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REVIEW ARTICLE

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Sensitive and accurate identification of PNH clones based on ICCS/ESCCA PNH Consensus Guidelines—A summary

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Abstract

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematopoietic stem cell disorder resulting from the somatic mutation of the X-linked phosphatidyl-inositol glycan complementation Class A (PIG-A) gene. Depending on the severity of the mutation in the PIG-A gene, there is a partial or absolute inability to make glycosylphosphatidyl-inositol (GPI)-anchored proteins including complement-defense structures such as CD55 and CD59 on RBCs and WBCs. Flow cytometric detection of PNH clones has become the gold standard and has played an increasingly important role in the diagnosis, monitoring, and clinical management of patients with PNH. Recently, a 4-part set of Consensus Guidelines have been published by flow experts in the field to address the key assay-specific considerations for the identification of PNH clones in RBC and WBC, how to report such data and a full validation document for the assays described. Below, we have summarized the most significant aspects of this International effort.

KEYWORDS
aplastic anemia (AA), CD59, FLAER, flow cytometry, myelodysplastic disorder (MDS), PNH

1 | INTRODUCTION

Prior to the advent of complement-inhibitory medications, PNH was often a severely debilitating disease with a 5-year morbidity of about 35%.[1] Detection of PNH and assessment of PNH clone size in RBC and WBC lineages by flow cytometric analysis has thus increased in importance due to the availability of novel therapies. The latter typically block the hemolysis of red blood cells and thus significantly lower the morbidities and mortality associated with PNH.

Medium and large PNH clones are associated with hemolysis and/or thrombosis in PNH patients, while the presence of rare GPI-deficient cells (<0.1%) in aplastic anemia (AA) and hypoplastic myelodysplastic syndrome (h-MDS) is predictive for better response to immunosuppressive therapy (IST), lower incidence of transformation to MDS, and/or acute myeloid leukemia (AML).[1,2] The previously reported significance of rare cells with PNH phenotype for the diagnostic exclusion of inherited bone marrow failure syndromes (IBMFS) has not been confirmed by other authors and remains unclear.[3,4] Current international recommendations for GPI-AP deficiency testing comprise cases with unexplained isolated anemia, unclear iron deficiency anemia, unexplained isolated neutropenia or thrombocytopenia, cytopenias, any subtype of MDS with evidence of hemolysis, venous or arterial thrombosis with atypical location and signs of hemolysis, cytopenia or in young patient as well as cases with acquired Coombs-negative hemolysis, intravascular hemolysis or recurrent abdominal pain or dysphagia. Depending on the absence or presence of PNH phenotypes, patients with AA should be further reassessed every 6 months for 2 years and every 3 months for 2 years, respectively.[5,6]

1.1 | PNH clone detection by flow cytometry

While the ability to rapidly detect GPI-deficient cells by flow cytometry has led to improved diagnosis, patient management, and prognosis in PNH and related disorders, earlier simple CD55/CD59 based approaches were neither accurate nor sensitive below the 1% to 4%

clone size, rendering them inadequate to detect small PNH clones present in PNH+ AA and MDS cases.[4,6] Since over 40% of samples positive for the presence of PNH cells contain GPI-deficient cells at a level of 1% or less (Figure 1), the development and validation of sensitive, standardized methodologies are essential to reliably detect small populations of GPI-deficient PNH phenotypes. To successfully develop highly sensitive, accurate, and reproducible assays, careful selection and titration of antibody clones/conjugates for lineage-specific gating (RBCs, neutrophils, and monocytes) and specific GPI-antigen detection within each cell lineage is required.[7-9] The detection of PNH phenotypes by flow cytometry represents a unique challenge in that the assays are designed to detect "negatives," that is, PNH phenotypes have "lost" the antigens of interest, namely GPI-linked structures. This has considerable implications for how instruments are set up and compensated because the target GPI-negative PNH cell populations (both RBCs and WBCs) need to be visible and fully on scale. A correct diagnosis of paroxysmal nocturnal hemoglobinuria (PNH) is essential for effective patient management and possible treatment if clinically indicated.[5,10,11] Although flow cytometry has become the gold standard for the diagnosis and monitoring of patients with PNH and related diseases,[5,8] it is important to emphasize that flow cytometry by itself does not establish the clinical diagnosis of overt/clinical PNH as this is determined by a number of other clinical factors in addition to the finding and quantification of GPI-deficient cells by flow cytometry.[1,12,13] High-sensitivity flow cytometric analysis can identify the presence of PNH phenotypes and is able to quantify the PNH clone size in red blood cells (RBCs) and white blood cells (WBCs, neutrophils, and monocytes) down to a lower limit of quantification (LLOQ) of 0.01% for RBC and 0.05%-0.1% for neutrophils.

