

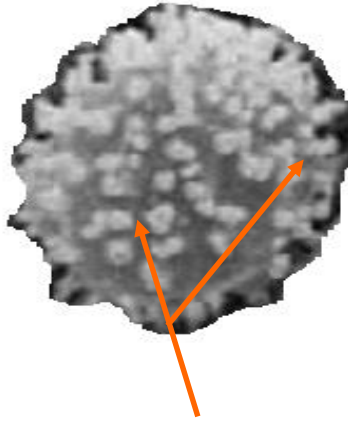
# Microparticles

A microscopic image of cells, likely from a blood smear, showing various cell types including a large, pale, spherical cell in the foreground and several smaller, more irregularly shaped cells in the background. The entire image is overlaid with a semi-transparent blue filter. A white, rounded rectangular bar is positioned in the upper left quadrant, containing the title text.

# Microparticles

## Origin

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MPs formed at surface of activated cell  
From 0.1 to 1  $\mu\text{m}$  diameter  
Heterogeneous in size - Small & Large MPs

1. Cellular events
  - Inflammation, thrombosis, cell activation, and apoptosis
2. Membrane lipid leaflet exchange
  - Negative phospholipids (PhosphatidylSerin PS) flip from inner to outer leaflet of cell membrane
3. Small vesicles are budded from the cell membrane
  - Microparticles release

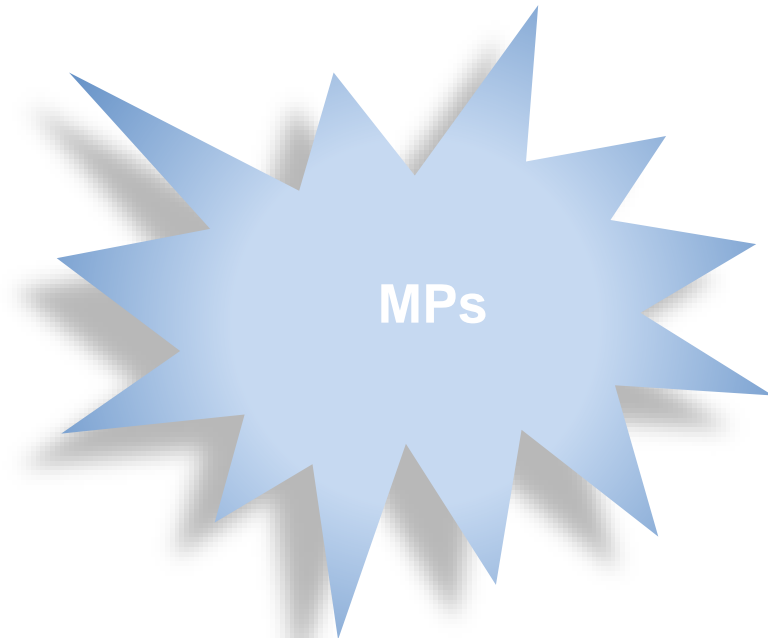
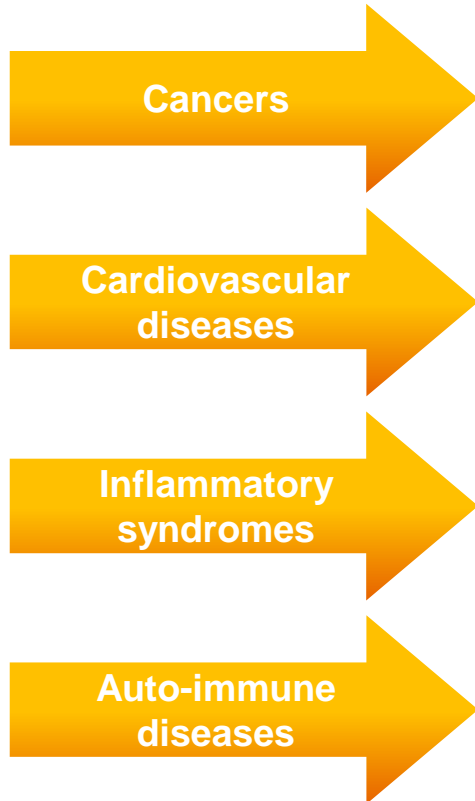
- Increase in MPs reflects **thrombotic** and/or **inflammatory events**
- MP **surface antigens** derived from cells of origin
- Procoagulant activity of MP due to negative Procoagulant PhosphoLipids (PPL), mainly **PhosphatidylSerin (PS)**, and **+/- Tissue Factor (TF)**

# Microparticles

*SMALL vesicles with LARGE clinical relevance*

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Elevated MPs levels occur in **many pathologic situations:**

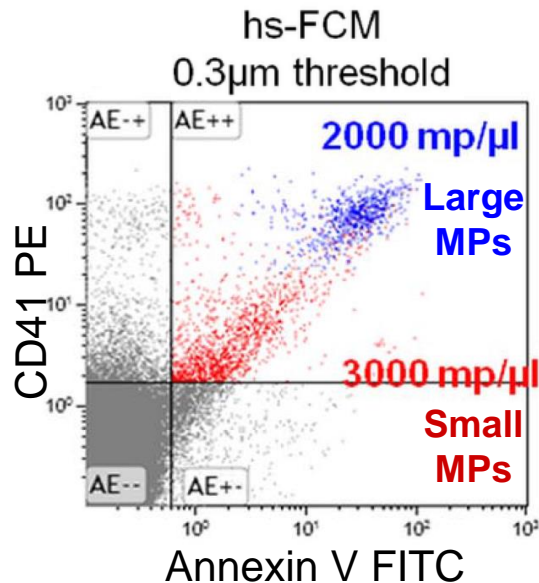


MPs quantification allows for:

