DryFlowEx PNH High-Sensitivity Assay Kit

25 tests | Cat. No. **ED7750**

IVD CE 2265

Instructions for Use (EN)

Version: ED7750_IFU_v4_EN

Date of Issue: 13-03-2026

Symbols used in the device labeling

IVD	In Vitro diagnostic medical device		Temperature limit
CE 2265	CE conformity mark Notified Body ID number		Keep away from sunlight
	Manufacturer		Keep Dry Keep away from rain
UDI	Unique Device Identifier		Caution
	Consult instructions for use		Do not re-use
	Contains sufficient for <n> tests	TUBE	Contains <n> tubes for single use test
REF	Catalogue number	CONC 10×	Concentrated solution (10x)
LOT	Batch code	CONTENTS	Contents
	Use by date	UK CA	UKCA mark

1. Intended Purpose

DryFlowEx PNH High-Sensitivity Assay Kit is intended for high sensitivity detection and enumeration of glycosyl-phosphatidyl-inositol (GPI)-deficient cells in human whole blood by flow cytometry.

What is detected and/or measured

The device DryFlowEx PNH High-Sensitivity Assay Kit detects and enumerates glycosyl-phosphatidyl-inositol (GPI)-deficient cells (PNH clones) as a percentage of:

- CD59 dim or CD59- cells from all erythrocytes (CD235a+)
- CD59 dim or CD59- cells from all iRBCs (CD235a+CD71+)
- CD14-, CD157- and GPI anchor- cells from all monocytes (CD45+CD64+)
- CD24-, CD157- and GPI anchor- cells from all neutrophils (CD45+CD15+)

Device function

The device is intended for diagnosis and monitoring of patients suffering, or suspected of suffering Paroxysmal Nocturnal Hemoglobinuria (PNH) and related disorders [(1)].

Context of a physiological or pathological state

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematopoietic stem cell disorder resulting as a consequence of the non-malignant clonal expansion of cells with somatic mutation of Phosphatidylinositol Glycan Anchor Biosynthesis Class A (PIGA) gene. Mutations of the PIGA gene result in the inability to express glycosyl-phosphatidyl-inositol (GPI)-anchored cell surface proteins.

The device is intended to detect GPI-deficient neutrophils and monocytes [(1)], together with complete (Type III) and partial (Type II) GPI-deficient erythrocytes [(2,3,4,5,6)] for evaluation of the PNH clone size.

Furthermore, the device detects GPI-deficient iRBCs (immature erythrocytes) in patients receiving blood transfusions when PNH erythrocytes are difficult to delineate [(7)].

Type of assay

Not automated

Quantitative

Type of specimen required

Human anticoagulated peripheral whole blood specimen (EDTA, heparin, citrate) [(1)]

Testing population

Patients with:

- laboratory markers of hemolysis, when other more common causes of hemolysis have been excluded,
- unexplained thromboses in young age,
- diagnosed thromboses in an unusual site,
- inherited or acquired aplastic anemia (AA),
- myelodysplastic syndrome (MDS),
- unexplained cytopenia in whom AA or MDS are differential diagnostic considerations [(1)]

2. Intended user

The device is intended for professional laboratory use only. Not for near-patient testing or self-testing.

Requirements on qualification

Intended user shall have state-of-the-art expertise in flow cytometry analysis of human cells, standard laboratory techniques including pipetting skills, safe and proper handling of specimens derived from the human body.

Intended user shall be compliant with standard EN ISO 15189 or other national provisions, where applicable.

3. Test principle

The test principle is based on the detection of GPI anchor and GPI-anchored proteins on the surface of human blood cells. Monoclonal antibodies and recombinant proaerolysin used in the test are labeled with different fluorochromes which are excited by a laser beam from a flow cytometer during acquisition of a stained blood specimen. Subsequent fluorescence (light emission) from each fluorochrome present on an acquired blood cell is collected and analyzed by the instrument. Fluorescence intensity is directly proportional to the antigen expression density in a cell allowing for separation of different cell subsets.

4. Reagent(s) provided

Contents

The device DryFlowEx PNH High-Sensitivity Assay Kit, sufficient for examination of 25 patients, is provided with the following reagents:

PNH High-Sensitivity Assay (25 pouches). Each pouch consists of 1 color-coded (Cyan strip) capped single-use tube **PNH WBC 7-color** (ED7750-1) and 1 color-coded (Red strip) capped single-use tube **PNH RBC 3-color** (ED7750-2), containing premixed combinations of fluorochrome-labeled reagents dried with the stabilizing ingredients as a layer at bottom of the test tubes (12 x 75 mm),

see Table 1 and 2.

Lysing Solution ED7750-3 (1 bottle) containing 15 ml of concentrated (10X) formaldehyde-based buffered solution.

PNH Compensation Set ED7750-4 (1 pouch) containing 10 capped single-use tubes, each containing single fluorochrome-labeled reagent dried with the stabilizing ingredients as a layer at the bottom of the tube (12 x 75 mm).

> **CAUTION:** PNH Compensation Set is intended for the compensation setup only. Single fluorochrome-labeled reagents (see Table 1 and Table 2) allow easy and accurate compensation procedure.

Composition

Table 1 Description of the PNH WBC 7-color active ingredients

Antigen	Fluorochrome	Clone	Isotype
GPI anchor (Proaerolysin)	Alexa Fluor®488	N/A	N/A
CD157	PE	SY11B5	IgG1
CD45	PerCP-Cy™5.5	2D1	IgG1
CD64	PE-Cy™7	10.1	IgG1
CD24	APC	SN3	IgG1
CD14	APC-Cy™7	MEM-15	IgG1
CD15	Pacific Blue™	MEM-158	IgM

Table 2 Description of the PNH RBC 3-color active ingredients

Antigen	Fluorochrome	Clone	Isotype
CD235a	FITC	JC159	IgG1
CD59	PE	MEM-43	IgG2a
CD71	APC	MEM-75	IgG1

5. Materials required but not provided

Deionized water (Reagent-grade)

Phosphate buffered saline (1X PBS), pH 7.2 – 7.4

Flow Cytometry Compensation Particles (Spherotech SPHERO™ COMPtrol Kit, Cat. No. CMIgP-50-3K or equivalent compensation particles)

6. Equipment required

Automatic pipette with disposable tips (100 µl – 5 ml) for pipetting specimen and reagents

Liquid dispenser or pipette with disposable tips (2 ml) for dispensing erythrocyte lysing solution

Vortex mixer

Conical polypropylene centrifuge tubes (15 ml or 50 ml) for specimen preparation

Centrifuge with appropriate rotor adaptors for 12 x 75 mm round bottom tubes

Flow cytometer with three laser excitation sources (488 nm, 635 nm and 405 nm), detectors for scatters, optical filters and emission detectors appropriate to collect signals from fluorochromes provided in Table 3

Table 3 Spectral characteristic of fluorochromes used in the device

Fluorochrome	Excitation [nm]	Emission [nm]
Alexa Fluor® 488	488	520
FITC	488	525
PE	488	576
PerCP-Cy™5.5	488	695
PE-Cy™7	488	780
APC	630 – 640	660
APC-Cy™7	630 – 640	780
Pacific Blue™	405	455

NOTICE: The device was tested on flow cytometers BD FACSCanto™ II (BD Biosciences), BD FACSLyric™ (BD Biosciences), Navios EX (Beckman Coulter), DxFLEX (Beckman Coulter).

7. Storage and handling

Store at 20-30 °C.

Avoid prolonged exposure to light.

Keep dry.

CAUTION: Moisture sensitive product. Do not open the foil pouch until the first use.

See Section 10 Procedure (Preparation of reagent(s) provided) for information about the storage conditions and stability of working solutions (where applicable).

8. Warnings, precautions and limitations of use

WARNING: Lysing Solution (ED7750-3) contains formaldehyde (CAS No. 50-00-0) and methanol (CAS No. 67-56-1) in concentrations classified as hazardous.

Label elements	Signal word
	**Danger**
H-phrases	H302: Harmful if swallowed. H315: Causes skin irritation. H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. H331: Toxic if inhaled. H335: May cause respiratory irritation. H341: Suspected of causing genetic defects. H350: May cause cancer. H371: May cause damage to organs. H373: May cause damage to the kidneys through prolonged or repeated exposure if swallowed. EUH071: Corrosive to the respiratory tract.
P-phrases	P201: Obtain special instructions before use. P260: Do not breathe vapours. P280: Wear protective gloves/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention. P403+P233: Store in a well-ventilated place. Keep container tightly closed.

Consult Safety Data Sheet (SDS) available on the product page at www.exbio.cz for the full information on the risks posed by chemical substances and mixtures contained in the Product and how they should be handled and disposed.

Biological Hazard

Human biological samples and blood specimens and any materials coming into contact with them are always considered as infectious materials.

Use personal protective and safety equipment to avoid contact with skin, eyes and mucous membranes.

Follow all applicable laws, regulations and procedures for handling and disposing of infectious materials.

Evidence of deterioration

Normal appearance of the reagent provided is a transparent dried layer at the bottom of the tube. Do not use the reagent if you observe any change in appearance, for example presence of moisture inside the tube.

Limitation of use

Do not use after the expiry date stated on the product labels.

Do not re-use test tubes.

9. Specimen

Use venous peripheral blood collected in specimen receptacle classified as a medical device, with EDTA, Heparin, or ACD (Acid Citrate Dextrose) anticoagulant [(1)].

Only use non-treated specimen. Do not use pre-lysed, washed or diluted specimen.

Process the blood specimen within 48 hours of collection. Keep the specimen at laboratory temperature (20–25°C) if it will be processed within 24 hours. If processing is delayed beyond 24 hours but within 48 hours, refrigerate the specimen [(1)].