The PNH clone size in WBCs is highly variable and although attempts have been made to determine a "threshold PNH clone size" for a diagnosis of clinical PNH, it should be noted that patients with smaller PNH WBC clones may show signs of clinical PNH while others with a larger WBC PNH clone size may not. Since more than 40% of PHN clone-containing samples do so at a level of 1% or less (Figure 1), the ability to detect and monitor such minor clones is critically important because some of them will progress to clinical PNH. Historically, the neutrophil clone size was used for this purpose based on the absence of at least two GPI-linked structures from neutrophils. However, more recently many laboratories have noted the additional value of assessing and reporting the PNH clone size in the monocytes as well.[14]

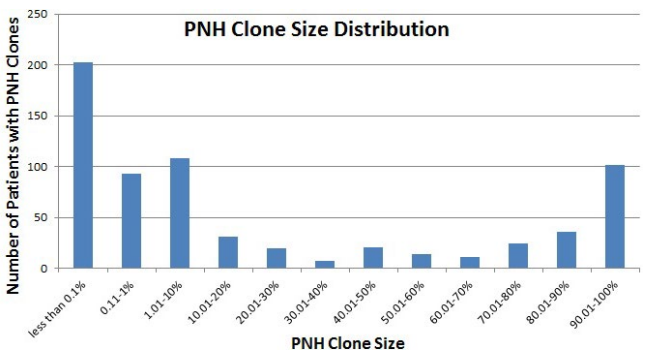


FIGURE 1 PNH Neutrophil clone size distribution based on 670 PNH+ patients based on a total of 10 338 screen patients based on the diagnostic pathway showing 44% of patients with a PNH clone size of <1% (data from Dahl-Chase Dx Services Dec 2008-Jan 2019) [Colour figure can be viewed at wileyonlinelibrary.com]

2 | PNH RBC ASSAY

2.1 | Antibody clone/conjugate selection

CD235a (glycophorin A) is the only RBC-specific gating reagent available and appropriately selected and titrated FITC-conjugated forms of specific clones minimize RBC aggregation. Appropriately selected/titrated CD59-PE conjugates allow best detection of PNH RBCs and separation of Type III (total loss of CD59 expression), from Type II (partial loss) and from normal (Type I) RBCs. A summary of recommended CD235a and CD59 clones/conjugates is shown in Table 1. Premixed "cocktails" of CD235a/CD59 combinations are strongly recommended to avoid the generation of false negatives.[7-9]

2.2 | Sample preparation and instrument setup

For instruments that do not automatically save "TIME" as a parameter, this parameter must be collected so that fluidics can be monitored if needed. Instrument setup for the RBC assay is performed using a fresh normal blood sample, diluted 1:100 with clean phosphate buffered saline (PBS). Light scatter voltages (in logarithmic format), photomultiplier tube voltages (PMTv) and compensation are optimized as detailed in several previous publications,[7-9] and recent examples can be viewed in Figures 1 and 2 of Ref. [8]). The RBC staining protocol has also been detailed elsewhere.[9,15]

