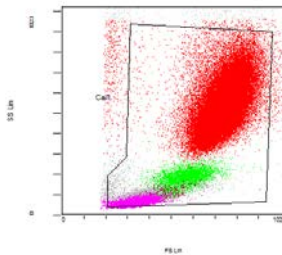# Gating procedure for white blood cells (WBC) on FC500

The French PNH diagnosis working group has developed an optimized gating strategy adapted to a two-tubes diagnostic test (PMN, MO), evaluated in a multicentric study. The combinations include CD45, CD15 and CD33 as gating markers and CD24, CD14 and FLAER as diagnostic markers

The gating procedure is composed of three parts:
- Firstly, the cell populations of interest among the leukocyte subtypes are gated based on their level of CD45 and CD15 expression for polymorphonuclear (PMN) and CD33 and CD45 for monocytes (MO).
- Secondly, the presence of a type III PNH clone is assessed using internal control cell populations.
- Finally, the precise size of the clone is evaluated.
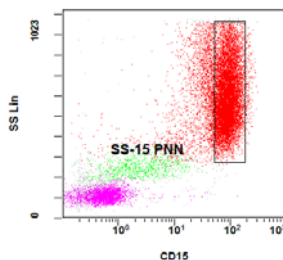
Tube 1 : FLAER/CD24/CD16/CD15/CD45

On FS/SS plot:

Adjust the **« Cell »** region to exclude dead cells, debris and doublets.
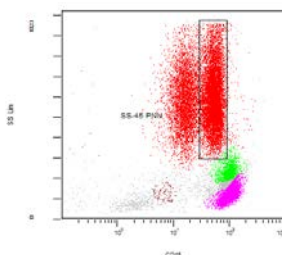
Identifying polymorphonuclear (PMN)
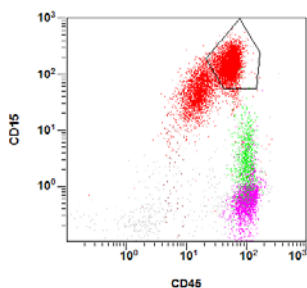
On CD15/SS plot: display "cell"

Adjust the « **SS-15 PMN** » region to include CD15 positive events, regardless of their level of SS intensity. Do not include events with low CD15/high SS intensity, so that immature granulocytes and eosinophils are excluded.
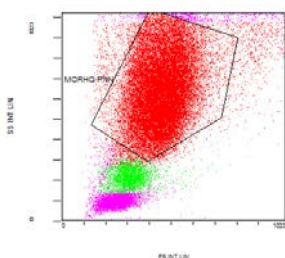
On CD45/SS plot : display "cell"

Adjust the « **SS-45 PMN** » region to include events with intermediate CD45 intensity. Do not include events with low SS intensity (blasts and basophils), events with intermediate CD45 intensity containing immature granulocytes, and events with high CD45 intensity and high SS intensity containing eosinophils.

On CD45/CD15 plot: display "cell"

Adjust the « **45-15 PMN** » region to include CD15 positive events and CD45 positive events. Do not include events with high CD45 intensity and low CD15 intensity containing eosinophils and events with low CD45 intensity and low CD15 intensity containing immature granulocytes.

On FS/SS plot: display the intersection of the 3 regions (SS-15, SS-45 and 45-15)

Adjust the MORPHO PMN region to exclude dead cells. Eosinophils are mostly excluded but not immature granulocytes.
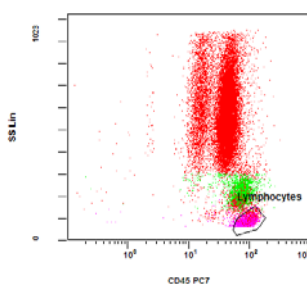

Identifying a PNH type III population using control cell populations

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The aim of this step is to assess either the presence of PNH type III cells or the absence of events with a full defect in GPI-linked proteins.
This approach consists in using internal control cell populations to define the threshold of positivity of the diagnostic markers.
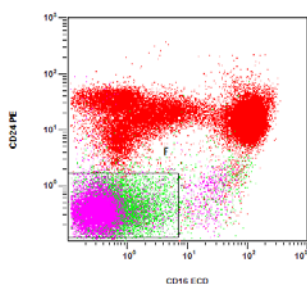The populations used are total lymphocytes, T-lymphocytes and NK-lymphocytes.

On CD45/SS plot : display "cell"

Adjust the « **lymphocytes** » region to include events with high CD45 intensity and low SS intensity.

On CD16/CD24 plot:

Identify **T-lymphocytes** on events with CD24 negative and CD16-negative.
Identify **NK-lymphocytes** on events with CD24 negative and CD16 positive.

Next, the PNH area is set according to the expression of the diagnostic markers on the control populations.

On FLAER/CD24 plot: determination of FLAER-CD24-region

The threshold of FLAER is set at the lowest expression of lymphocytes (positive control limit). Similarly, the threshold of CD24 is set using non-B-cell lymphocyte populations (CD24 negative).