Endogenous Interference

Based on scientific literature research endogenous interference sources are identified in Table 4.

Table 4 Endogenous Interference of the device

Endogenous interference	Impact	Reference
Albumin	Albumin may interfere in high concentrations due to its ability to bind as well as to release large quantities of ligands.	9, 10, 11
Bilirubin (icterus) (unconjugated)	Bilirubin increases the fluorescence background of cells due to its high autofluorescence.	12, 13, 14
Cell debris (after lysis)	Cell debris may provide inaccurate cell counts and deplete the antibody within the device.	15, 16
Erythrocytes	Insufficient lysis, red blood cells present in the sample may yield erroneous cell counting.	17
Hemoglobin	Hemolyzed samples may produce unreliable results.	18
Human anti-Murine antibodies	May affect device functionality (ability to bind to cell surface antigens).	19

Immunoglobulins	It can affect lymphocyte subset count.	12
Rheumatoid factors	The presence of RF does interfere with MIA (multiplex immunoassays).	20
Triglycerides	High circulating levels of lipids may affect flow cytometry analysis of certain blood cell populations.	21

Exogenous Interference

According to published papers by Sutherland et al. [7], red blood cells from blood transfusions significantly interfere with PNH analysis in patients with a large PNH clone experiencing extravascular hemolysis. Healthy erythrocytes received through transfusion considerably impact the monitoring of the PNH clone in the red blood cell (RBC) lineage. The addition of CD71 greatly improves the ability to analyze PNH clone sizes in RBCs, regardless of whether the patient is hemolytic or has received transfusions.

The DryFlowEx PNH High-Sensitivity Assay Kit addresses the mentioned issue by including CD71 antigen for identifying immature red blood cells (iRBCs), which also express GPI-anchored CD59 antigen. In transfusion bags, iRBCs are typically found in very low amounts [22]. However, in patients with extravascular hemolysis caused by PNH, the iRBC count increases due to the rapid destruction of erythrocytes [23]. Therefore, adding a CD71 monoclonal antibody for iRBC detection enables more accurate monitoring of PNH progression, as indicated by the loss of CD59 antigen on iRBCs, while overcoming interference from exogenous RBCs in transfusion bags. The DryFlowEx PNH High-Sensitivity Assay Kit includes anti-human CD71 antibody and allows adherence to the latest guidelines for PNH clone detection and quantification.

10. Procedure

Preparation of reagent(s) provided

PNH High-Sensitivity Assay

No reagent preparation is necessary, supplied in test tubes for single use only.

Lysing Solution

Bring the reagent to room temperature prior to use.

The reagent is 10X concentrated and must be diluted with deionized water prior use (1 volume of the concentrated solution and 9 volumes of deionized water). Following the first opening, the reagent retains its performance characteristics until the expiry date when stored under the stated conditions in its original primary container.

The diluted lysing solution (1X) is stable for 1 month when stored in a liquid dispenser or closed container at room temperature.

Preparation of materials required but not provided

Compensation particles

Prepare working solution of flow cytometry compensation particles according to manufacturer´s instructions.

Compensation setup

Acquire Compensation Set tubes using the same flow cytometer set-up, prior to the analysis of PNH RBC 3-color and PNH WBC 7-color stained tubes.

CAUTION: PNH RBC 3-color and PNH WBC 7-color compensation setup procedures differ in type of specimen preparation and sample staining.

PNH RBC 3-color compensation tubes (Red strip)

1. Add **SPHERO™ COMPtrol Kit or equivalent compensation particles** into the bottom of each single-color compensation tube.
2. Vortex and incubate for 20 minutes at room temperature in the dark.
3. Add 4 ml of 1X PBS to each compensation tube. Centrifuge for 5 minutes at 300×g.
4. Discard supernatant without disturbing the compensation particles and add 0.1 ml of 1X PBS to each compensation tube.
5. Set voltages on fluorescence detectors of interest prior to stained specimen analysis. Voltage on a PMT detector should be set high enough, so that minimum of negatively stained events interferes with 0th channel on the fluorescence axis. Also, PMT detector voltage should not exceed values at which positive events are pressed to the right axis.
6. Acquire the stained compensation tubes immediately using flow cytometer.
7. Calculate PNH RBC 3-color compensation matrix either in cytometer software developed by manufacturer or software dedicated for offline cytometry data analysis. Use this compensation matrix for all test tubes of this lot of PNH RBC 3-color.

   CAUTION: Once set for the specific PNH RBC 3-color lot, do not change fluorescent detectors settings in order to retain the same compensation matrix acquisition settings and compensation results.

PNH WBC 7-color compensation tubes (Cyan strip)

1. Add 50 µl deionized water into the bottom of each single-color compensation tube and vortex vigorously for 7-10 seconds.
2. Add **100 µl of peripheral whole blood to each single-color compensation tube** and vortex vigorously.

3. Incubate for 20 minutes at room temperature in the dark.
4. Add 2 ml of dilute (1X) Lysing Solution to each compensation tube.
5. Incubate for 10 minutes at room temperature in the dark.
6. Centrifuge for 5 minutes at 300×g, discard supernatant and resuspend the cell pellet in 2 ml of 1X PBS.
7. Centrifuge for 5 minutes at 300×g, discard supernatant and resuspend the cell pellet in 0.2 ml of 1X PBS.
8. Set voltages on fluorescence detectors of interest prior to stained specimen analysis. Voltage on a PMT detector should be set high enough, so that minimum of negatively stained events interferes with 0th channel on the fluorescence axis. Also, PMT detector voltage should not exceed values at which positive events are pressed to the right axis.
9. Acquire the stained compensation tubes immediately using flow cytometer.
10. Calculate PNH WBC 7-color compensation matrix either in cytometer software developed by manufacturer or software dedicated for offline cytometry data analysis. Use this compensation matrix for all test tubes of this lot of PNH WBC 7-color.

    CAUTION: Once set for the specific PNH WBC 7-color lot, do not change fluorescent detectors settings in order to retain the same compensation matrix acquisition settings and compensation results.

Specimen preparation

Detection and differentiation of PNH clones in erythrocytes using PNH RBC 3-color tube requires specimen preparation prior to the staining procedure.

NOTICE: Before processing the specimen, ensure that the cytometer has been properly set up.

1. Label a polypropylene conical tube with the identification of examined blood specimen.
2. Pipette 10 µl of well-mixed blood specimen to the bottom of the labeled conical tube.
3. Dilute blood specimen 1:100 with 1 ml of 1X PBS and mix by hand swaying for 5 seconds.

   CAUTION: Classic form of PNH is dominated by intravascular hemolysis. Prior to diluting blood specimen, refer to RBC counts from hematology analyzer in order to achieve RBC count in diluted blood specimen in the range of 3 – 5 × 10^7 / ml of diluted blood and adjust dilution factor as required in order to

acquire sufficient count of RBCs in flow cytometer.

4. Proceed to Specimen staining procedure immediately after specimen dilution.

Detection of GPI-deficient cells in neutrophils and monocytes using PNH WBC 7-color tube requires no specimen preparation prior to the staining procedure.

Specimen staining – PNH RBC 3-color tube (Red strip)

1. Label PNH RBC 3-color tube with the identification of examined blood specimen.
2. **Pipette 50 µl of well-mixed diluted blood specimen** into the bottom of the PNH RBC 3-color tube.

   CAUTION: Avoid pipetting blood on the side of the test tube. If blood smear or droplet remains on the side of the tube, it will not be stained with the reagent and the test results can be invalid.
3. Vortex vigorously for 7-10 seconds.

   CAUTION: Shortening the vortex time may affect the test results.
4. Incubate PNH RBC 3-color tube for 20 minutes at room temperature in the dark.
5. Add 4 ml of 1X PBS to PNH RBC 3-color tube.
6. Centrifuge the PNH RBC 3-color tube for 5 minutes at 300× g.
7. Discard supernatant without disturbing the cell pellet and add 0.5 ml of 1X PBS to the PNH RBC 3-color tube.
8. Vortex shortly to resuspend the cell pellet.

Acquire the stained sample using flow cytometer. If the stained sample will not be acquired immediately, cap the test tube, store at 2-8 °C in the dark and analyze within 2 hours.

CAUTION: Disrupt cell aggregates in the stained sample by sliding the test tube 6-10 times against the top of the tube rack immediately before acquisition on the flow cytometer. Excessive amount of RBC aggregates may affect the test results.

Specimen staining – PNH WBC 7-color tube (Cyan strip)

1. Label PNH WBC 7-color tube with the identification of examined blood specimen.
2. Add **50 µl of deionized water** to the PNH WBC 7-color test tube. Vortex vigorously for 7-10 seconds.

CAUTION: Shortening the vortex time may affect the test results.

3. Pipette **100 µl of well-mixed blood specimen** into the bottom of the PNH WBC 7-color tube and vortex vigorously.

   CAUTION: Avoid pipetting blood on the side of the test tube. If blood smear or droplet remains on the side of the tube, it will not be stained with the reagent and the test results can be invalid.

4. Incubate for 20 minutes at room temperature in the dark.
5. Add 2 ml of 1X working erythrocyte Lysing Solution to PNH WBC 7-color tube.
6. Incubate for 10 minutes at room temperature in the dark.
7. Centrifuge the PNH WBC 7-color tube for 5 minutes at 300× g.
8. Discard supernatant without disturbing the cell pellet and add 2 ml of 1X PBS to the test tube.
9. Centrifuge the PNH WBC 7-color tube for 5 minutes at 300× g.
10. Discard supernatant without disturbing the cell pellet and add 0.2 ml of 1X PBS to the test tube.
11. Vortex shortly to resuspend the cell pellet.

Acquire the stained sample using flow cytometer. If the stained sample will not be acquired immediately, cap the test tube, store at 2-8 °C in the dark and analyze within 24 hours.