2.3 | RBC analysis and interpretation

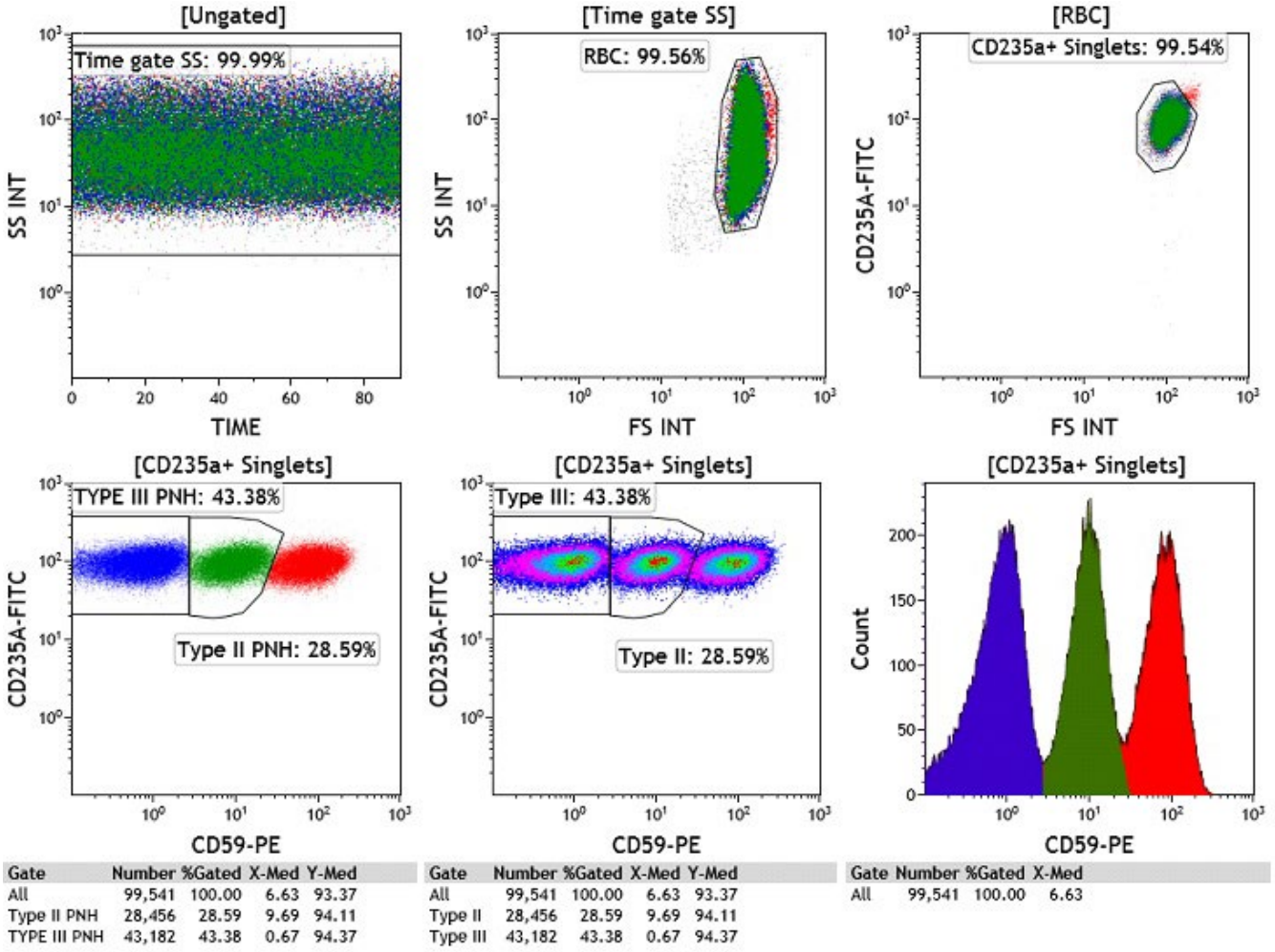
Red blood cells are analyzed by a series of gating dotplots beginning with TIME vs side scatter (SS-log), forward (FS-log) vs SS-log and CD235a-FITC vs FS-log to gate singlet RBCs and to quantify and exclude any remaining RBC aggregates (Figure 2, top row). The threshold (discriminator) is set on FS-log ensuring that no RBCs are excluded from acquisition. The diagnostic plots include a bivariate CD59 vs CD235a dot plot, a bivariate CD59 vs CD235a density plot, and a single parameter histogram of CD59 staining (Figure 2, bottom row). Bivariate dot plots and/or density plots are recommended over single-parameter histograms, especially for samples containing small numbers of PNH phenotypes, for identifying poorly stained samples that need to be re-stained, and for detecting media contamination and troubleshooting instrumentation issues (see Supplementary Data of Ref. [6]). However, while data regarding clone sizes comes predominantly from the two-dimensional plots, in which the gating regions are linked across the dotplot and density plots, the single-parameter histogram can also be useful in some situations, and when comparing lot-to-lot of RBC reagent cocktails. All three plots work in concert for optimal adjustment of the regions for Type III PNH cells and Type II PNH cells (see Figure 2).

TABLE 1 Recommended antibody clones and conjugates for High-Sensitivity PNH RBC and WBC assays

Target	Antibody conjugates	Purpose	Clone and vendor
RBC (all platforms)	CD235a-FITC	Gating on RBC	10F7MN (eBio),
			YTH 89.1 (Cedarlane)
			KC16 (BC), JC159 (DAKO)
	CD59-PE	GPI-linked for RBC	OV9A2 (eBio), MEM-43 (Invitrogen)
			MEM-43 (EXBIO/Cedarlane)
WBC (BC Cytometers)	FLAER-Alexa488	GPI-linked (Neuts+Monos)	NA (Cedarlane)
	CD24-PE	GPI-linked (Neuts)	SN3 (eBio), ALB9 (BC)
	CD24-APC		SN3 (eBio, EXBIO)
	CD14-PE	GPI-linked (Monos)	61D3 (eBio), RMO52 (BC)
	CD14-APC700		Tuk4 (Invitrogen) RMO52 (BC)
	CD157-PE	GPI-linked (Neuts+Monos)	SY11B5 (eBio, EXBIO, BD, BC, Sysmex)
	CD64-PC5	Gating on Monocytes	22 (BC)
	CD64-ECD		22 (BC)
	CD64-PC7		22 (BC), 10.1 (EXBIO)
	CD15-PC5	Gating on Neutrophils	80H5 (BC)
			MMA (eBio)
	CD15-PerCP-eF710		MEM158 (EXBIO)
	CD15-PerCPCy5.5		
	CD45-PC7	Debris/unlysed RBC exclusion+pattern recognition	J33 (BC)
			J33 (BC)
	CD45-KO		2D1 (eBio)
	CD45-eF450		
WBC (BD Cytometers)	FLAER-Alexa488	GPI-linked (Neuts+Monos)	NA (Cedarlane)
	CD24-PE	GPI-linked (Neuts)	SN3 (eBio), ML5 (BD)
	CD24-APC		SN3 (eBio, EXBIO)
	CD14-PE	GPI-linked (Monos)	61D3 (eBio),
			Tuk4 (Invitrogen)
	CD14-APC		MoP9 (BD)
	CD157-PE	GPI-linked (Neuts+Monos)	SY11B5 (eBio, EXBIO, BD, BC, Sysmex)
	CD64-APC	Gating on Monocytes	10.1 (BD, eBio)
	CD64-PECy7		10.1 (EXBIO), 22 (BC)
	CD15-APC	Gating on Neutrophils	HI98 (BD)
	CD15-PerCP-eF710		MMA (eBio)
	CD15-PerCPCy5.5		MEM 158 (EXBIO)
	CD45-eF450	Debris/unlysed RBC exclusion+pattern recognition	2D1 (eBio)
	CD45-PerCP		2D1 (BD)
	CD45-APC-H7		2D1 (BD)