- **Diagnosis** of pathologies
- **Monitor** & follow-up
- **Evaluate** drug efficacy

# Microparticles





*SMALL vesicles with LARGE clinical relevance*



\* $\mu$ m MP-equivalents

- Recent improvements in FCM provide access to **previously undetectable MP**
- Small/Large MP ratio calculation
  - ❖ MP gate from 0.3 to 0.5 $\mu$ m-eq\* **→ Small MPs**
  - ❖ MP gate from 0.5 to 1 $\mu$ m-eq\* **→ Large MPs**
- **Questions about small MPs :**
  - Same mechanism of formation than large MPs?
  - Same biological function?
  - Clinical relevance?

**Table. Small-to-Large MP Ratio According to Clinical Situation and MP Subset**

Small/Large MP Ratio	PMP	Ery-MP	Leu-MP	EMP
Healthy subjects	2.4 (1–3.4)	1.9 (1–4.2)	1.6 (1.2–3.4)	1.7 (1.3–3.9)
Coronary patients	6.5 (3.4–30) 	3.2 (2–9.4) 	7.3 (3.6–11) 	44 (6.3–111) 

Data expressed as median (25th–75th percentile). EMP indicates endothelial cell–derived microparticle; EryMP, erythrocyte-derived microparticle; LeuMP, leukocyte-derived microparticle; MP, microparticle; PMP, platelet-derived microparticle.

- Access to small-size MP resulted in a **8 to 20 fold increase** in the number of enumerated MP in **pathological samples**
- Small/Large MP ratio varies according to the **clinical status** and **MP subset**

#### Bibliography

ROBERT S. *et al.*, High-Sensitivity Flow Cytometry Provides Access to Standardized Measurement of Small-Size Microparticles. *Arterioscler Thromb Vasc Biol.* 2012

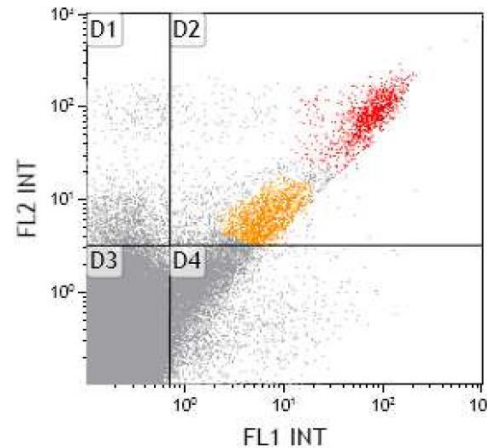
# Microparticles

## Analysis methods

### Quantitative Analysis

#### By Flow Cytometry

- Quantification
- Cell origin



### Qualitative Analysis

#### By Functional Assays

- Procoagulant potential through PPL/PS
- Procoagulant potential through TF

#### MP characteristics

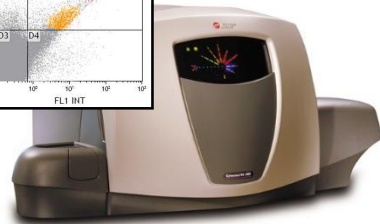
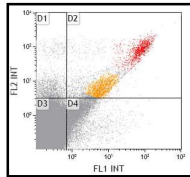
Size	0.1 to 1 $\mu$ m
Procoagulant Phospholipid exposure	PS + (annexin V)

#### MP origin Antigens/Antibodies

Platelet-MP	CD41
	CD42 a or b
	CD61
Erythrocyte-MP	CD235a
Leukocyte-MP	CD45
	CD11b
	CD14b
	CD66b
Endothelial-MP	CD144
	CD146-LeuMP
	CD105-LeuMP
	CD31-PMP
	CD62E

# Microparticles

*A complete Stago offering for MP analysis*



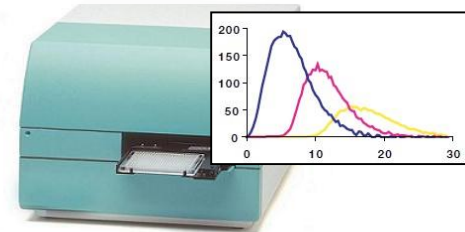
Megamix Line

Quantitative  
Assays



STA Procoag PPL

Functional  
Assays



Thrombin  
Generation (CAT)



# Quantitative Assays

## *Megamix Line - Calibration beads for MP analysis*

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- Because of their small size, MPs analysis push the Flow Cytometer (FCM) to its optical sensitivity limits
- Megamix calibration beads allow to:
  - ❖ Set up a standardised MP gate
  - ❖ Ensure stability of settings over time
  - ❖ Ensure standardisation between all brands & models of FCM
- These features are critical to the success of multicentric studies, and all other cases in which protocol transfers are needed

Megamix calibration beads are a **QC tool for MP analysis**, ensuring FCM protocol stability and reproducibility