On FLAER/CD16 plot: determination of FLAER- CD16- region

We use the same threshold for FLAER. Determination of the threshold of CD16 is set according to the lymphocytes.
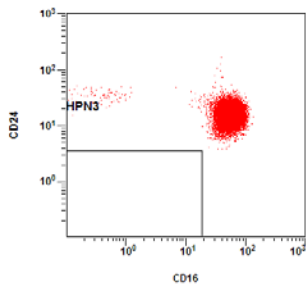
On CD24/CD16 plot : determination of CD24 - CD16- region

Determination of the size of the PNH clone
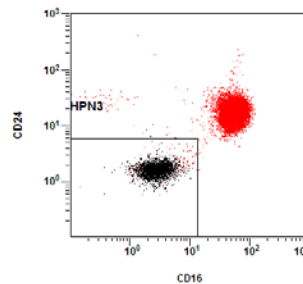

Optimization of the gating:

In this final step, the PNH area is optimized in order to include type II cells on the plots displaying PMN.
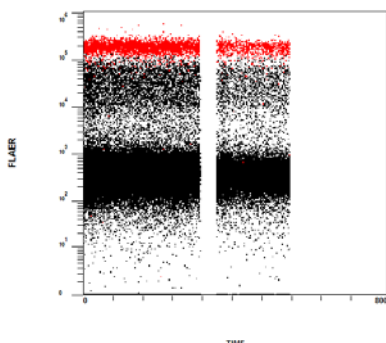
Normal: PNH:

In the presence of PMN with low expression of GPI-linked proteins (Type II cells), create a second region to determine the percentage of these cells.
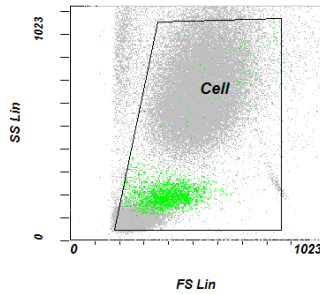
Finally, additional control such as time plot can be used to ensure a reliable result.

Check the distribution of PNH cells during data acquisition. It is valid only if the number of PNH cells detected follows the same variations as normal PMN.
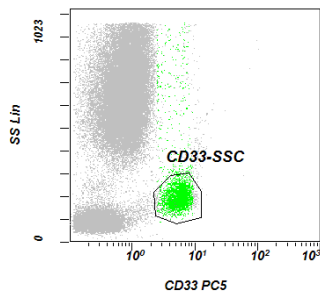
Tube 2 : FLAER/-/CD14/CD33/CD45

On FS/SS plot:

Adjust the **« Cell »** region to exclude dead cells, debris and doublets.
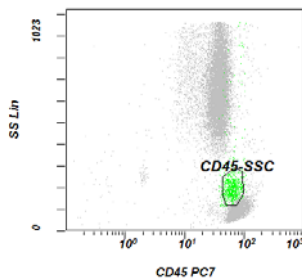
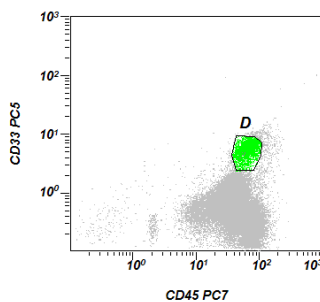## Identifying monocytes

On CD33/SS plot : display "cell"

Adjust the « **CD33-SS MO** » region to include events with bright CD33 intensity and low SS intensity. Do not include events with intermediate CD33 intensity and low SS intensity – this population contains basophils and dendritic cells.

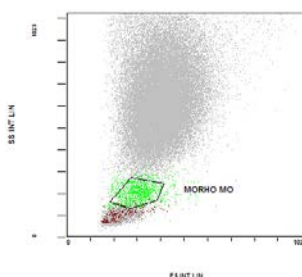**On CD45/SS plot :** display "cell"

Adjust the « **CD45-SS MO** » region to include CD45 positive events, without overlapping with the SS-45 PMN events.

On CD45/CD33 plot:

Adjust the « **33-45 MO** » region to include events with high CD33 intensity and intermediate CD45 intensity.

On FS/SS plot :

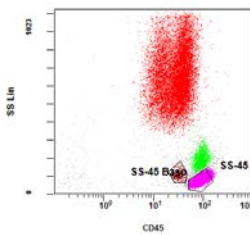Adjust the « **MORPHO MO »** region to exclude dead cells.

Identifying a PNH type III population using control cell populations

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The aim of this step is to assess either the presence of PNH type III cells or the absence of events with a full defect in GPI-linked proteins.

This approach consists in using internal control cell populations to define the threshold of positivity of the diagnostic markers.

The populations used are total lymphocytes and basophils.

On CD45/SS plot:

Adjust the « **SS-45 lympho »** region to include events with high CD45 intensity and low SS intensity (blue).
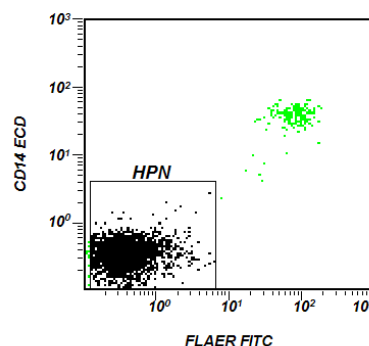
Adjust the « **SS-45 baso »** region to target basophils.

On CD33/SS plot:

Identify basophils on events with intermediate CD33 intensity and low SS intensity.

On FLAER/CD14 plot:

The threshold of FLAER is set at the lowest expression of lymphocytes and/or basophils (positive control limit). Determination of the threshold of CD14 is set according to the lymphocytes.
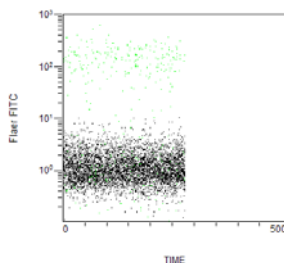
<div style="border: 1px solid black; text-align: center;">

Determination of the size of the PNH clone

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<u>Optimization of the gating:</u>

In this final step, the PNH area is optimized in order to include type II cells on the plots displaying monocytes.

In the presence of monocytes with low expression of GPI-linked proteins (Type II cells), create a second region to determine the percentage of these cells.

Finally, additional control such as time plot can be used to ensure a reliable result.

Check the distribution of PNH cells during data acquisition. It is valid only if the number of PNH cells detected follows the same variations as normal monocytes.