Flow cytometry analysis

The flow cytometer selected for use with the device DryFlowEx PNH High-Sensitivity Assay Kit shall be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors according to the cytometer manufacturer's instructions.

If not maintained properly the flow cytometer may produce false results.

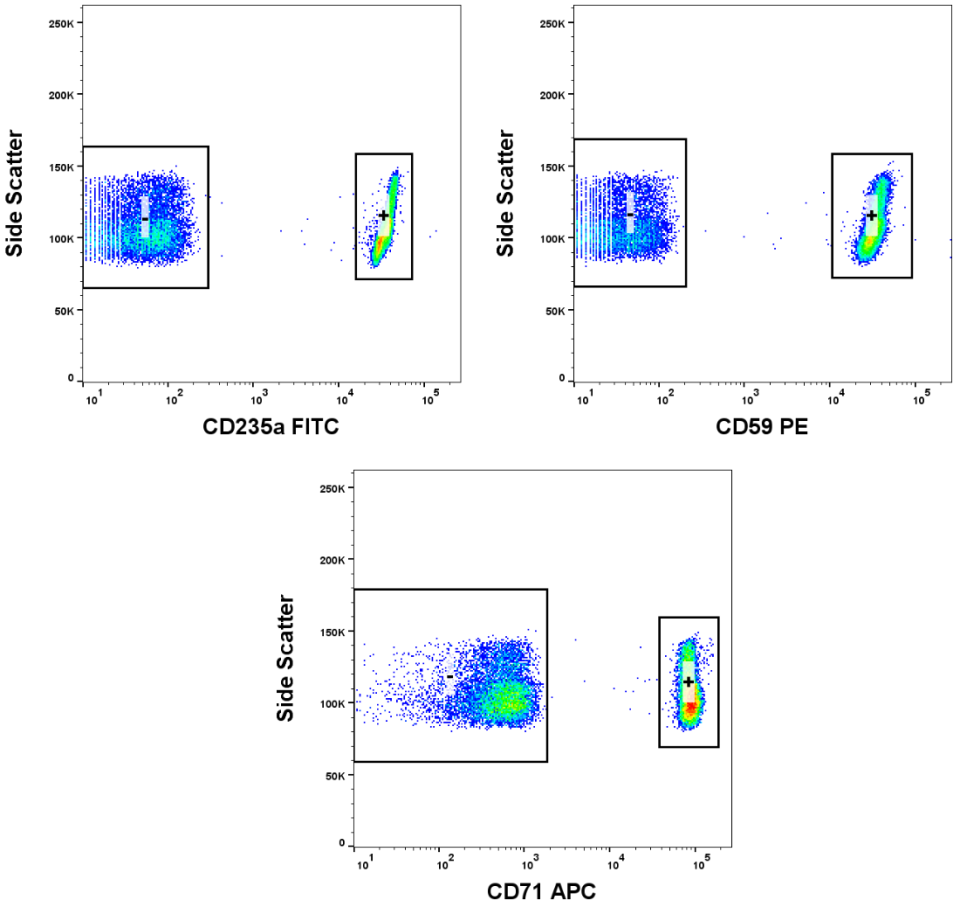
Refer to the manufacturer's cytometer specifications for lasers and fluorescence detectors according to the excitation and emission characteristics of the fluorochromes in Section 6 Equipment required.

For measured data analysis, it is possible to use cytometer software developed by the manufacturer, or software dedicated for offline cytometry data analysis (for example FlowJo™, VenturiOne®, Infinicyt™).

Analysis of PNH RBC 3-color compensation tubes (Red strip)

Visualize non-compensated data for each compensation tube in a side-scatter (SSC) versus “fluorochrome to be compensated” dot-plot. Set the gates for positive (+) and negative (-) cytometry compensation particles as shown in Figure 1.

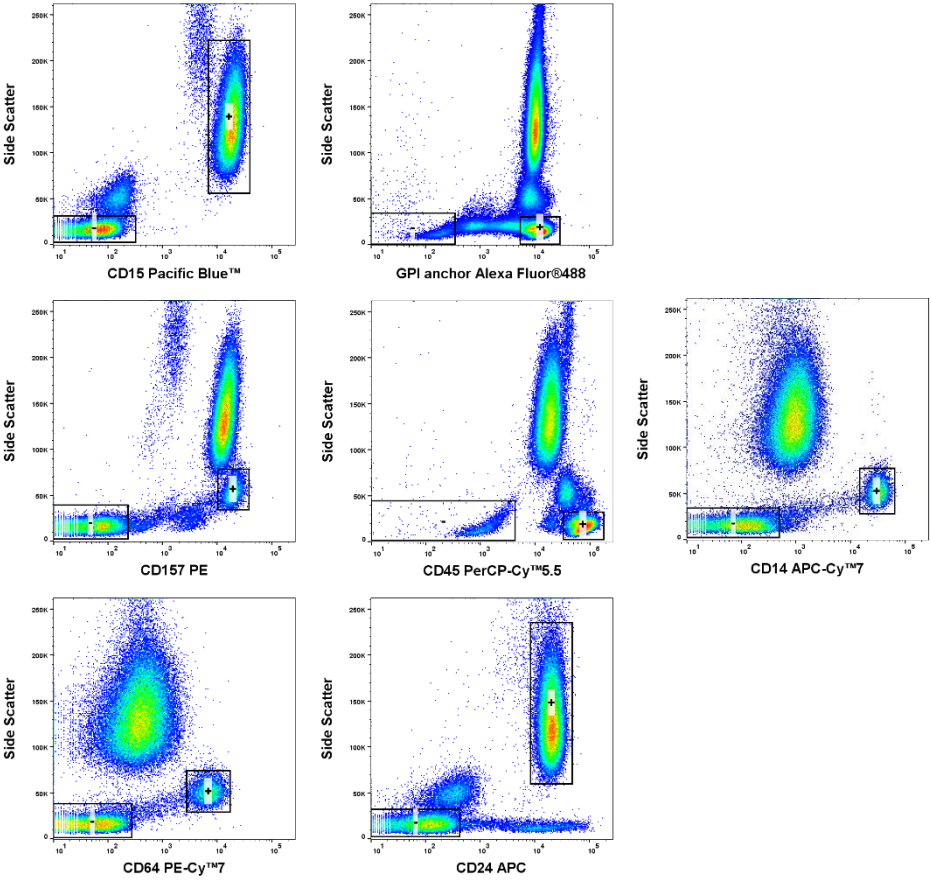
Figure 1 Identification of positive (+) and negative (-) cytometry compensation particles in compensation tubes (data acquired on BD FACSCanto™ II).



Analysis of PNH WBC 7-color compensation tubes (Cyan strip)

Visualize non-compensated data for each compensation tube in a side-scatter (SSC) versus “fluorochrome to be compensated” dot-plot. Set the gates for the most positive (+) and the most negative (-) populations as shown in Figure 2.

Figure 2 Identification of the most positive (+) and the most negative (-) events in compensation tubes (data acquired on BD FACSCanto™ II).



PNH RBC 3-color tube (Red strip)

Due to low iRBC count in diluted blood specimen, acquire a minimum of 500.000 erythrocyte events for analysis. Acquisition of ≥ 500.000 events results in long acquisition times. This may affect antibody-antigen binding complex equilibrium and the decrease of CD235a FITC fluorescence. Always monitor the stability of fluorescence intensity over the acquisition time (Figure 3).

Figure 3 Check all acquired events in a dot-plot, Time vs. CD235a FITC (data acquired on BD FACSLyric™).

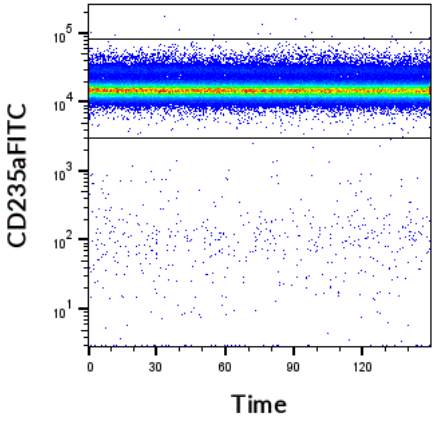
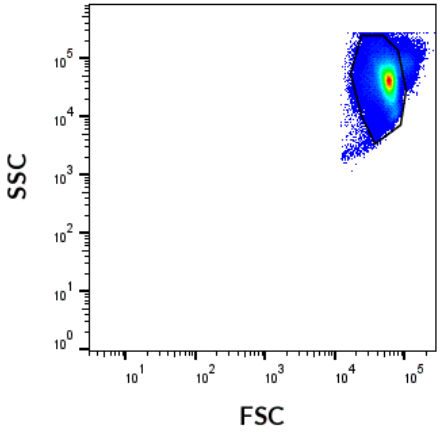
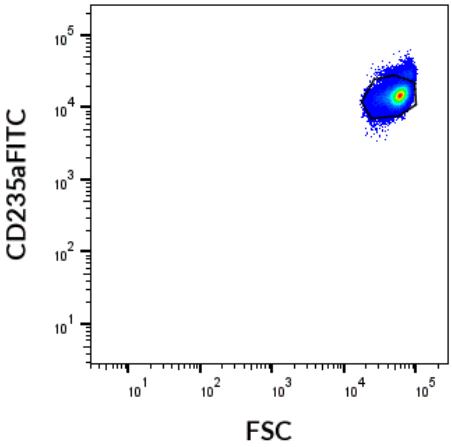


Figure 3a Display all events from the Time gate in a dot-plot FSC-A vs. SSC-A in logarithmic mode (data acquired on BD FACSLyric™).



