

Classical PNH

About this case study

Details:	Classical PNH
----------	---------------

Flow cytometry

Details of flow cytometry instrument	BD FACSCanto
--------------------------------------	--------------

Cell populations evaluated for PNH clones:	Granulocytes, monocytes, RBCs
--	-------------------------------

External control:	None
-------------------	------

Internal control:	None
-------------------	------

Antibodies/reagents and gating strategies used:	FLAER-Alexa 488, CD14 (61D3)-PE, CD15 (H198)-APC, CD24 (SN3)-PE, CD64 (10.1)-PE, CD45 (2D1)-PerCP-Cy5.5, CD59 (MEM43)-PE, CD235a (KC16)-FITC Practical Guidelines 2012
---	--

Clone sizes in all populations tested:	Granulocytes: 98.9% (Type II + III) Monocytes: 99.8% (Type II + III) RBCs: 29.7% (Type III), 1.5% (Type II)
--	--

LDH level:	58.5 μ kat/L (2.2–3.6)
------------	----------------------------

Ferritin level:	335 μ g/L (10–291)
-----------------	------------------------

Creatine level:	239 μ mol/L (44–104)
-----------------	--------------------------

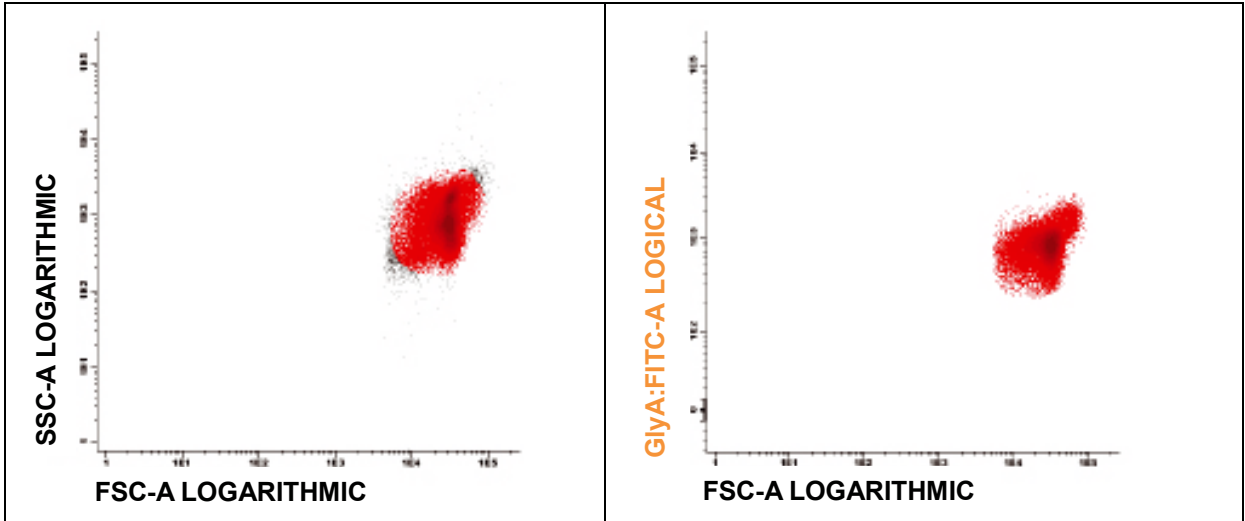
RBC count:	4.01×10^{12} /L (4.70–6.10)
------------	--------------------------------------

WBC and neutrophil counts:	4.3×10^9 /L (4.00–10.80)
----------------------------	-----------------------------------

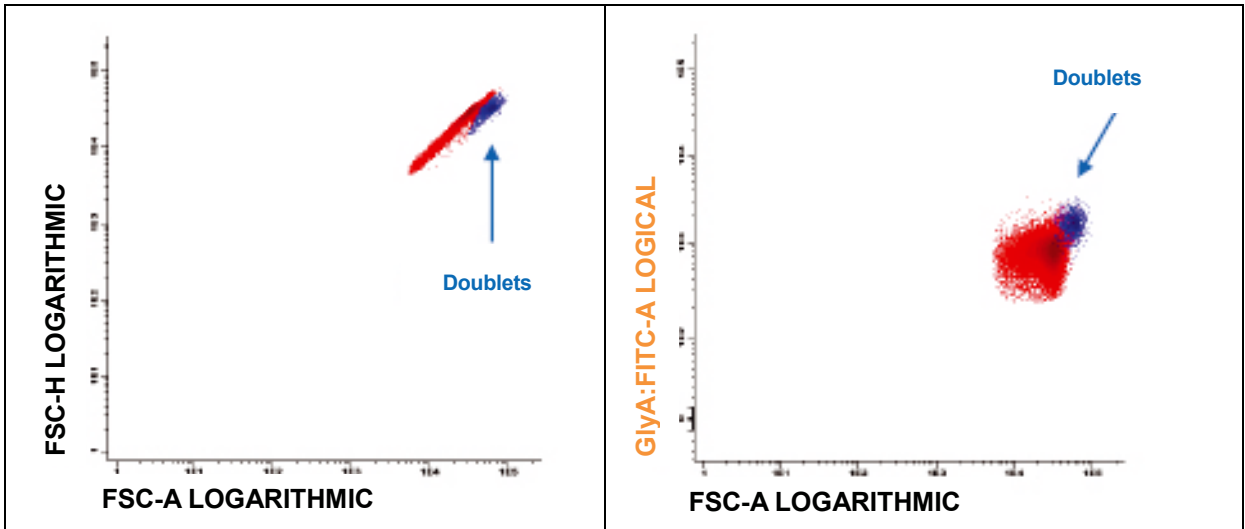
Download .fcs for RBCs, Monocytes and Granulocytes:

https://www.alexion.de/-/media/alexion_de/ws_alexion_germany/download/netflowconnect

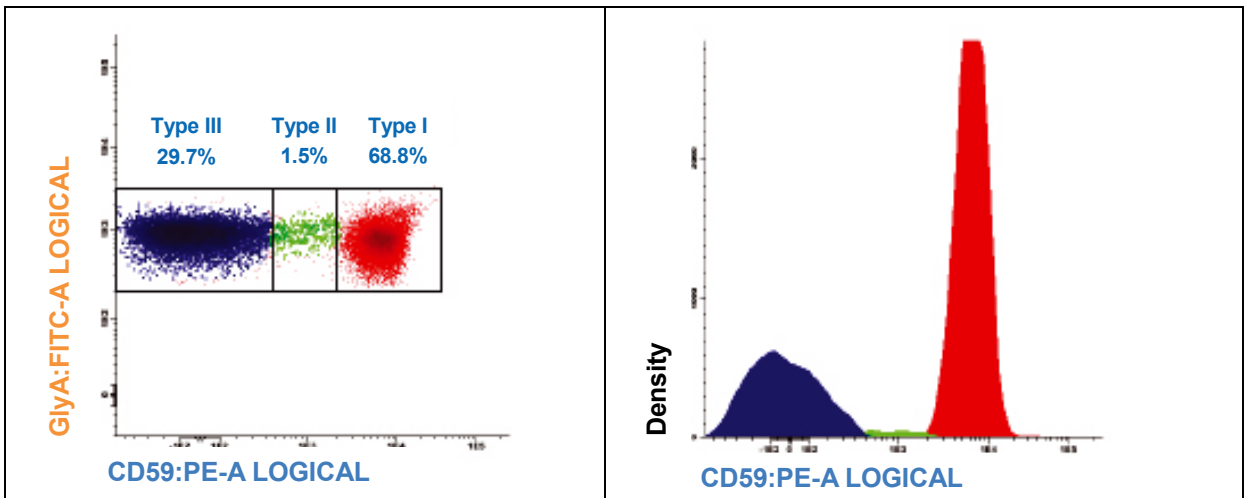
Evaluation of PNH RBCs



Step 1: Gate RBCs on FS log vs SS log. **Step 2:** Gate RBCs on FS log vs CD235a.

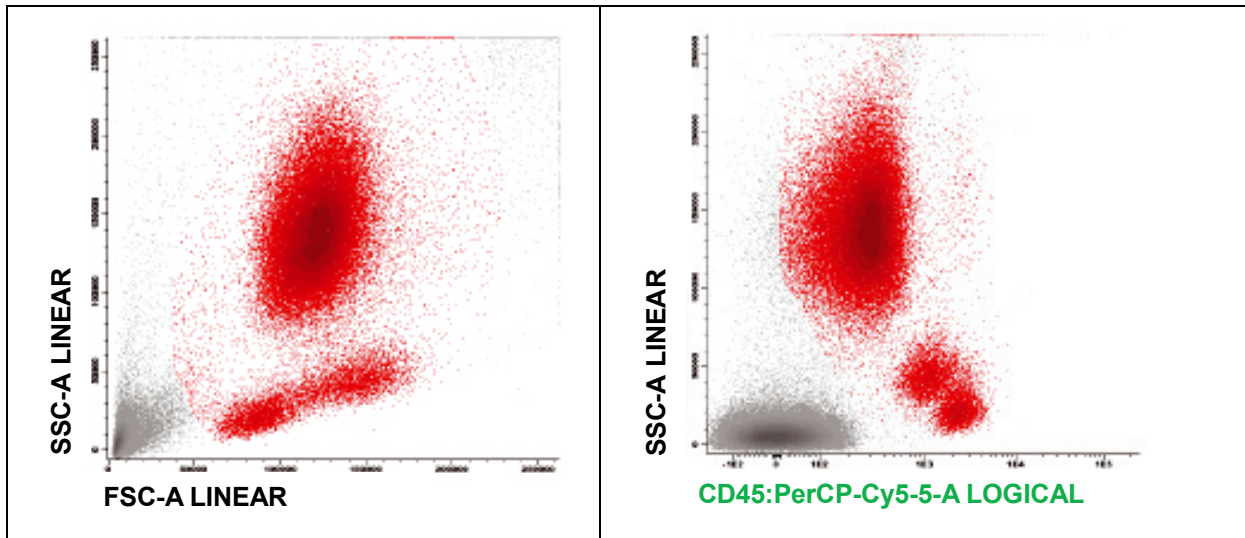


Step 3: Check for doublets. If >2%, rack rigorously again.



