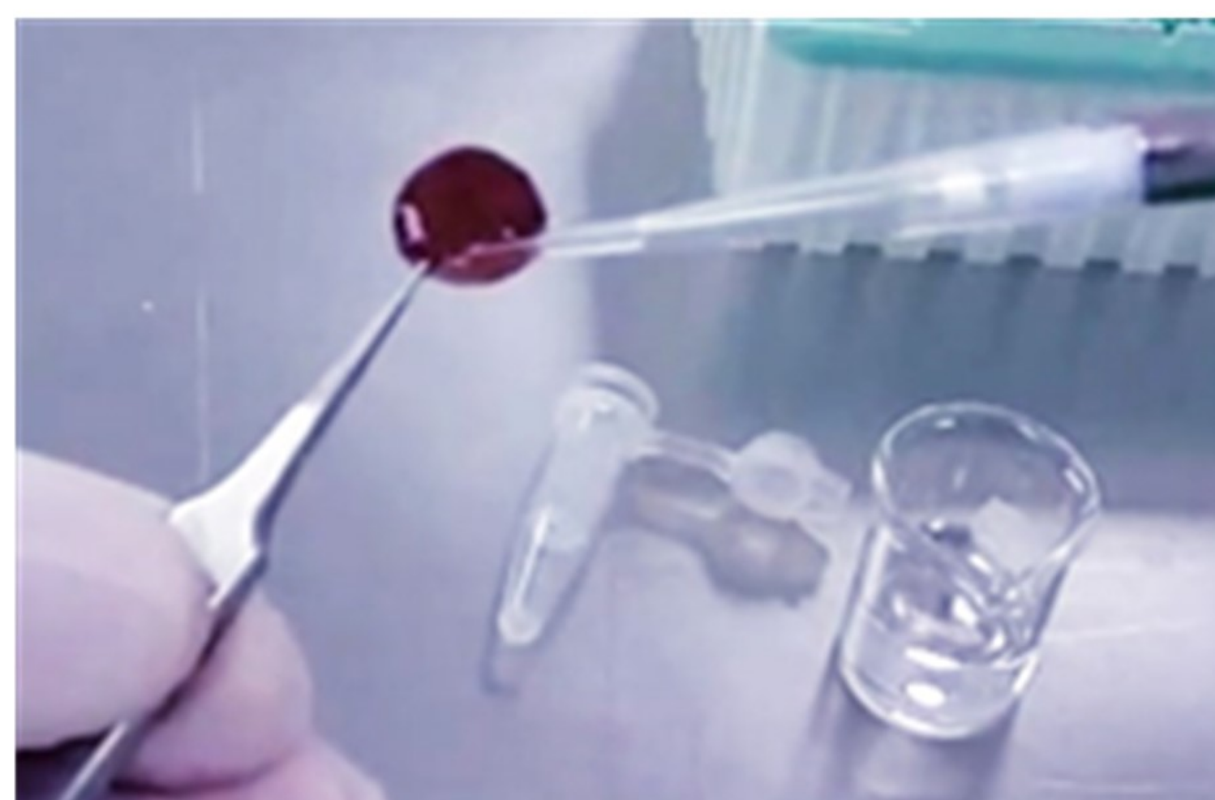
## Extracellular Vesicles

All living cells produce extracellular vesicles (EVs), which are **nano-sized compartments**. They are considered as a pivotal part of the intercellular environment and act as important players in cell-to-cell communication. The fact, that EVs are involved in the development and progression of several diseases, has formed the basis for the use of EV analyses in a **clinical setting** and envisions a great potential for using EVs as **disease-related biomarkers**.

EVs are a heterogeneous population of membrane -enclosed vesicles that can be divided into a number of subpopulations based on specific characteristics such as size, biogenesis, cellular origin, protein composition, and biological function. The two major subtypes of EVs are exosomes and microvesicles.