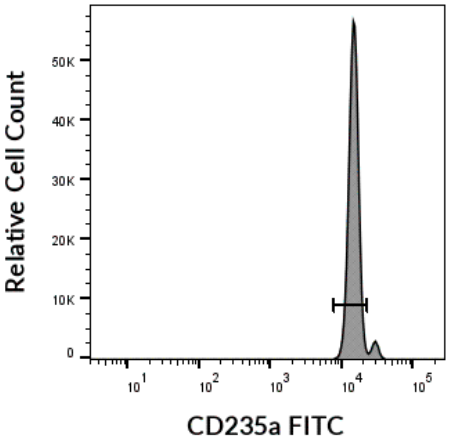
Visualize compensated data from the SSC vs. FSC gate in a dot-plot in logarithmic mode, where the X-axis represents FSC and the Y-axis represents fluorescence intensity in the FITC channel. Set “CD235a+ RBC singlets” gate (Figure 4).

Figure 4 Delineation of CD235a+ RBC singlets (data acquired on BD FACSLyric™).



Optional:
For better differentiation of singlets is possible to visualize compensated data as a histogram where the X-axis represents fluorescence intensity in the FITC channel. Set “CD235a+ RBC singlets” gate (Figure 4a).

Figure 4a Delineation of CD235a+ RBC singlets (data acquired on BD FACSLyric™).



Erythrocytes

Visualize CD235a+ RBC singlets in a dot-plot CD59 PE versus CD235a FITC. Separate events into three populations using three appropriate gates (Figure 5a, 5b) and calculate the percentage of events in the regions of Type I, Type II and Type III. For better adjustment of regions between Type II and III in some situations, a histogram can be useful.

Figure 5a Patient with PNH clone: A) CD235a+ RBC singlets in a dot-plot CD59 PE vs. CD235a FITC; B) CD235a+ RBC singlets in a CD59 PE vs. histogram (data acquired on BD FACSLyric™).

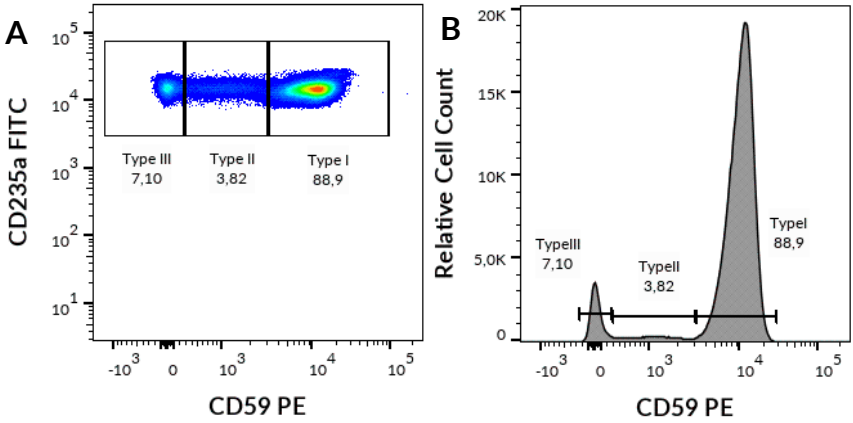
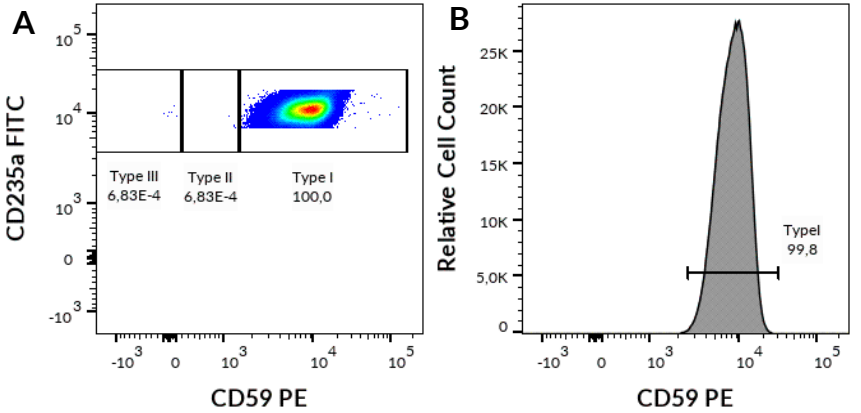


Figure 5b Healthy donor: A) CD235a+ RBC singlets in a dot-plot CD59 PE vs. CD235a FITC; B) CD235a+ RBC singlets in a CD59 PE vs. histogram (data acquired on BD FACS Lyric™).

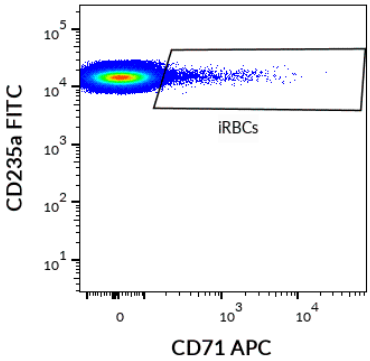


iRBCs (immature reticulocytes)

In patients receiving blood transfusions, it may be difficult to determine the true percentage of PNH type II and III RBCs. Healthy RBCs from a blood donor circulating in PNH patient`s bloodstream impact accurate PNH clone size percentage. The addition of CD71 may be helpful to gate out the donor's transfused cells [6].

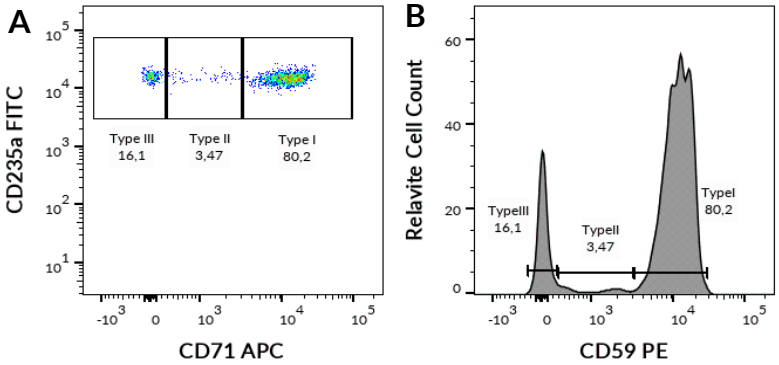
Visualize CD235a+ RBC singlets in a dot-plot CD71 APC versus CD235a FITC and separate CD71+ iRBCs (Figure 6).

Figure 6 CD235a+ RBC singlets in a dot-plot CD71 APC vs. CD235a FITC. Delineation of CD71+ iRBCs (data acquired on BD FACSLyric™).



Visualize CD71+ iRBCs in a dot-plot CD59 PE versus CD235a FITC and in a CD59 PE vs. histogram (Figure 7). Separate events into three populations using three appropriate gates and calculate the percentage of events in the regions of Type I, Type II and Type III.

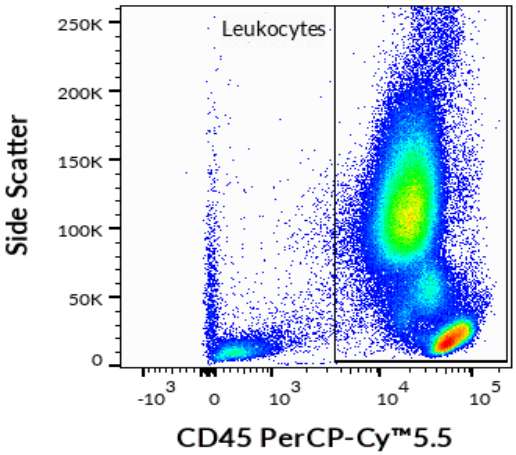
Figure 7 Patient with PNH clone, but without transfusion: A) CD71+ iRBCs in a dot-plot CD59 PE vs. CD235a FITC; B) CD71+ iRBCs in a CD59 PE vs. histogram (data acquired on BD FACSLyric™).



PNH WBC 7-color tube (Cyan strip)

Acquire at least 50,000 neutrophils and 10,000 monocytes, which means at least 200.000 events for analysis are acquired. Visualize compensated data in a dot-plot side-scatter versus fluorescence intensity in PerCP-Cy™ 5.5. Set CD45+ leukocytes gate as shown in Figure 8.

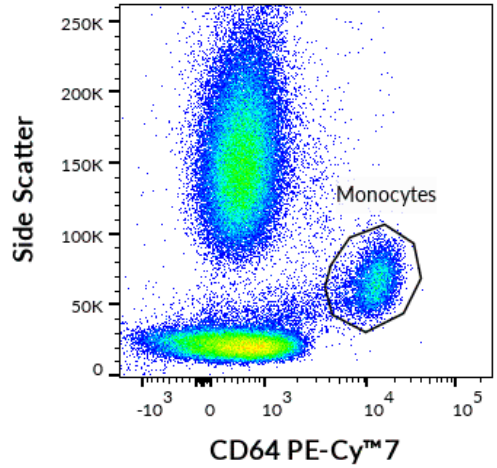
Figure 8 Delineation of CD45+ Leukocytes (data acquired on BD FACSLyric™).



