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Extending the Capabilities of a High-Parameter Immunophenotyping Assay with Cytoplasmic Staining Applications for Mass Cytometry

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Abstract

Mass cytometry, powered by CyTOF® technology, utilizes monoisotopic metal-tagged antibodies and a high-sensitivity mass cytometer to allow high-dimensional single-cell analysis in complex biological samples. The 30-marker panel Maxpar® Direct™ Immune Profiling Assay™ (Cat. No. 201325) for suspension mass cytometry provides an unprecedented sample-to-answer solution for detecting and analyzing 30 surface markers to identify 37 immune cell subsets (Figure 1A).[1,2] The 18 open mass channels (Figure 1B) in the Maxpar Direct Assay facilitate panel expansion and enable flexibility for higher multiplexity and applications. Among the potential applications with the Maxpar Direct Assay, intracellular cytokine staining (ICS) is of particular interest as it may be used to assess antigen-specific immune responses. However, for assessing cell viability in ICS, the effectiveness of the Cell-ID™ Intercalator-Rh (103Rh, Cat. No. 201103) is in question, since cell permeabilization during ICS can potentially damage the DNA-intercalator bond.

In this study, we investigated the compatibility of 103Rh with ICS. To do this, we stained either human peripheral blood mononuclear cell (PBMC) or whole blood samples (FLDM-400287) with the Maxpar Direct Assay, which includes 103Rh, followed by ICS for the detection of expressed cytokines. 103Rh was evaluated for its ability to discriminate live and dead cells when the sample undergoes surface antibody staining. We demonstrate that 103Rh provides equivalent functionality as a cell viability indicator during ICS compared to the benchmark reagent Cell-ID monoisotopic Cisplatin-194Pt (Cat. No. 201194) or Cisplatin-198Pt (Cat. No. 201198). This work was designed to support the use of the Maxpar Direct Assay in combination with additional intracellular cytoplasmic markers. Overall, these findings expand the applicability of 103Rh to processes that involve cytoplasmic or secreted antigen staining.

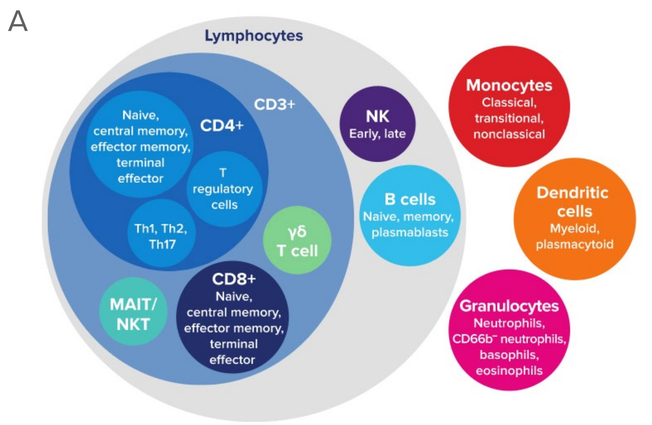

Figure 1. A) A comprehensive immune profile. The 37 immune cell subsets identified using the Maxpar Direct Immune Profiling Assay and Maxpar Pathsetter™ software.[3] B) The 30 markers plus 18 open channels in the Maxpar Direct Immune Profiling Assay.[3]

Materials and Methods

Catalog Number	Antibody (clone)	Isotope
201103A/B	Cell-ID Intercalator-Rh	^{103}Rh
N/A	TNFα (MAb11)	^{114}Cd
N/A	IFNγ (B27)	^{116}Cd
N/A	Perforin	^{196}Pt
N/A	Granzyme B	^{198}Pt
201192A/B	Cell-ID Intercalator-Ir	^{191}Ir/^{193}Ir
201194	Cell-ID Cisplatin-^{194}Pt	^{194}Pt
201198	Cell-ID Cisplatin-^{198}Pt	^{198}Pt
201325	Maxpar Direct Immune Profiling Assay	Various

Table 1. List of Maxpar cell staining reagents used in this work

Surface staining by the Maxpar Direct Immune Profiling Assay

- Cell staining by the Maxpar Direct Immune Profiling Assay was performed following Maxpar Direct Immune Profiling Assay in Whole Blood (FLDM-400287) and Maxpar Direct Immune Profiling Assay in PBMC (FLDM-400288).

Cell-ID Intercalator-Rh in cytoplasmic staining of whole blood samples

- ^{114}Cd-TNFα and ^{116}Cd-IFNγ were selected as the intracellular cytoplasmic (IC) markers for intracellular staining (FLDM-400279) of whole blood samples following surface staining by the Maxpar Direct Immune Profiling Assay.
- The staining procedure is shown in Figure 2. Whole blood samples were stimulated by PMA/ionomycin for 5 hours. Heat-kill PBMC, labeled with Cell-ID ^{198}Pt, were spiked into the whole blood samples prior to surface staining by the Maxpar Direct Assay, which contains Cell-ID Intercalator-Rh for cell viability identification.
- For each whole blood donor, 4 samples were stained and acquired on a Helios™ instrument:
  - Unstimulated whole blood sample stained by Maxpar Direct Immune Profiling Assay
  - Stimulated whole blood sample stained by Maxpar Direct Immune Profiling Assay
  - Unstimulated whole blood sample stained by Maxpar Direct Immune Profiling Assay and IC markers
  - Stimulated whole blood sample stained by Maxpar Direct Immune Profiling Assay and IC markers
- Whole blood samples from 2 donors were tested.
- Applicability of Cell-ID Intercalator-Rh as cell viability indicator in intracellular cytoplasmic staining of whole blood samples was assessed by comparing the percentages of live cells identified by Cell-ID Cisplatin-^{198}Pt and Cell-ID Intercalator-Rh.