2.4 | Separation between normal Type I RBC, PNH Type II, and PNH Type III RBCs

In most cases, the PNH clusters are evident and the initial region settings can be used for the quantification of the Type II and Type III PNH RBC based on the combination of bivariate plots (Figure 2). However, in some patients the regions may need to be adjusted (dynamic region setting) based on slight patient-specific differences in the CD59 expression rather than strictly adhering to the initial region setting. It is often difficult to delineate PNH Type II from PNH Type III and



Gate	Number	%Gated	X-Med	Y-Med
All	99,541	100.00	6.63	93.37
Type II PNH	28,456	28.59	9.69	94.11
TYPE III PNH	43,182	43.38	0.67	94.37

Gate	Number	%Gated	X-Med	Y-Med
All	99,541	100.00	6.63	93.37
Type II	28,456	28.59	9.69	94.11
Type III	43,182	43.38	0.67	94.37

Gate	Number	%Gated	X-Med
All	99,541	100.00	6.63

FIGURE 2 Patient with 28.6% PNH RBC Type II and 43.4% PNH RBC Type III clone, resulting in a total PNH clone size is 72.0%. Sample stained with CD235a-FITC/CD59-PE [Colour figure can be viewed at wileyonlinelibrary.com]

between Type II from normal cells which renders the interpretation challenging and the PNH clone size assessment and reporting problematic and potentially misleading. The addition of CD71, which stains immature RBCs (mainly reticulated RBCs in peripheral blood) allows a much more objective assessment (see below) as "PNH clone size" in immature RBCs is not affected by hemolysis or prior RBC transfusions.

2.5 | Assessment of PNH in immature RBCs

A recent study performed with CD235aFITC/CD59PE and CD71-APC[16] allowed both mature RBCs to be assessed for PNH clone content as well as the CD71-gated immature RBCs (mainly reticulocytes). The results showed the CD71-based assay is better able to delineate Type III, Type II, and normal reticulocytes in PNH cases with WBC clones of 5% or larger. Total reticulocyte clone sizes (Type II plus Type II) were in almost all cases, very similar to the WBC clone sizes (whether measured in neutrophils or monocyte lineages). The assay retained the ability to detect minor PNH RBC clones in other BMFS cases in which the lack of sufficient reticulocytes does not allow the assessment of the latter.

3 | PNH WBC ASSAY

3.1 | Antibody clone/conjugate selection

The inclusion of a pan-CD45 conjugate in the WBC staining cocktail is recommended as subcellular debris, platelets, and unlysed RBCs can be removed from analysis. As detailed elsewhere,[7-9,17] appropriately selected, validated, and titrated conjugates of CD15 and CD64 can accurately delineate neutrophils and monocytes. As outlined previously,[5,7-9] two GPI-linked structures must be analyzed per WBC lineage assessed. Combinations of FLAER and CD24, or FLAER and CD157 represent the most tested combination of reagents to detect GPI-deficient neutrophils, while FLAER in combination with either CD14 or CD157 represent the most validated combination of reagents to detect GPI-deficient monocytes.[8,9]

After much empirical testing of multiple clones and instrument-specific conjugates, recommended clones/conjugates of GPI-specific reagents for use on instruments equipped with 4-, 5-, and 6- (or more) PMTs are shown in Table 1 for Beckman and BD Biosciences platforms respectively,[9] and in Table 1 of Ref. [17] for recently developed cross-platform assays.

3.2 | Sample preparation, instrument setup, and compensation

To analyze white blood cells, 100 µL of anti-coagulated peripheral blood is stained with the reagent cocktail of choice and subject to a RBC lysis step. Regardless of lysing agent used, the cells need to be washed thereafter with phosphate buffered saline supplemented with 1% bovine serum albumin before acquisition. A multi-step Boolean gating approach has been adopted to efficiently gate neutrophils and monocytes and lymphocytes (used as internal staining controls). Light scatter voltages in linear mode and PMTv are optimized as detailed in several previous publications.[7-9] The WBC staining protocol has also been detailed elsewhere.[9,15]

The use of antibody capture beads is recommended for setting the compensation matrix but for FLAER-based assays an Alexa488 conjugate of CD3 (or similar) is required. Some manual optimization will still be required using a blood sample stained with the reagent cocktail in use. In the example shown in Figure 3 for a FLAER-based 6-color assay on a 10-color Navios cytometer, the compensation matrix was established with individual aliquots of VersaComp™ capture beads stained with CD3-Alexa488 (eBioscience/Thermo Fisher), CD24-PE, CD15-PC5, CD64-PC7, CD14-APCA700 and CD45-KO (all Beckman Coulter).