Monocytes

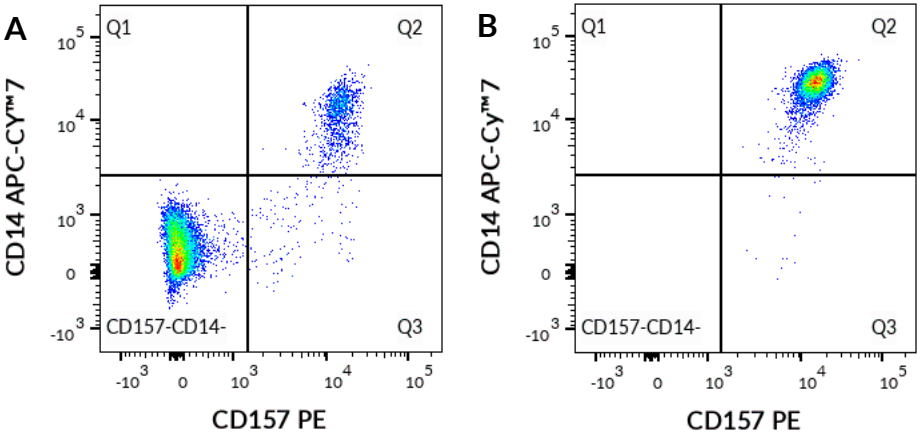
Visualize CD45+ leukocytes in a dot-plot side-scatter versus CD64 PE-Cy™7 and delineate CD64+ monocytes as shown in Figure 9.

Figure 9 Delineation of CD64+ Monocytes from Leukocytes (data acquired on BD FACSLyric™).



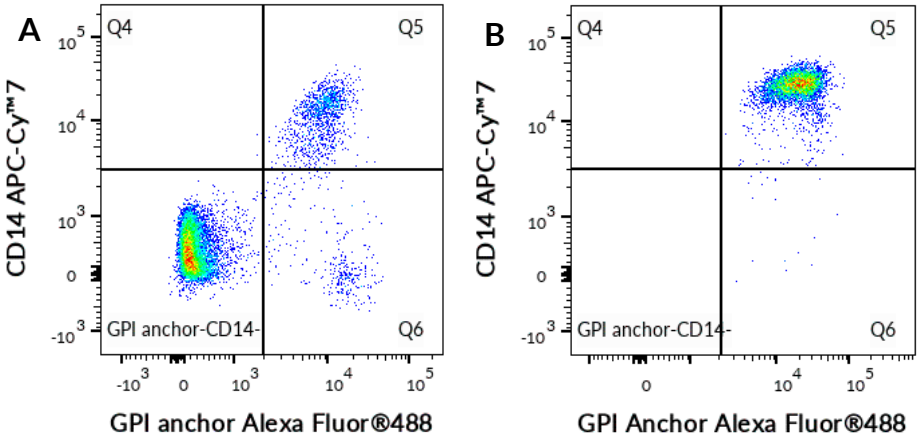
Visualize CD64+ monocytes in a dot-plot CD157 PE versus CD14 APC-Cy™7 (Figure 10). Set appropriate gates and calculate the percentage of CD157-CD14- population.

Figure 10 CD64+ Monocytes in a dot-plot CD157 PE vs. CD14 APC-Cy™7 (data acquired on BD FACSLyric™). A) patient with PNH clone; B) healthy donor



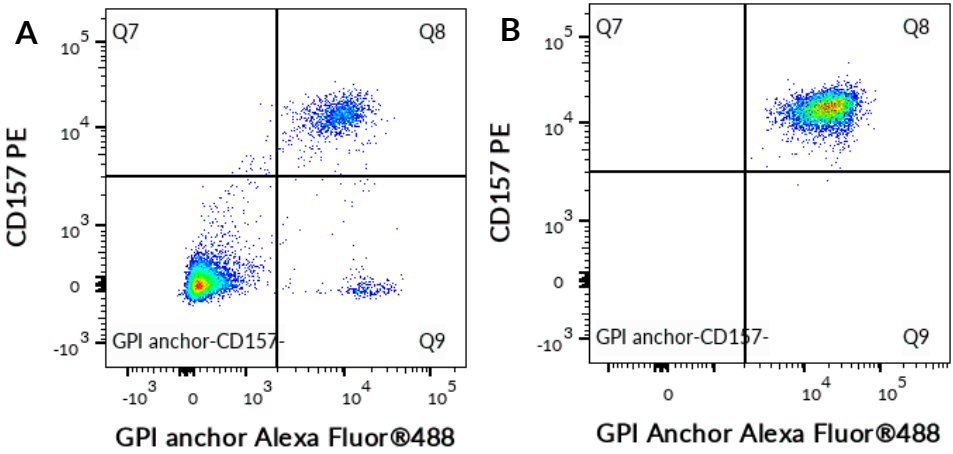
Then visualize the same CD64+ monocytes in a dot-plot GPI anchor Alexa Fluor® 488 (Proaerolysin) versus CD14 APC-Cy™7 (Figure 11). Set appropriate gates and calculate the percentage of GPI anchor-CD14- population.

Figure 11 CD64+ Monocytes in a dot-plot GPI anchor Alexa Fluor® 488 vs. CD14 APC-Cy™7 (data acquired on BD FACSLyric™).
A) patient with PNH clone; B) healthy donor



Then visualize the same CD64+ monocytes in a dot-plot GPI anchor Alexa Fluor® 488 (Proaerolysin) versus CD157 PE (Figure 12). Set appropriate gates and calculate the percentage of GPI anchor- CD157- population.

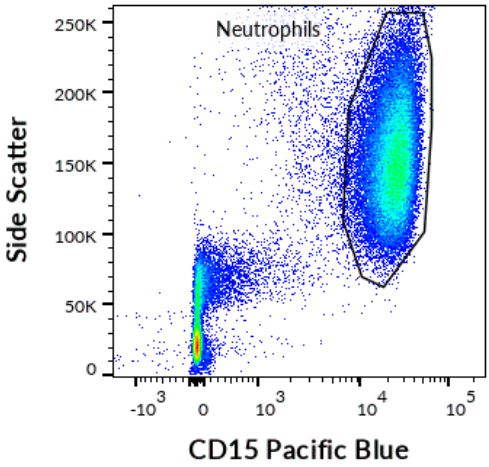
Figure 12 CD64+ Monocytes in a dot-plot GPI anchor Alexa Fluor® 488 vs. CD157 PE (data acquired on BD FACSLyric™). A) patient with PNH clone; B) healthy donor



Neutrophils

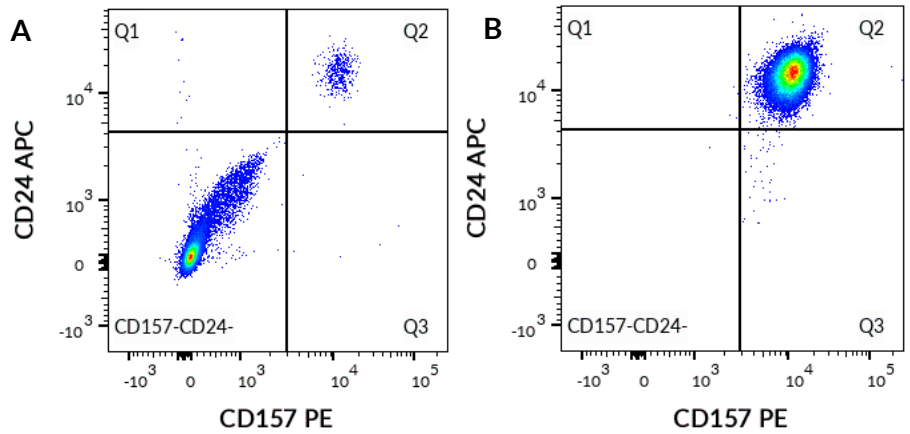
Visualize CD45+ leukocytes in a dot-plot side-scatter versus CD15 Pacific Blue™ and separate CD15+ neutrophils as shown in Figure 13.

Figure 13 Delineation of CD15+ Neutrophils from Leukocytes (data acquired on BD FACSLyric™).



Visualize CD15+ neutrophils in a dot-plot CD157 PE versus CD24 APC as shown in Figure 14. Set appropriate gates and calculate the percentage of CD157-CD24- population.

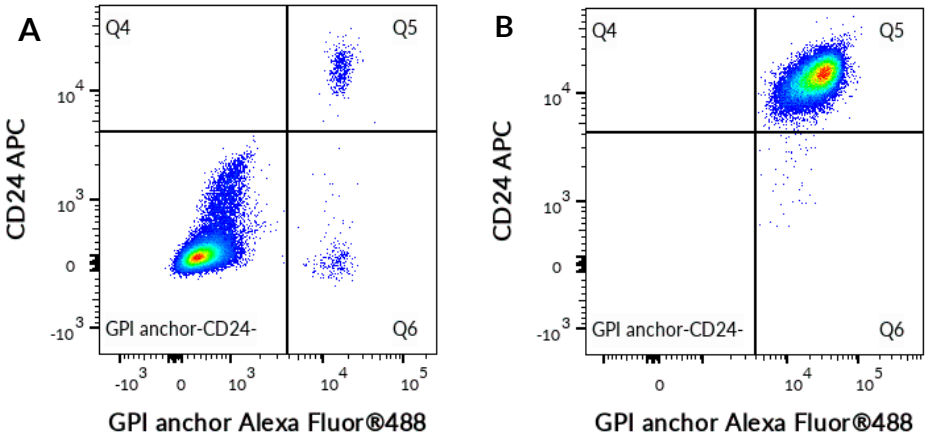
Figure 14 CD15+ Neutrophils in a dot-plot CD157 PE vs. CD24 APC (data acquired on BD FACSLyric™). A) patient with PNH clone; B) healthy donor



Then visualize the same CD15+ neutrophils in a dot-plot GPI anchor Alexa Fluor® 488 (Proaerolysin) versus CD24 APC. Set appropriate gates and calculate the percentage of GPI anchor-CD24- population as shown in Figure 15.