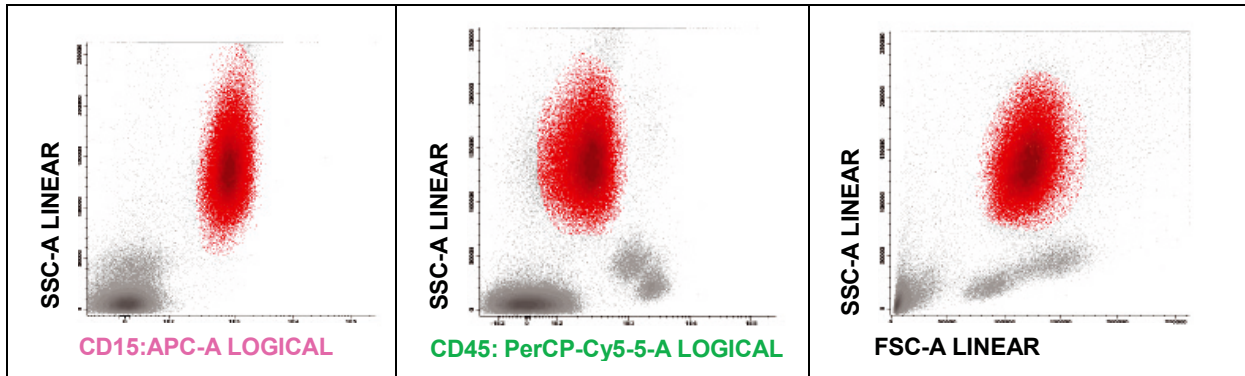
Step 4: Display RBCs on CD59 vs CD235a dot plot and determine type III (CD59 negative) and type II (CD59 partially negative) PNH RBCs.

Evaluation of PNH granulocytes

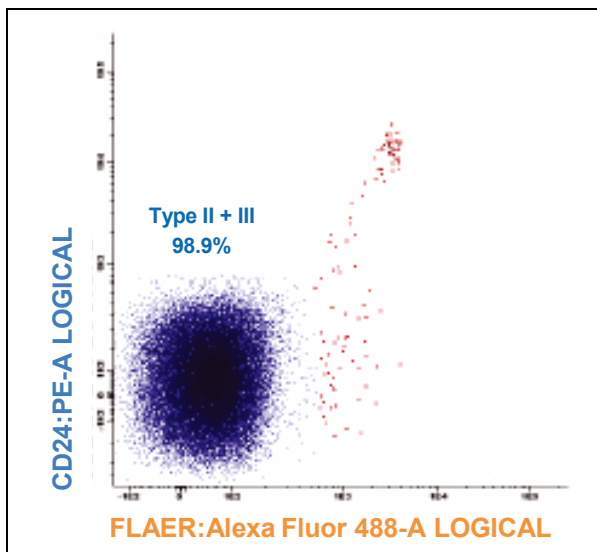


Step 1: Gate WBCs on FS lin vs SS lin.

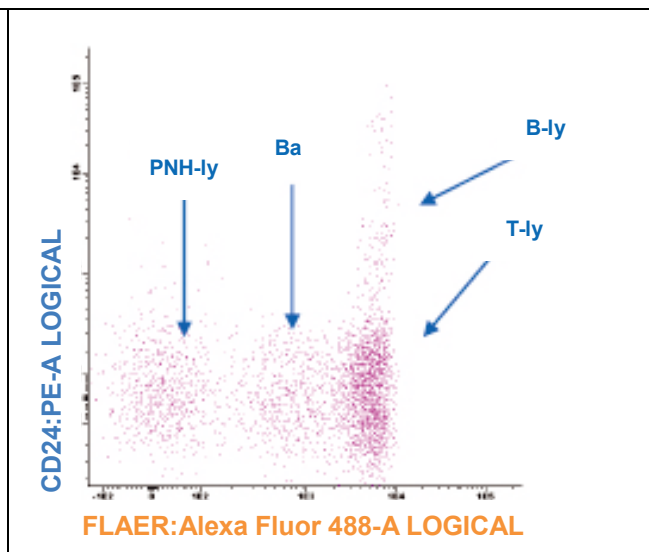
Step 2: Gate WBCs on CD45 vs SS lin.



Step 3: Gate granulocytes on CD15 strong vs SC lin and backgate on CD45 vs SS lin and FS lin vs SS lin to discriminate immature granulocytes and eosinophils.



Step 4: Gate PNH granulocytes on FLAER vs CD24.



Step 5: Check compensation on FLAER vs CD24 gated on Ly (we should discriminate B, T, NK, Ba and PNH-Ly).

Evaluation of PNH monocytes