3.3 | Reagent selection

A large number of specific clones/conjugates have been assessed for lineage-specific gating and detection of GPI-deficient neutrophils and monocytes. Optimal titration ranges were established and

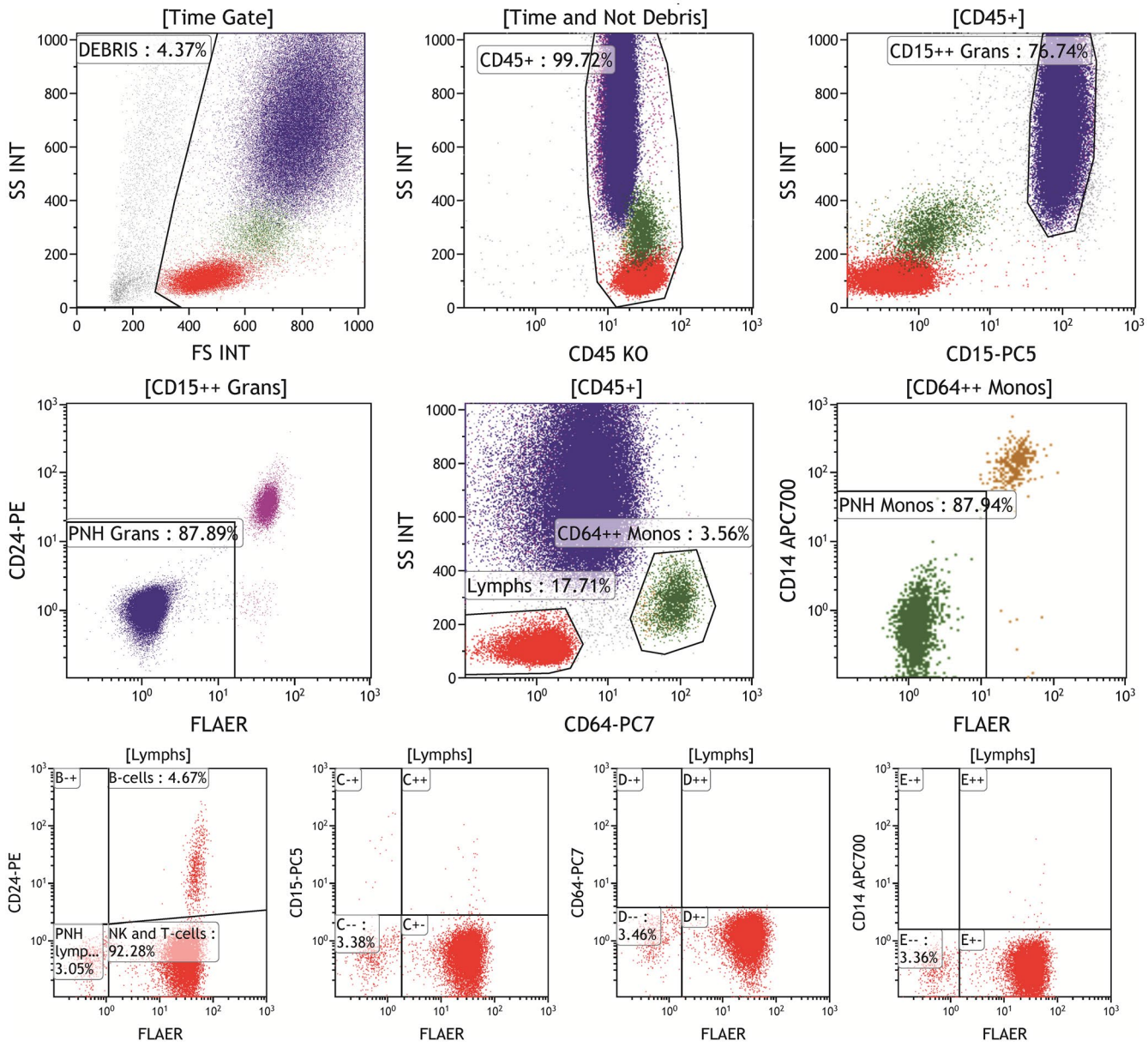


FIGURE 3 FLAER/CD24/CD14-based WBC assay with PNH clone present in neutrophils and monocytes. Sample stained with FLAER-Alexa 488/CD24-PE/CD15-PC5/CD64-PC7/CD14-APC700/CD45-KrO [Colour figure can be viewed at wileyonlinelibrary.com]

selected conjugates validated both individually and in combination with other reagents on both normal and PNH samples. Using this information, high-sensitivity assays were developed using 4-color cocktails based on FLAER, CD24, CD15, and CD45 (for neutrophils) and FLAER, CD14, CD64, and CD45 (for monocytes) ([7] and Supplementary data,[8,9]). These assays and reagent sets were reliable and reproducible across FC500 and FACSCalibur instrument platforms in subsequent studies.[18,19]

Thereafter, 5-color single tube assays capable of simultaneous high-sensitivity detection of both PNH neutrophils and PNH monocytes based on FLAER, CD157, CD15, CD64, and CD45 were developed for a variety of clinical cytometers with 5 or more PMTs,[8,20,21] and independently validated.[22] Instrument-specific FLAER/CD24/CD14-based 6-color[9,14,15,21] assays have been also developed for clinical instruments with 6 or more PMTs.