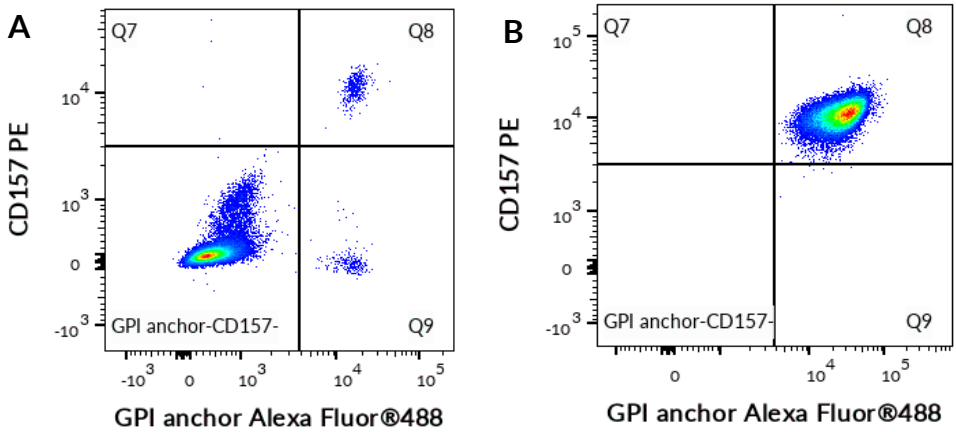
Figure 15 CD15+ Neutrophils in a dot-plot GPI anchor Alexa Fluor® 488 vs. CD24 APC (data acquired on BD FACSLyric™).

A) patient with PNH clone; B) healthy donor



Then visualize the same CD15+ neutrophils in a dot-plot GPI anchor Alexa Fluor® 488 (Proaerolysin) versus CD157 PE. Set appropriate gates and calculate the percentage of GPI anchor-CD157- population as shown in Figure 16.

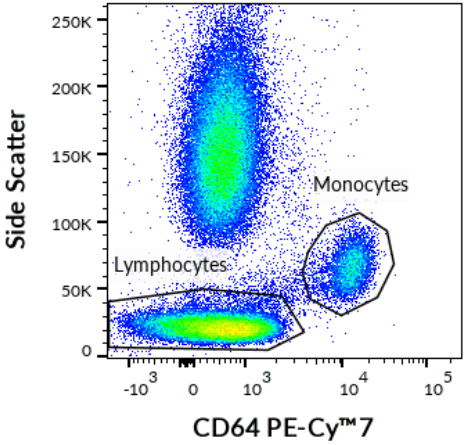
Figure 16 CD15+ Neutrophils in a dot-plot GPI anchor Alexa Fluor® 488 vs. CD157 PE (data acquired on BD FACSLyric™). A) patient with PNH clone; B) healthy donor



Internal control

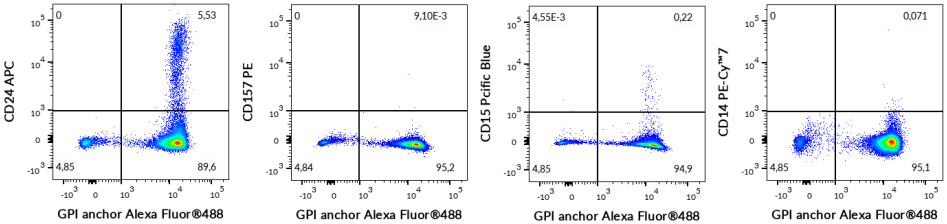
In order to verify fluorescence detector voltage and compensation, it is necessary to check the internal control (Figure 18). In the CD64 PE-Cy™7 versus side scatter plot, gate lymphocytes as the population for internal control (Figure 17).

Figure 17 Delineation of CD64- Lymphocytes from Leukocytes (data acquired on BD FACSLyric™).



Then visualize lymphocytes in dot-plots GPI anchor Alexa Fluor® 488 (Proaerolysin) versus CD24 APC/CD157 PE/CD15 Pacific Blue™/CD14 PE-Cy™7 for verification of voltage settings, compensation, and antibody performance (Figure 18).

Figure 18 Internal controls (data acquired on BD FACSLyric™).



Calculation and interpretation of analytical results

Enumerate the percentage of GPI-deficient (having PNH phenotype) cells, see Table 5.

Table 5 PNH phenotypes

Parent cell population		PNH phenotype according to the gating strategy
PNH RBC 3-color Tube	Erythrocytes (Type III)	CD59- CD235a+ (Fig. 5)
	Erythrocytes (Type II)	CD59 dim CD235a+ (Fig. 5)
	iRBCs (Type III)	CD59- CD235a+CD71+(Fig. 7)
	iRBCs (Type II)	CD59 dim CD235a+CD71+(Fig. 7)
PNH WBC 7-color Tube	Monocytes	CD14- CD157- CD64+ (Fig. 10)
		CD14- GPI anchor- CD64+ (Fig. 11)
		CD157- GPI anchor- CD64+ (Fig. 12)
	Neutrophils	CD24- CD157- CD15+ (Fig. 14)
		CD24- GPI anchor- CD15+ (Fig. 15)
		CD157- GPI anchor- CD15+ (Fig. 16)

Table 6 Results interpretation

Limit of detection (LOD) for the WBC and RBC tubes reported as frequency of parent (%), calculated from 100 measurements of n=25 normal patient samples on n=4 different cytometer platforms				
PNH phenotype	Cytometer			
	BD FACS Lyric™	BD FACS Canto™ II	Beckman Coulter NAVIOS EX	Beckman Coulter DX Flex
PNH RBC 3-color tube				
CD59- Type II and Type III RBCs	0.005	0.002	0.029	0.049
CD59- Type II and Type III iRBCs	0.240	0.320	0.388	0.562
PNH WBC 7-color Tube				
CD157- CD14- Monocytes	0.20	0.19	0.14	0.30
GPI anchor- CD14- Monocytes	0.08	0.04	0.10	0.17
GPI anchor- CD157- Monocytes	0.07	0.06	0.04	0.03
CD157- CD24- Neutrophils	0.02	0.02	0.06	0.03
GPI anchor- CD24- Neutrophils	0.03	0.03	0.02	0.02
GPI anchor- CD157- Neutrophils	0.01	0.01	0.01	0.01

GPI deficiency reporting algorithm rules [6]:

1. Report a PNH clone in the RBC tube

Cell population	Frequency	Resulting statement and action [6]
CD59- Type II + Type III RBCs	< LOD	"No PNH cells detected" Rare PNH phenotype cells recorded below the level of detection
	> 1 %	"PNH clone present" Cell percentage can be reported
	> LOD (Table 6) < 0.01 % [6]	"PNH cells present" Rare PNH phenotype cells recorded below the level of quantification

2. Report a PNH clone in the WBC tube

Cell population	Frequency (%)	Resulting statement and action [6]
GPI anchor- CD14- Monocytes	< LOD	"No PNH cells detected" Rare PNH phenotype cells recorded below the level of detection
	> 1 %	"PNH clone present" Cell percentage can be reported
	> LOD (Table 6); <LLOQ (Table 13, 14)	"PNH cells present" Cell percentage can be reported
GPI anchor- CD24- Neutrophils	< LOD	"No PNH cells detected" Rare PNH phenotype cells recorded below the level of detection
	> 1 %	"PNH clone present" Cell percentage can be reported
	> LOD (Table 6); <LLOQ (Table 13, 14)	"PNH cells present" Cell percentage can be reported

3. Report the PNH clone size in the RBCs.
Report the total PNH clone size as well as the percentages for the Type II and Type III PNH population, together with the cell population count acquired according to Table 7.

4. Report the PNH clone size in the WBCs.
Report the PNH clone size in both lineages – neutrophils and monocytes. WBC PNH clones appear clustered and less scattered than random double-negative events. Monocytes may show larger PNH clone sizes than neutrophils [2]. GPI-deficiency (presence of PNH clone) should be expressed in all PNH phenotypes

within the given leukocyte subset. All monocyte PNH phenotypes (Table 5) are expected to stain GPI-deficient cells similarly [6]. The same applies to neutrophil PNH phenotypes.

Terminology of reporting PNH clone (according to CLSI H52-A2):

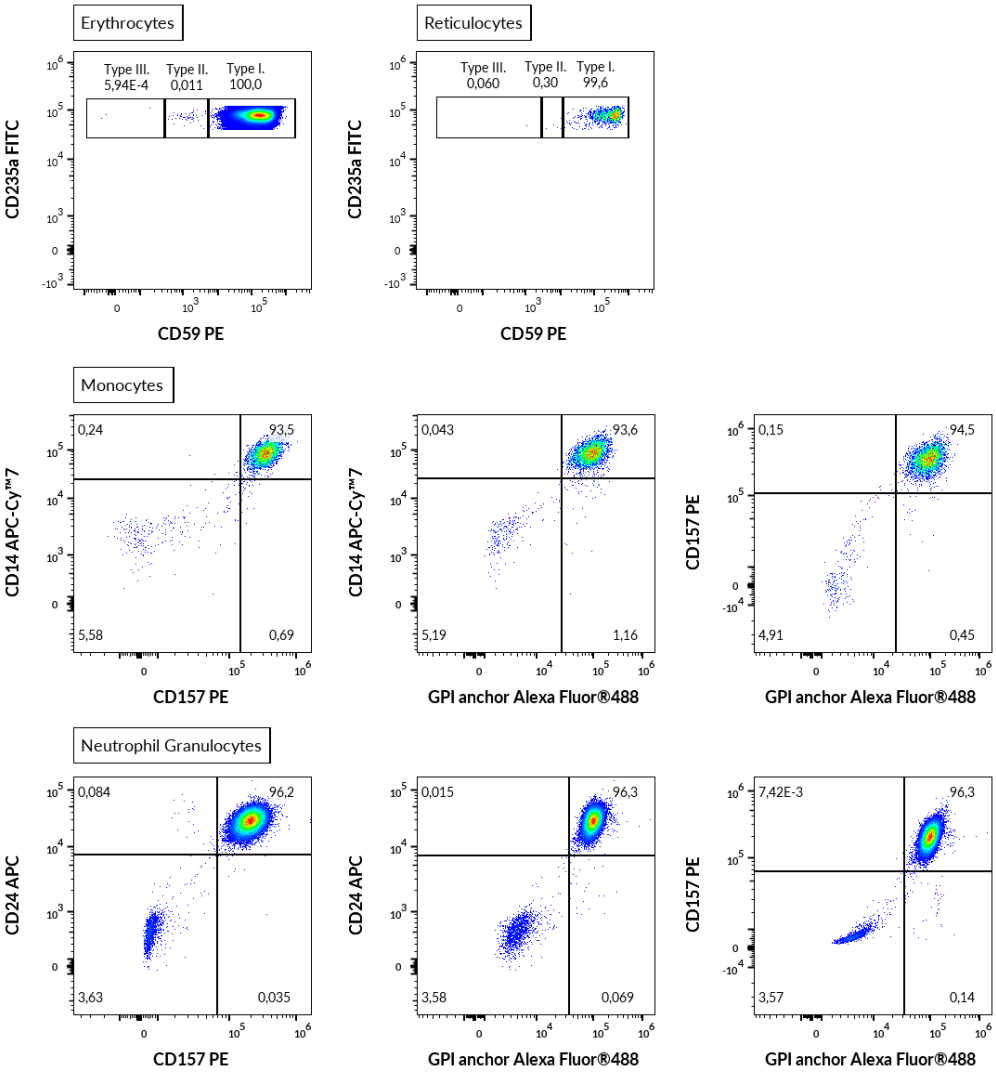
a. PNH population >1%: "PNH clone"
b. PNH population 0.1% to 1%: "minor PNH clone"
c. PNH population <0.1%: "rare cells with PNH phenotype"

