Extraction and analysis of intact EVs collected from dried blood spots

Jørgensen, Malene Møller; Bæk, Rikke ; Varming, Kim

Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

Extraction and analysis of intact EVs collected from dried blood spots

Malene Møller Jørgensen, Rikke Bæk & Kim Varming

EVSearch.dk; Department of Clinical Immunology, Aalborg University Hospital

Contact information: maljoe@rn.dk

EV SEARCH

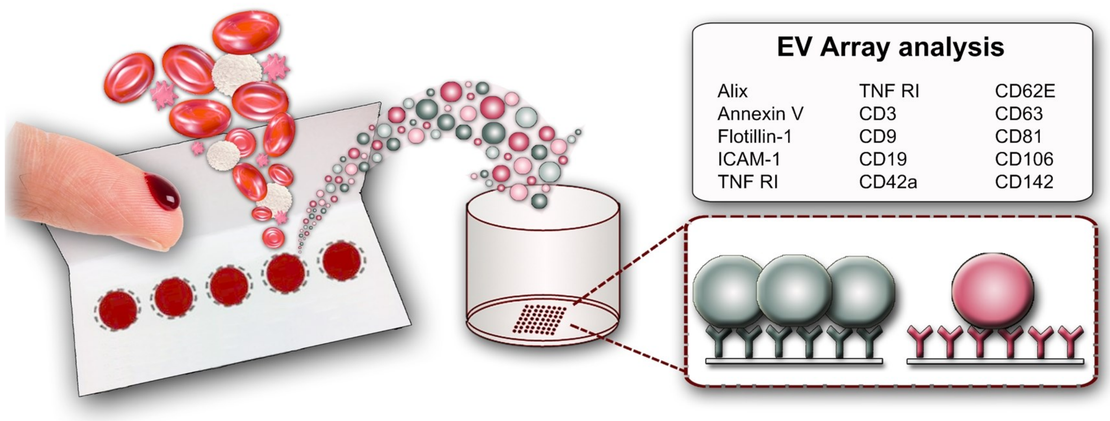
EXTRACELLULAR VESICLE RESEARCH CENTER DENMARK

WWW.EVSEARCH.DK

Introduction

Venous blood is a convenient source of circulating 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.



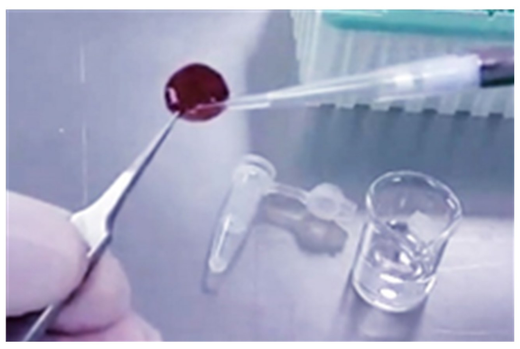
Methods

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 two series of experiments.

Test #1: Venous peripheral blood was drawn from three healthy donors (EDTA, CPDA, heparin and serum) and compared with blood applied on the dried blood cards. The extraction procedures were optimized and eight different buffer compositions were tested.

Test #2: Blood from 20 healthy volunteers were used to test the effect of prolonged storage of the dried blood cards. The most optimal extraction procedure found in the first experiment, buffer composition #8, was used to compare the EV contents after 1 hour, 7, 14 and 21 days after collection.

Protocol in general



- 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

Test #1 - Buffer composition

Comb.	Elution Buffer	Analysis Buffer
# 1	*Commercial no. 1*	*Commercial no. 2*
# 2	*Commercial no. 1*	*PBS*
# 3	*Commercial no. 1*	*PBS + 0.05% Tween®20*
# 4	*Commercial no. 1*	*PBS + 0.2% Tween®20*
# 5	*Commercial no. 2*	*Commercial no. 2*
# 6	*PBS*	*PBS*
# 7	*PBS + 0.05% Tween®20*	*PBS + 0.05% Tween®20*
# 8	*PBS + 0.2% Tween®20*	*PBS + 0.2% Tween®20*

