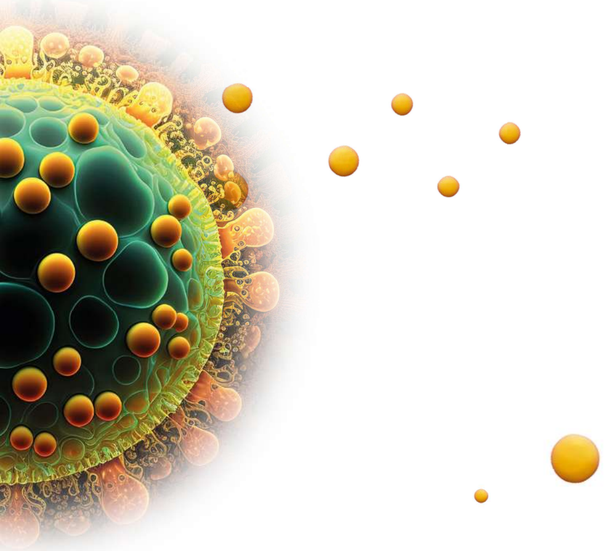
vesicles

WHY ARE THEY OF **INTEREST**?

The literature has clearly established the role of microvesicles in **activating coagulation**. **Overproduction of MVs** has been linked with various physiological and pathophysiological conditions.



HOW TO **ANALYSE** THEM?

Microvesicles can be analyzed using **two complementary methods:**

- **Quantitative tests:** Flow cytometry (FMC) is used to count microvesicles and determine their cellular origin.
- **Functional tests:** Various methods can be used to assess procoagulant properties resulting from the action of procoagulant phospholipids and/or tissue factor.

Preanalytical recommendations

Step	Recommended	Acceptable	Not recommended
Sampling	- 19-21 g needle - Tourniquet only to locate the vein (<1 min) - Discard the first mL - Collection at the same period	- Butterfly needle with short tube - Tourniquet >1 min - No discard of the first mL	- Needle <18 g or >21 g
Anticoagulant	Citrate	CTAD	EDTA, heparin, hirudin
Type of citrate	105-109 mmol/l trisodium citrate dihydrate ($Na_3C_6H_5O_7, 2H_2O$), buffered or unbuffered	129 mmol/L	Pentahydrate form (less stable)
Transport	- Without agitation or in special transport boxes to prevent the formation of microvesicles - Room temperature (20-25°C)	- Gentle agitation - Other temperatures	- Strong agitation - Ice
Delay before analysis or first centrifugation	1 h	2 h	>2 h
Centrifugation (if indicated)	- Double centrifugation - 2 x 15 min at 2500 g - Light brake possible - Room temperature (20-25 °C) - Centrifuge check required	- Other strategies for double centrifugation if platelet count is controlled - Brake - Other temperatures	- Single centrifugation - Ice
Storage (if indicated)	- Rapid freeze in liquid nitrogen before storage at -80 °C - Stable for up to 1 year - All samples should be stored for similar length of time - Controls treated in same conditions as patients	- Direct freeze at -80 °C - Samples stored for different length of time but < 1 year	>1 year

Proposed guidance on the preanalytical steps for MV characterization - Adapted from Mullier et al. (2013)

Diagnostica Stago
Always read the label and instructions for use – follow the instructions for use

BioCytex
Always read the label and instructions for use – follow the instructions for use

Stago
Diagnostics is in our blood.

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Microvesicles Line

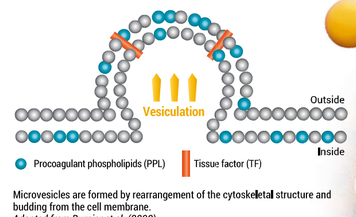
EXPAND THE LIMITS **OF MICROVESICLE ANALYSIS**

Stago

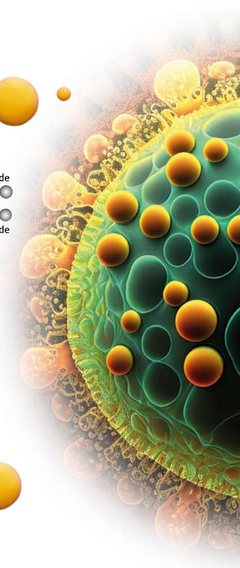
Micro

WHAT IS A **MICROVESICLE?**

A microvesicle (MV) is a **vesicle originating via a cell budding mechanism**, has no nucleus and contains a membrane skeleton. It measures between **0.1 and 1 µm**. Microvesicles have a **procoagulant activity** due to the presence of negative procoagulant phospholipids – mainly phosphatidylserine – and/or tissue factor at their surface. They reflect blood cell **activation** and **apoptosis processes**.