## General Protocol



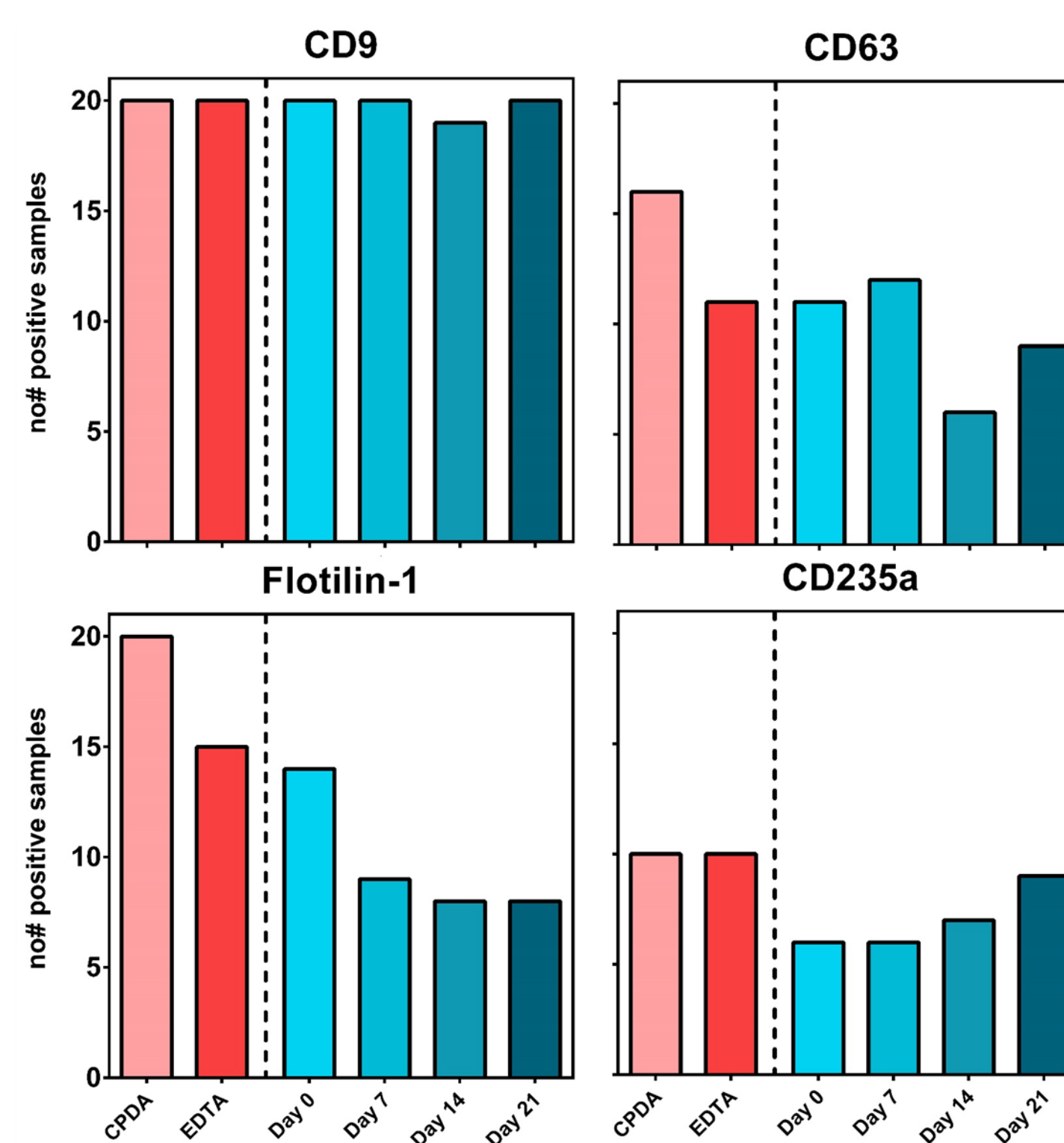
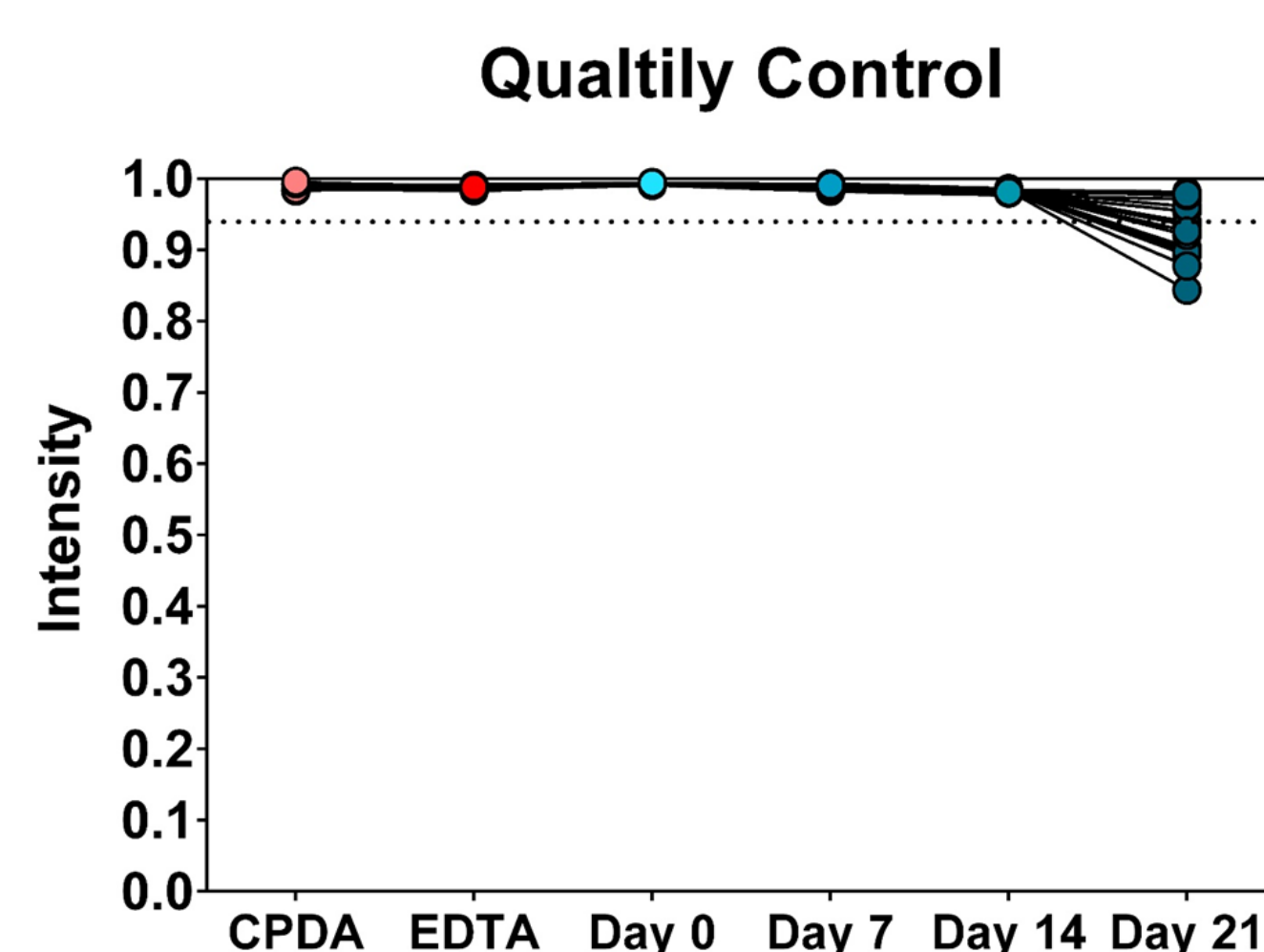
- Excision of disc containing sample
- Wetting of disc with **elution buffer**
- Placing of disc in spin column
- Incubation: 1 hour, RT
- Centrifugation: 20.000 x g for 5 min into **analysis buffer**
- EV Array analysis