Most recently, 7-, 6-, and 5-Color reagent sets have been developed that can be analyzed on either/both BC Navios and BD Biosciences Canto II cytometers.[23] Thus for large reference flow laboratories that are often equipped with instruments from multiple manufacturers, a single reagent set can be employed and analysis performed on whichever instrument platform is available at the time.

Cocktailing of reagents is very important for WBC assays, just as it is for the RBC assay. For laboratories that run the assay infrequently, it is recommended that the laboratory stain a normal sample at least once per month to validate the performance of all reagents used in the assay.[7,24]

3.4 | Analysis and reporting

High-sensitivity methodologies to detect PNH phenotypes in neutrophils and monocytes have been published extensively.[5,7,24] Initial methods were based on a 4-color neutrophil tube (FLAER, CD24, CD15, and CD45) with a reflex monocyte tube (FLAER, CD14, CD64, and CD45). Newer flow cytometers with 5, 6, or more PMTs allow the simultaneous detection and quantification of both neutrophils and monocytes. Currently recommended WBC panels based on

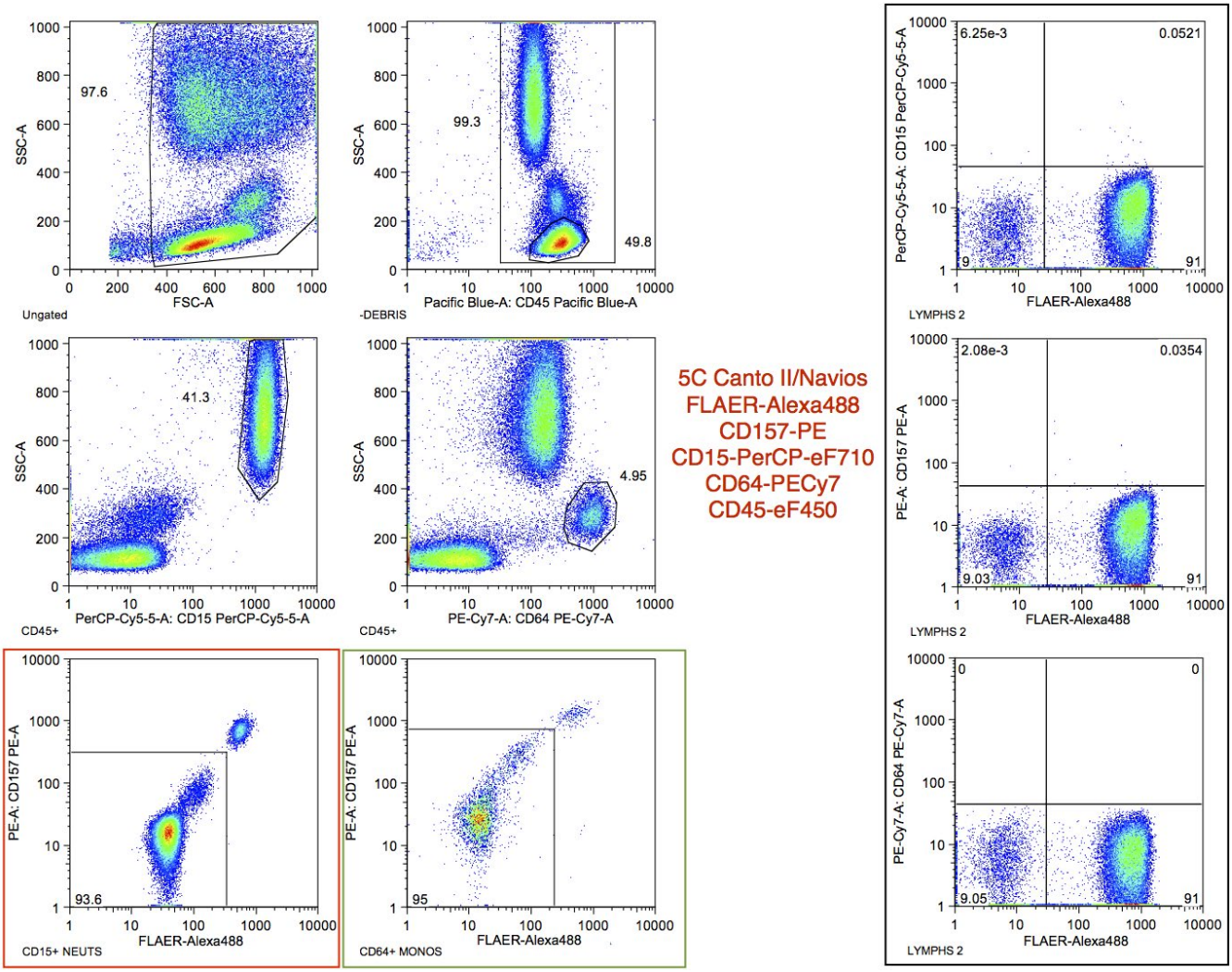


FIGURE 4 CD157 and FLAER-based cross-platform assay with PNH clone present in neutrophils and monocytes. Sample stained with FLAER-Alexa488/CD157-PE/CD15PerCPeFluor710/CD64-PECy7/CD45-eFluor450 [Colour figure can be viewed at wileyonlinelibrary.com]