Microvesicles are formed by rearrangement of the cytoskeletal structure and budding from the cell membrane.
Adapted from Burnier et al. (2009)



WHERE DO THEY COME FROM?

Circulating microvesicles are derived mainly from **platelets**, but also from **red blood cells, white blood cells** and **endothelial cells**. Microvesicles express antigens from their parent cells on their surface, allowing for determination of cell origin by the use of specific antibodies.

Quantitative tests

Because microvesicles (MVs) are so **small**, their analysis pushes flow cytometry (FMC) to its optical **sensitivity limits**. Megamix calibration beads can be used to:

- Ensure the accurate **intrinsic performance** of the cytometer for MV analysis
- Ensure setting stability over time with **daily quality control**
- Set-up a **standardized MV measurement gate**
- **Standardization** between different FCM manufacturers and models

MP-Count Beads (Ref. 01169): a suspension of beads designed for the **absolute count of microvesicles**

- Allow an **optimized and precise** MVs count
- Compatible with **all cytometers**
- **Ready to use**

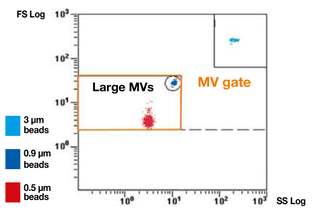
Megamix Range: a calibration tool optimized for each type of cytometer*

FSC optimized cytometer

Standard cytometer (e.g. Life Science)

Megamix** (Ref. 00420)

- Mixture of fluorescent beads **0,5 / 0,9 / 3 µm**
- Allows for setting up a **0.5 to 1.0 µm-MV equivalent standard gate**

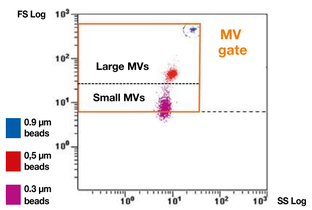


High-sensitivity cytometer (e.g. Gallios or Navios)

FSC and SSC optimized cytometer (e.g. DxFLEX or CytoFLEX)

Megamix-Plus FSC** (Ref. 01077)

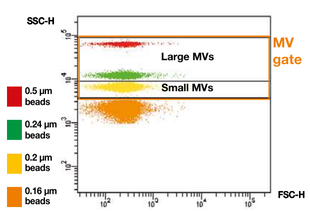
- Mixture of fluorescent beads **0,1 / 0,3 / 0,5 / 0,9 µm**
- Allows for setting up a **0.3 to 1.0 µm-MV equivalent standard gate**



SSC optimized cytometer (e.g. Becton Dickinson range)

Megamix-Plus SSC** (Ref. 01078)

- Mixture of fluorescent beads **0,16 / 0,2 / 0,24 / 0,5 µm**
- Allows for setting up a **0.3 to 1.0 µm-MV equivalent standard gate**



* For further details, please consult your local Stago contact ** Products manufactured by BioCytex (a Stago Group company)

Functional tests

Microvesicles (MVs) have **procoagulant properties** due to the **procoagulant phospholipids (PPL)** and/or **tissue factor (TF)** found at their surface. These activities can be measured using functional tests.

Determination of the procoagulant activity of microvesicles: a reagent for every situation

STA line systems

Microplate

Procoagulant Phospholipids (PPL) activity

STA-Procoag-PPL (Ref. 00429)

- Determination of the **PPL-dependent procoagulant activity** of mainly platelet MVs
- Fully **automated**
- **Fast results** (under 4 minutes)
- **Simple, reproducible** assay
- Two levels of **control**

Tissue factor (TF) activity

Cy-Quant MV/TF activity** (Ref. 01514)

- **Determination of the TF-dependent procoagulant activity of MVs**
- **All-in one** and **standardized** assay
- **Improved specificity** Anti-TF antibody used
- **Optimized sensitivity** low limit of detection (4fM)
- In line with **international recommendations**

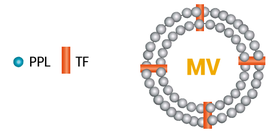
Measurement of thrombin generation induced by microvesicles

CAT system (Calibrated Automated Thrombogram)

Procoagulant Phospholipids (PPL) activity

PRP-Reagent (Ref. 86196)

- Measurement of thrombin generation induced **solely by PPL-bearing MVs**
- Contains a small amount of TF, with no added PPL



Tissue factor (TF) activity

MP-Reagent (Ref. 86222)

- Measurement of thrombin generation induced **solely by TF-bearing MVs**
- Contains PPL, with no added TF

All products mentioned in this document are for research use only - Not to be used in diagnostic procedures