Table 7 Examples of possible examination outcomes (not from a single patient) for a BD FACLyric™ flow cytometer.

PNH Phenotype	PNH event count	Parent population event count	Frequency	LLOQ	Evaluation
CD59-CD235a+ RBCs	3 events	521,023 singlet RBCs	0.0006%	0.01 % [6]	"No PNH cells detected" Rare PNH phenotype cells recorded below the level of detection
CD24-GPI anchor-CD15+	69,851 events	75,849 neutrophils	92.1 %	0.088 % (Table 13)	"PNH clone detected" 92.1 % neutrophil PNH clone size
CD14-GPI anchor-CD64+	13 events	12,497 monocytes	0.103 %	0.141 % (Table 13)	"PNH cells present; Minor PNH clone" 0.103 % monocyte PNH clone size

5. **Histograms and dot plots** can be optionally included in the report.

Figure 19 Example of a case with presence of a PNH clone in a WBC tube, while not being detected in a RBC tube (data acquired on Beckman Coulter DxFLEX).



11. Analytical performance

Specificity

Proaerolysin Alexa Fluor® 488 is a fluorescently-labeled variant of bacterial aerolysin protoxin that specifically binds to GPI anchors of surface membrane proteins in human cells [1, 2, 5, 8]. The specificity of Proaerolysin Alexa Fluor® 488 was verified by staining GPI-deficient Jurkat cells in comparison with the wild type phenotype of this cell line using flow cytometry.

The antibody SY11B5 recognizes an extracellular epitope on CD157 antigen of the CD157 protein expressed mainly on monocytes and granulocytes. Specificity of the antibody has been confirmed by HCDM Council (HLDA X workshop).

The antibody 2D1 recognizes all leukocyte isoforms of human CD45 (Leukocyte Common Antigen). Specificity of the antibody has been confirmed by HCDM Council (HLDA III workshop).

The antibody 10.1 recognizes of human CD64 antigen, that is expressed on monocytes. Specificity of the antibody has been confirmed by HLDA workshops (HLDA III: WS Code M-250 workshop).

The antibody SN3 reacts with CD24 antigen, expressed by granulocytes. Specificity of the antibody has been confirmed by HLDA workshop (HLDA IV: WS Code B 136; HLDA V: WS Code B CD24.7).

The antibody MEM-15 reacts with CD14, a GPI (glycosylphosphatidylinositol)-linked extracellular membrane glycoprotein expressed on monocytes. Specificity of the antibody has been confirmed by HCDM Council (HLDA III: WS Code M 252; HLDA IV: WS Code M 113; HLDA IV: WS Code NL 90; HLDA IV: WS Code T 53; HLDA V: WS Code M MA086; HLDA VI: WS Code M MA94 workshop).

The antibody MEM-158 reacts with CD15, strongly expressed on the surface of granulocytes. Specificity of the antibody has been confirmed by HCDM Council (HLDA VI: WS Code AS A053 workshop).

The antibody JC159 recognizes an epitope of the extracellular portion of CD235a (glycophorin A), a sialoglycoprotein expressed on early erythroblasts, late erythroblasts, erythroblasts and mature erythrocytes.

The antibody MEM-43 reacts with well defined epitope on CD59 (Protectin), (GPI)-anchored glycoprotein expressed on the surface of all hematopoietic cells. Specificity of the antibody has been confirmed by HLDA workshop (HLDA IV: WS Code NL 705; HLDA V: WS Code AS S013; HLDA V: WS Code BP BP345; HLDA V: WS Code T T-103 workshop).

The antibody MEM-75 reacts with an extracellular epitope of CD71 antigen expressed on immature iRBCs. Specificity of the antibody has been confirmed by HLDA workshop (HLDA IV: WS Code A 45; HLDA V: WS Code T T-165 workshop).

Accuracy

The accuracy of the method was assessed by comparing the device DryFlowEx PNH High-Sensitivity Assay Kit with an accredited clinical laboratory's in-house method (a cocktail of single-color conjugated antibodies from different manufacturers and analysed using BD FACSCanto™ II) by parallel staining of 13 patients with confirmed presence of PNH phenotype. Linear regression analysis parameters are provided in Table 8.

Table 8 Linear regression analysis for relative counts of GPI-deficient cell populations in patients with confirmed presence of PNH phenotypes. Comparison of the device DryFlowEx PNH High-Sensitivity Assay kit analysed using BD FACSCanto™ II with an accredited clinical laboratory in-house method.

PNH phenotype	n	Slope	Intercept	R^2	Range [%]
CD59- CD235a+ Type III erythrocytes	13	0.99	-0.026	1.00	1.28 – 83.79
CD59- CD235a+ Type III RBCs	13	0.99	-0.384	1.00	5.97 – 97.78
CD59- CD235a+ Type II erythrocytes	13	1.00	-0.059	1.00	0.13 – 89.92
CD59- CD235a+ Type II iRBCs	13	0.98	0.141	1.00	0.33 – 74.67
CD157- GPI anchor- CD64+ monocytes	13	1.00	0.060	1.00	2.07 – 99.95
CD157- GPI anchor- CD15+ neutrophils	13	0.99	0.294	1.00	0.80 – 99.82
CD14- GPI anchor- CD64+ monocytes	13	Not determined			2.04 – 99.96
CD14- CD157- CD64+ monocytes	13	Not determined			2.17 – 99.96
CD24- CD157- CD15+ neutrophils	13	Not determined			0.80 – 99.83
CD24- GPI anchor- CD15+ neutrophils	13	Not determined			0.81 – 99.80

n = number of blood samples

Limit of detection

Limit of detection (LOD) has been determined for each target population (see Table 5) as a mean value of results from 25 healthy blood donors, increased by the addition of three standard deviations from the mean for 4 different flow cytometer platforms (Tables 9, 10, 11, and 12).

Table 9 DryFlowEx PNH High-Sensitivity Assay Kit LOD values for each PNH phenotype acquired on BD FACSLyric™ flow cytometer.

PNH phenotype	BD FACSLyric™				
	n	Mean [%]	SD [%]	**PNH phenotype incidence**	**LOD (Mean + 3*SD)**
RBC tube (1.000.000 events acquired; min. 80% singlet RBC events)*					
CD59- Type II and Type III RBCs	25	0.003	0.001	**5 – 48 events per 1,000,000 events**	**0.005 %**
CD59- Type II and Type III iRBCs	25	0.054	0.061	**0 – 5 events per 3,000 iRBCs**	**0.240 %**
WBC Tube (50,000 neutrophils and 10.000 monocytes acquired)					
CD157- CD14- Monocytes	25	0.076	0.041	**2 - 24 events per 10,000 Monocytes**	**0.20 %**
GPI anchor- CD14- Monocytes	25	0.021	0.018	**0 - 5 events per 10,000 Monocytes**	**0.08 %**
GPI anchor- CD157- Monocytes	25	0.014	0.020	**0 - 4 events per 10,000 Monocytes**	**0.07 %**
CD157- CD24- Neutrophils	25	0.006	0.006	**0 - 10 events per 50,000 Neutrophils**	**0.02 %**
GPI anchor- CD24- Neutrophils	25	0.006	0.008	**0 - 15 events per 50,000 Neutrophils**	**0.03 %**
GPI anchor- CD157- Neutrophils	25	0.002	0.002	**0 - 4 events per 50,000 Neutrophils**	**0.01 %**

NOTICE*: A minimum of 98 % singlet events is advised for analysis in a liquid antibody cocktail [24]. A dried antibody cocktail in DryFlowEx PNH High-Sensitivity Assay usually allows for lower singlet event frequency and 98% singlet frequency may not be achieved.

Table 10 DryFlowEx PNH High-Sensitivity Assay Kit LOD values for each PNH phenotype acquired on BD FACSCanto™ II flow cytometer.