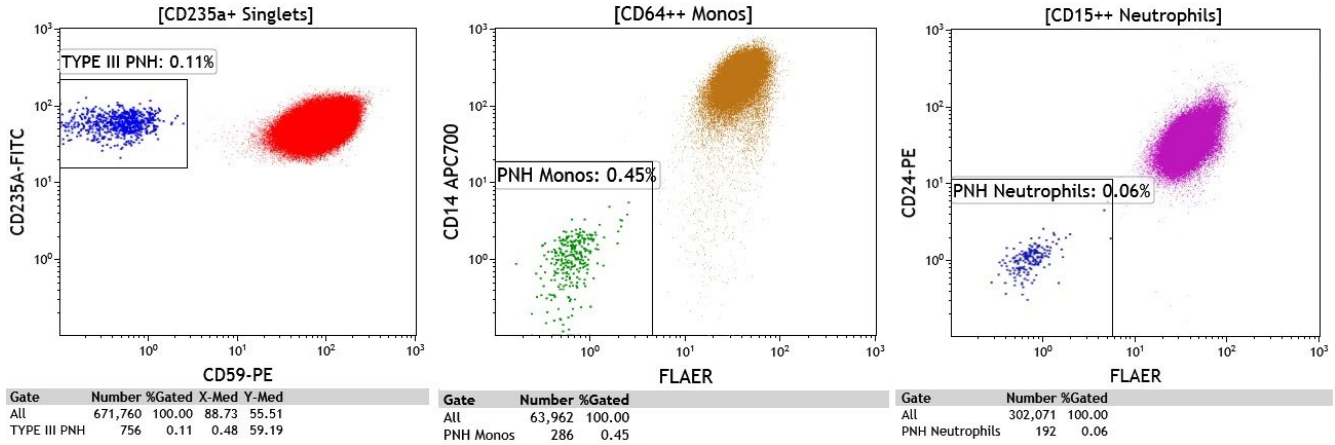


FIGURE 5 Minor clone in all 3 lineages showing a quantifiable PNH clone (>50 PNH cells) [Colour figure can be viewed at wileyonlinelibrary.com]

established PNH guidelines include a FLAER/CD24/CD14 based panel and a FLAER/CD157 based panel.[9,14]

3.4.1 | FLAER/CD24/CD14 based single tube assay for neutrophils and monocytes

In this panel (Figure 3), neutrophils are gated on CD45 and CD15 and a PNH clone can be detected based on the absence of the GPI markers FLAER and CD24. Monocytes are gated on CD45 and CD64 and a PNH clone can be detected based on the absence of FLAER and CD14. Analysis begins with review of TIME vs CD45 or Side Scatter on ungated events (not shown) to confirm that no fluidics irregularities are present. The lymphocytes gated on the CD64-negative/low SS plot are not a suitable target population for the PNH clone quantification due to their long life span. However, they serve as internal controls for verification of antibody specificity and compensation settings.

3.4.2 | FLAER/CD157 based single tube cross-platform assay for neutrophils and monocytes

In this panel (Figure 4), neutrophils are gated on CD45 and CD15 and monocytes are gated on CD45 and CD64. A PNH clone can be detected in both the neutrophils and monocytes based on the absence of the GPI markers FLAER and CD157. CD157 is highly expressed on both mature neutrophils and monocytes[25] leading to the possibility that CD157 could replace both CD24 and CD14, allowing the development of a single tube, high-sensitivity 5C assay to identify and quantify both PNH neutrophils and PNH monocytes on cytometers with 5 or more PMTs.[20] It is important to note that several CD157-negative, non-PNH cases have been observed in the authors' laboratories.[26] For these rare cases, the inclusion of the second GPI reagent (FLAER) as part of the built-in robustness of the assay prevents the misinterpretation of the data as a PNH clone-containing sample; the loss of two GPI-linked structures is required to identify *bona fide* PNH phenotypes.

4 | MINOR PNH CLONES

Regardless of lineage, when less than 1% of the target population is GPI deficient (see Figure 5), PNH clone sizes have been historically referred to as "minor".[24] Such small populations of GPI-deficient cells are often encountered in patients with AA and some subsets of MDS.[5] Clinically, these patients do not usually show symptoms of hemolysis but the presence of minor clones in AA has been associated with better response to immunosuppressive therapy.[27] AA patients must be monitored at specific intervals for possible clone size expansion to identify patients, who progress to clinical PNH.[6] From a technical perspective, the same PNH RBC and WBC reagent panels can be used to screen all patient samples. However, for those containing only small numbers of PNH phenotypes, the number of events acquired will need to be increased depending on the sensitivity of the assay as established by the laboratory. The generally accepted smallest number of events required to reproducibly detect a PNH population and determine the limit of detection (LOD) is 20 PNH events, the generally accepted smallest number of events required to reproducibly quantify a PNH population and determine lower limit of quantification (LLOQ) is 50 PNH events. Lower levels should be validated in each laboratory.