Blood samples for the dried blood cards was obtained from the fingertips of the donors using a lancet. The blood drops thoroughly saturated the paper (blood card especially designed for whole cells) and was allowed to air dry before storage and isolation of small EVs according to the protocol below. To evaluate the EV concentration and composition, the samples were analyzed using the EV Array (Jørgensen et al., 2013, JEV) using antibodies against 15 selected surface-markers. This proof-of-concept study persisted of an experiment with blood from 20 healthy volunteers. The blood samples were used to test the effect of prolonged storage of the dried blood cards. The most optimal extraction procedure was used to compare the EV contents after 1 hour, 7, 14 and 21 days after collection.



**More Information**  
Detailed information about the technology can be found at:  
[WWW.EVARRAY.DK](http://WWW.EVARRAY.DK)

## Storage Testing



The qualitative tests revealed that, for most of the markers (11 out of 15), the samples from DBS showed similar results as for blood drawn using EDTA or CPDA collection tubes. **After 21 days of storage at room temperature**, a higher degree of hemolysis were observed in the extracted samples (see top graph for Quality Control). The increase in free hemoglobin generated a higher background signal, but the samples were still acceptable for analysis with the EV Array.

The analysis of small EVs carrying CD9 showed that all 20 persons were tested positive both with CPDA, EDTA and DBS. Furthermore, it was still possible to get a positive signal after 21 days of storage at room temperature. For CD63, the responses from DBS were equal to EDTA plasma although a decrease were seen after 2 weeks of storage.

A refinement of the extraction procedure will be performed to obtain the most optimal condition for a future qualitative analysis of small EVs sampled as DBS.

## Value proposition / USP

The method is based on a combination of the dry blood sample (DBS) on a blood card and extracellular vesicle (EV) array. DBS is a minimally invasive and widespread method for collecting blood samples, where samples are transported, stored and processed easily. Combined with the EV Array's ability to detect extracellular vesicles from unpurified blood makes this combination promising with regard to find biomarkers for cancer, inflammatory, metabolic, cardiovascular or neurodegenerative diseases. Another application is diagnosing, screening and monitoring of drug resistance, making drug trials cheaper, as less blood is needed.

## Business Opportunity / Objective / Commercial Perspectives

DBS combined with the EV Array, utilizes a proven, cost-effective tool with a strong diagnostic tool making handling and processing of blood samples promising in developed and developing countries alike. The DBS-EV Array combination would reduce the cost of acquiring blood samples, and opens the possibility for development of home test kits.

## Technology Description / Technology Summary

The proposed method is a combination of a known method for collecting blood samples used for more than 50 years, where samples are easily transported and stored as significantly less blood is required, with the EV Array, a new method for characterization of extracellular vesicles directly from plasma without purification requiring only 10  $\mu$ L of sample. The EV Array is a protein-based microarray enabling classification of multiple surface markers simultaneously.

## Development Phase / Current state

A test setup with 20 subjects has proven that it is possible to measure vesicles from DBS, as a proof of concept. Several parameters can be optimized such as time, temperature, buffer and quantities. A series of optimization experiments must be performed with more donors before commercialization; this is estimated to take 2-3 months without extensive costs.

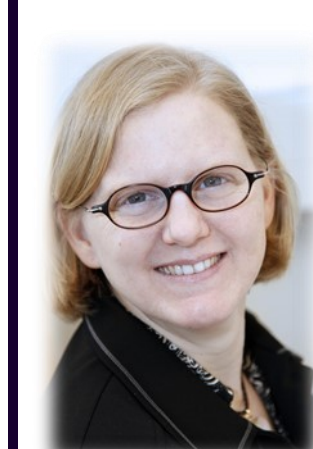
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