PNH phenotype	BD FACSCanto™ II				
	n	Mean [%]	SD [%]	**PNH phenotype incidence**	**LOD (Mean + 3*SD)**
RBC tube (1.000.000 events acquired; min. 80% singlet RBC events)					
CD59- Type II and Type III RBCs	25	0.0006	0,0004	**1 – 12 events per 1,000,000 events**	**0.002 %**
CD59- Type II and Type III iRBCs	25	0.0657	0.0847	**0 – 5 events per 1,000 iRBCs**	**0.320 %**
WBC Tube (50,000 neutrophils and 10,000 monocytes acquired)					
CD157- CD14- Monocytes	25	0.085	0.035	**2 - 16 events per 10,000 Monocytes**	**0.19 %**
GPI anchor- CD14- Monocytes	25	0.086	0.096	**0 - 3 events per 10,000 Monocytes**	**0.04 %**
GPI anchor- CD157- Monocytes	25	0.084	0.019	**0 - 7 events per 10,000 Monocytes**	**0.06 %**
CD157- CD24- Neutrophils	25	0.004	0.052	**0 - 8 events per 50,000 Neutrophils**	**0.02 %**
GPI anchor- CD24- Neutrophils	25	0.006	0.010	**0 - 16 events per 50,000 Neutrophils**	**0.03 %**
GPI anchor- CD157- Neutrophils	25	0.002	0.002	**0 - 4 events per 50,000 Neutrophils**	**0.01 %**

Table 11 DryFlowEx PNH High-Sensitivity Assay Kit LOD values for each PNH phenotype acquired on Beckman Coulter Navios EX flow cytometer.

PNH phenotype	Beckman Coulter Navios EX				
	n	Mean [%]	SD [%]	**PNH phenotype incidence**	**LOD (Mean + 3*SD)**
RBC tube (1.000.000 events acquired; min. 80% singlet RBC events)					
CD59- Type II and Type III RBCs	25	0.007	0.007	**4 – 236 events per 1,000,000 events**	**0.029 %**
CD59- Type II and Type III iRBCs	25	0.087	0.100	**0 – 6 events per 1,000 iRBCs**	**0.388 %**
WBC Tube (50,000 neutrophils and 10,000 monocytes acquired)					
CD157- CD14- Monocytes	25	0.062	0.027	**0 - 23 events per 10,000 Monocytes**	**0.14 %**
GPI anchor- CD14- Monocytes	25	0.024	0.006	**0 - 10 events per 10,000 Monocytes**	**0.10 %**
GPI anchor- CD157- Monocytes	25	0.007	0.011	**0 - 6 events per 10,000 Monocytes**	**0.04 %**
CD157- CD24- Neutrophils	25	0.012	0.015	**0 - 21 events per 50,000 Neutrophils**	**0.06 %**
GPI anchor- CD24- Neutrophils	25	0.005	0.005	**0 - 6 events per 50,000 Neutrophils**	**0.02 %**
GPI anchor- CD157- Neutrophils	25	0.002	0.002	**0 - 5 events per 50,000 Neutrophils**	**0.01 %**

Table 12 DryFlowEx PNH High-Sensitivity Assay Kit LOD values for each PNH phenotype acquired on Beckman Coulter DxFLEX flow cytometer.

PNH phenotype	Beckman Coulter DxFLEX				
	n	Mean [%]	SD [%]	**PNH phenotype incidence**	**LOD (Mean + 3*SD)**
RBC tube (1.000.000 events acquired; min. 80% singlet RBC events)					
CD59- Type II and Type III RBCs	25	0,015	0.012	**5 – 48 events per 1,000,000 events**	**0.049 %**
CD59- Type II and Type III iRBCs	25	0.106	0.152	**0 – 5 events per 1,000 iRBCs**	**0.562 %**
WBC Tube (50,000 neutrophils and 10,000 monocytes acquired)					
CD157- CD14- Monocytes	25	0.092	0.068	**0 - 27 events per 10,000 Monocytes**	**0.30 %**
GPI anchor- CD14- Monocytes	25	0.053	0.040	**0 - 16 events per 10,000 Monocytes**	**0.17 %**
GPI anchor- CD157- Monocytes	25	0.005	0.009	**0 - 1 events per 10,000 Monocytes**	**0.03 %**
CD157- CD24- Neutrophils	25	0.010	0.008	**0 - 14 events per 50,000 Neutrophils**	**0.03 %**
GPI anchor- CD24- Neutrophils	25	0.008	0.006	**0 - 10 events per 50,000 Neutrophils**	**0.02 %**
GPI anchor- CD157- Neutrophils	25	0.002	0.002	**0 - 3 events per 50,000 Neutrophils**	**0.01 %**

Limit of quantification

The lower limit of quantification (LLOQ) has been determined by serial dilutions (1:10, 1:100, 1:1000 and 1:10000) of a fresh PNH sample with a fresh normal blood sample and evaluated by triplicate staining and analysis using BD FACSLyric™ and Beckman Coulter DxFlex flow cytometers. A maximum CV of 10 % has been selected as the sensitivity criteria for LLOQ.

Table 13 DryFlowEx PNH High-Sensitivity Assay Kit LLOQ frequency values acquired on BD FACSLyric™ flow cytometer.

PNH phenotype	Dilution	No. of PNH cells	LLOQ	CV
CD59- Type II and Type III RBCs	1:10,000	101	0.029 %	3.9 %
CD157- CD14- Monocytes	1:10,000	28	0.677 %	2.3 %
GPI anchor- CD14- Monocytes	1:10,000	7	0.130 %	7.7 %
GPI anchor- CD157- Monocytes	1:10,000	2	0.018 %	9.9 %
CD157- CD24- Neutrophils	1:10,000	3	0.012 %	3.6 %
GPI anchor- CD24- Neutrophils	1:10,000	1	0.009 %	8.7 %
GPI anchor- CD157- Neutrophils	1:10,000	1	0.010 %	1.2 %

Table 14 DryFlowEx PNH High-Sensitivity Assay Kit LLOQ frequency values acquired on Beckman Coulter DxFLEX flow cytometer.

PNH phenotype	Dilution	No. of PNH cells	LLOQ	CV
CD59- Type II and Type III RBCs	1:10,000	101	0.005 %	9.1 %
CD157- CD14- Monocytes	1:10,000	22	0.143 %	4.0 %
GPI anchor- CD14- Monocytes	1:10,000	4	0.026 %	2.2 %
GPI anchor- CD157- Monocytes	1:10,000	11	0.068 %	5.9 %
CD157- CD24- Neutrophils	1:10,000	29	0.024 %	4.2 %
GPI anchor- CD24- Neutrophils	1:10,000	8	0.007 %	7.1 %
GPI anchor- CD157- Neutrophils	1:10,000	9	0.007 %	8.5 %

NOTICE: For flow cytometry analysis following flow cytometers including software version were used:

BD FACSCanto™ II	BD FACSDiva Software – version 8.0.2
BD FACSLyric™	BD FACSuite™ Software – version v1.5.0.925
Beckman Coulter DxFLEX	CytExpert for DxFLEX – version 2.0.2.18
Beckman Coulter Navios EX	Navios EX Software – version 2.2

For evaluation of measured data following analysis platform was used:
FlowJo™ (Becton, Dickinson and Company) - version 10.9.0

12. Clinical performance

Patients with GPI deficiency

Clinical data was collected at a clinical site from 19 patients, both healthy [6] and patients with confirmed GPI deficiency [13]. Clinical performance was determined as a comparison of the device DryFlowEx PNH High-Sensitivity Assay Kit with an accredited clinical laboratory in-house method (a cocktail of single color conjugated antibodies from different manufacturers and analysed using BD FACSCanto™ II).

GPI deficiency in pacients has been evaluated in regard to the method used (Table 15) by detection of GPI deficient cells (PNH clones).

Table 15 Clinical performance of the device DryFlowEx PNH High-Sensitivity Assay Kit

		GPI deficiency assessment using accredited clinical laboratory in-house method	
		GPI deficiency	**Normal condition**
GPI deficiency assessment using the device DryFlowEx PNH High-Sensitivity Assay Kit	**GPI deficiency**	13 patients	0 patients
	Normal condition	0 patients	6 patients

13. Expected values

Reference Interval

In normal population no GPI-deficiency is detected and all PNH phenotype percentage values are expected to be lower than assay Cut-off (LOD) [6].

14. Limitations

No limitations for use in specific types of diseases, like anemias, have been identified.

GPI deficiency reporting is limited as per the current state-of-the-art published guidelines [6].

The PNH clone is not detected in lymphocytes because it occurs more frequently in neutrophils and monocytes than in mature lymphocytes, suggesting that lymphoid cells are long-lived and may exist before the formation or expansion of PNH clones.

15. References

1) Borowitz, MJ et al. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. Cytometry B Clin Cytom. 2010 Jul;78(4):211-30. doi: 10.1002/cyto.b.20525.
2) Sutherland, DR et al. Practical guidelines for the high-sensitivity detection and monitoring of paroxysmal nocturnal hemoglobinuria clones by flow cytometry. Cytometry Part B 2012; 82B: 195–208.
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16. Summary of safety and performance

The summary of safety and performance will be available in the Eudamed database at https://ec.europa.eu/tools/eudamed/#/screen/home. Until then the summary of safety and performance is available upon request.

17. Use of Third Party Trademarks

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18. Revision History

Version 4, ED7750_IFU_v4

1) Chapter „Specimen“ was updated.

2) Cautions in Chapter 10 were updated.

3) Sections „PNH RBC 3-color tube“ and „PNH WBC 7-color tube,“ including figures in chapter 10, were updated.

4) Section „Internal Controls“ in chapter 10 was added.

5) Caution in the section „Calculation and interpretation of analytical results" was added.

6) Section „GPI deficiency reporting algorithm rules" was updated.

7) Section 11 „Analytical performance" was updated.

8) Section „Limitations" was updated.

9) Section „References" article numbering has been corrected.

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NOTICE: Any serious incident that has occured in relation to the device shall be reported to the manufacturer and the local competent authority.