Test #2 - Storage time

Of blood cards before analysis

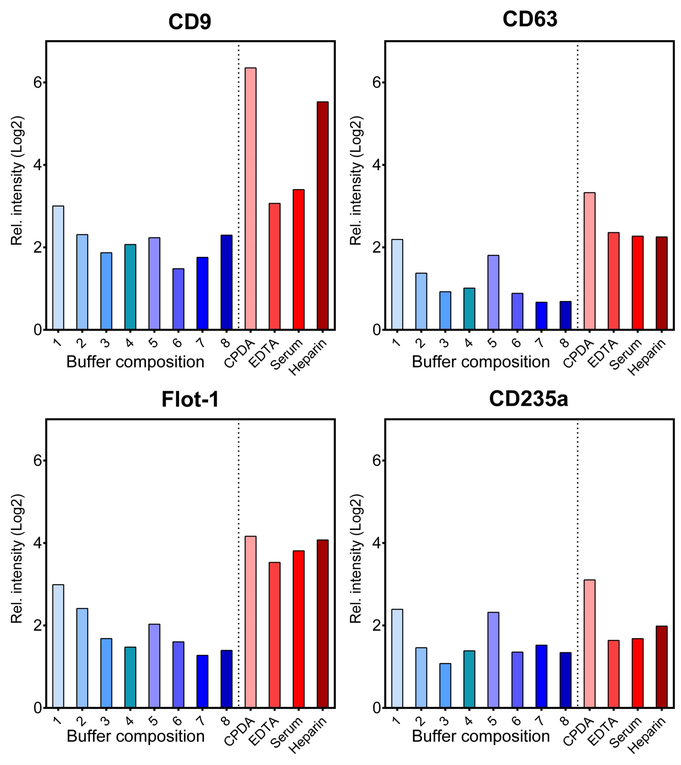
1 hour
7 days
14 days
21 days

→ Buffer composition #8

Results - Buffer Composition

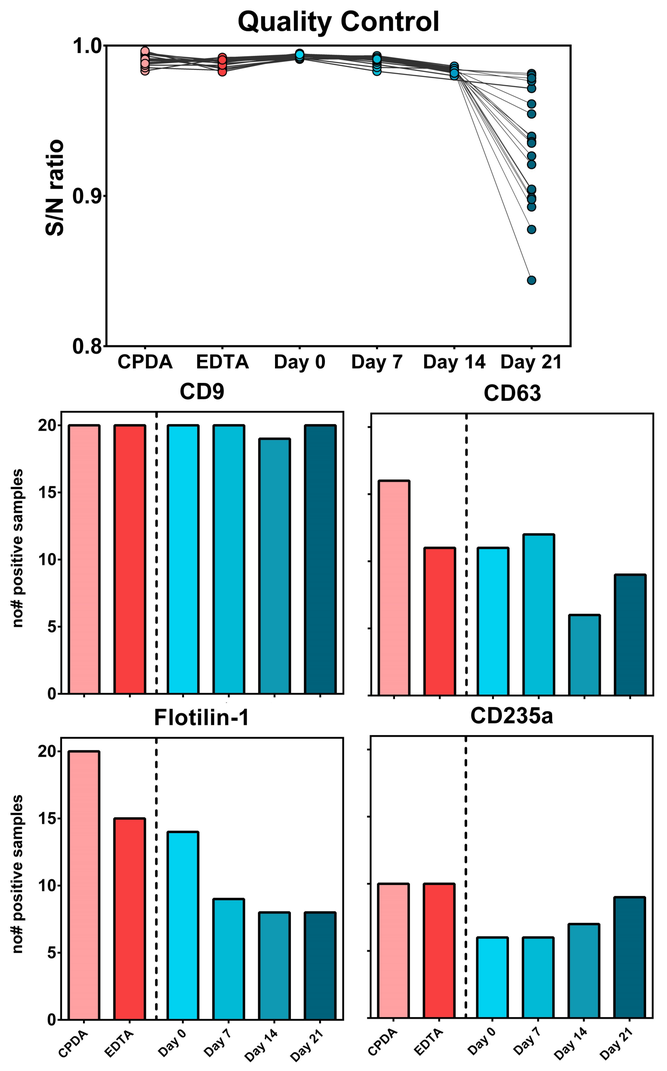
Elution of EVs from dried blood spots were found to be possible when a soaking procedure was used and followed by a short centrifugation. During the centrifugation, the EVs were collected in a specific sample buffer. The composition of the buffers were found to be very important for the outcome of the extraction which were compared to blood collected the traditional way in tubes.

It has to be kept in mind that the results obtained from samples collected in tubes and from samples collected from DBS cannot be compared directly since the amount of blood in each DBS varies greatly. However, the results from the different buffer compositions display the same tendencies (shown for one representative donor). Together with the measures for the signal-to-noise ratio it was chosen to carry on with buffer composition #8.



Results - Storage Time

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.

Intellectual property rights: Patent pending EP17206867.8, filed on Dec.13th, 2017

NORTH DENMARK REGION

AALBORG UNIVERSITY HOSPITAL