The characteristics of quasi-quantitative assays and the different relevant parameters are described in more detail in the ICCS/ESCCA PNH Consensus Guidelines, data analysis,[14] and validation section.[28]

5 | REPORTING

Based on the recent ICCS/ESCCA PNH consensus guidelines,[14] the following components are recommended for a PNH report:

1. Report if a *PNH clone present or absent*. It is important to be clear and to avoid potentially misleading ambiguous terminology. A report stating that a CD59 test is negative may imply to some providers that the target population is negative for the

GPI marker CD59 (thus indicating a PNH clone) or that no CD59 absence is seen (thus indicating the absence of a PNH clone).

2. Report the *PNH clone size in the RBCs* (total PNH clone size as well as the percentages for Type II and Type III PNH populations). There is a clinical significance associated with Type II and Type III RBCs. Type I RBCs are normal red blood cells with bright CD59 expression and a lifespan of approximately 120 days. Type III PNH RBCs have complete CD59 deficiency, which results in no protection from complement-mediated lysis and a shortened lifespan of 10-15 days. Type II PNH RBCs have partial CD59 deficiency resulting in partial protection from complement-mediated lysis. Just as the expression of CD59 on Type II RBCs varies considerably from patient to patient, the lifespan of Type II cells reflects this being intermediate between Type I normal RBCs and Type III PNH RBCs. Since the clinical significance of Type II PNH RBCs and Type III PNH RBCs is well established, it is recommended to report them separately and combined as the total PNH RBC clone. Since a recent transfusion will result in a decrease in RBC clone size, a comment such as "Any potential difference in clone size between the white blood cells and the red blood cells may be due to hemolysis and/or recent transfusion" should be included.
3. Report the *PNH clone size in both lineages for the WBCs* (neutrophils and monocytes). The PNH monocyte clone is often larger than the neutrophils PNH clone and reporting only the PNH neutrophil clone may underestimate the PNH clone size in the WBCs. Neutrophils and monocytes may also show the presence of Type II populations but the clinical and biological significance of these populations has not been established at this time. It is therefore recommended to report only the total PNH clone size in the neutrophils and monocytes.
4. *Standardized terminology of reporting PNH clones based on CSLI H52-A2*[29]:
   a PNH population >1%: "PNH clone"
   b PNH population 0.1% to 1%: "minor population of PNH cells" or "minor PNH clone"
   c PNH population <0.1%: " rare cells with GPI deficiency" or " rare cells with PNH phenotype
5. List *all gating and diagnostic markers* used for the PNH assay
6. State the *lower limit of quantification (LLOQ) for the neutrophil/granulocyte assay and the RBC assay* on the report, stating the recommended LLOQ of 0.05% or better for RBCs (100 000 gated cells) and 0.1% or better for neutrophils (50 000 gated cells). It is important to include this information to the provider as an LLOQ of 1% means that the possibility of a minor clone (<1%) cannot be excluded based on this LLOQ.
7. *Retesting recommendations*: see current recommendations and requirements based on International Guidelines for retesting frequencies in PNH[5] and related diseases.[6] The frequency of testing is dictated by clinical and hematological parameters: repeat testing is indicated upon any significant change in clinical or laboratory parameters and is suggested at least annually for routine monitoring[5,30] . In the setting of aplastic anemia, international guidelines recommend screening for PNH at diagnosis, and every 3 to 6 months initially, reducing the frequency of testing if the proportion of GPI-deficient cells has remained stable over an initial 2 year period.[6]

Examples of informative PNH reporting templates are shown in reference 13 and Appendix A of its Supplementary Data. An interactive reporting template that contains the above recommendations has recently been developed under the auspices of the Canadian PNH Network and can be downloaded at http://www.pnhnetwork.ca/pnh-resources.

6 | VALIDATION

The high-sensitivity PNH assay is intended to evaluate the presence or absence of a PNH clone with respect to the LOD as well as to quantify the PNH clone size if present with respect to the LLOQ. As a quasi-quantitative assay, the assay validation should meet criteria for accuracy, analytical and clinical specificity, analytical, functional and clinical sensitivity, repeatability, reproducibility and stability.[28,29]

7 | SUMMARY

High-sensitivity testing of all three lineages (RBC, neutrophils, and monocytes) has become the standard of care for patients with suspected PNH. Since this is a rare disease and often overlooked as a diagnostic possibility, it is important for the ordering physician to test the appropriate high-risk patient groups for the presence of PNH clones. The laboratories are often technically challenged with some of the assay-specific challenges, including the validation and ongoing quality control of this assay, selection of best antibody clones/conjugates, panel design and targeted acquisition and interpretation of data. We hope that these latest PNH consensus guidelines provide comprehensive guidance for best practices in PNH testing to all laboratories. Standardized reporting based on currently available guidelines is important to communicate to the physician the size of the PNH clone, which aids him/her in the decision-making for optimal treatment of the patient.

COMPETING INTEREST

The authors have no competing interests.

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