Materials and Methods

Cell-ID Intercalator-Rh in cytoplasmic staining of whole blood samples

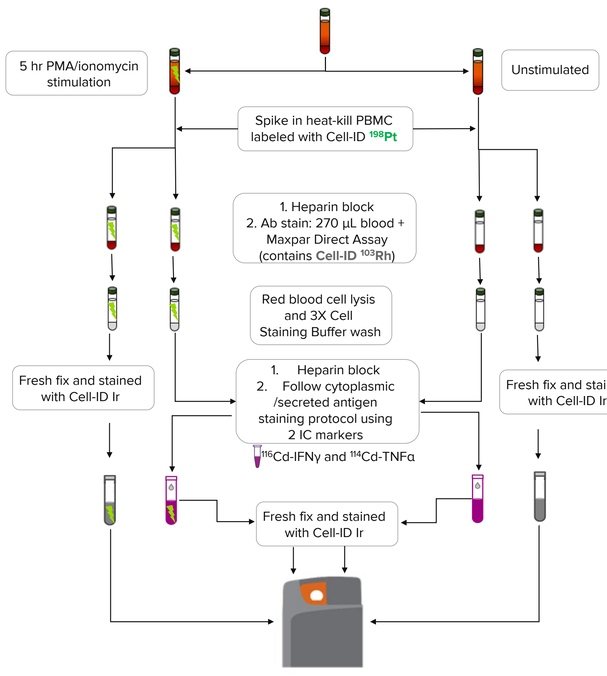

Figure 2. Experimental procedure for assessing the applicability of using Cell-ID Intercalator-Rh in intracellular cytoplasmic staining of whole blood samples

Cell-ID Intercalator-Rh in cytoplasmic staining of human PBMC samples

- ^{196}Pt-perforin and ^{198}Pt-granzyme B were selected as the IC markers for intracellular staining (FLDM-400279) of human PBMC samples following surface staining by the Maxpar Direct Immune Profiling Assay. Neither of the two IC markers requires stimulation for expression in human PBMC.
- The staining procedure is shown in Figure 3. Human PBMC were either stained by anti-human B2M antibodies or heat-killed. The heat-kill PBMC and the B2M-labeled PBMC were combined and split into 3 samples:
  - PBMC stained by Maxpar Direct Immune Profiling Assay (which contains Cell-ID Intercalator-Rh)
  - PBMC stained by Cell-ID ^{194}Pt and then stained by Maxpar Direct Immune Profiling Assay
  - PBMC stained by Maxpar Direct Immune Profiling Assay with 1X amount of Cell-ID Intercalator-Rh added to the assay (total 2X)
- Each sample was stained in duplicate.
- Compatibility of Cell-ID Intercalator-Rh as cell viability indicator in intracellular cytoplasmic staining of PBMC samples was assessed by comparing the percentage of live cells identified by Cell-ID Cisplatin-^{194}Pt and Cell-ID Intercalator-Rh as well as co-staining of Cell-ID Cisplatin-^{194}Pt and Cell-ID Intercalator-Rh.

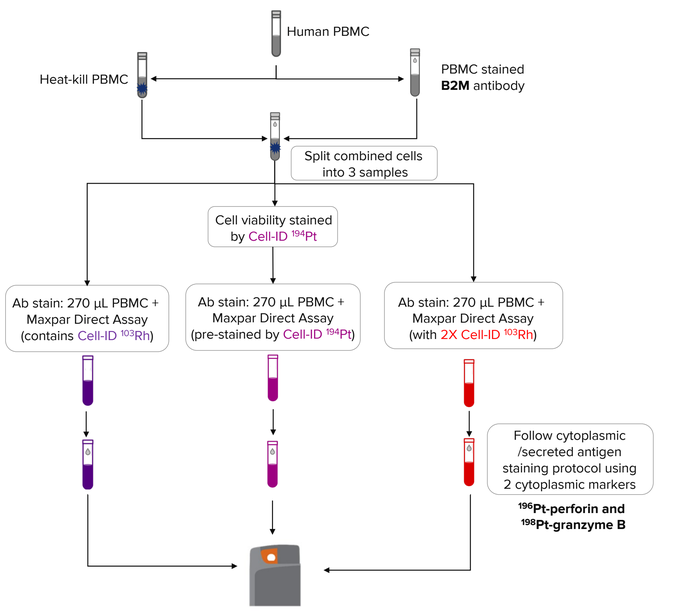

Figure 3. Experimental procedure for assessing the applicability of using Cell-ID Intercalator-Rh in intracellular cytoplasmic staining of human PBMC samples

Results

Cell-ID Intercalator-^{103}Rh is compatible with cytoplasmic staining of whole blood samples

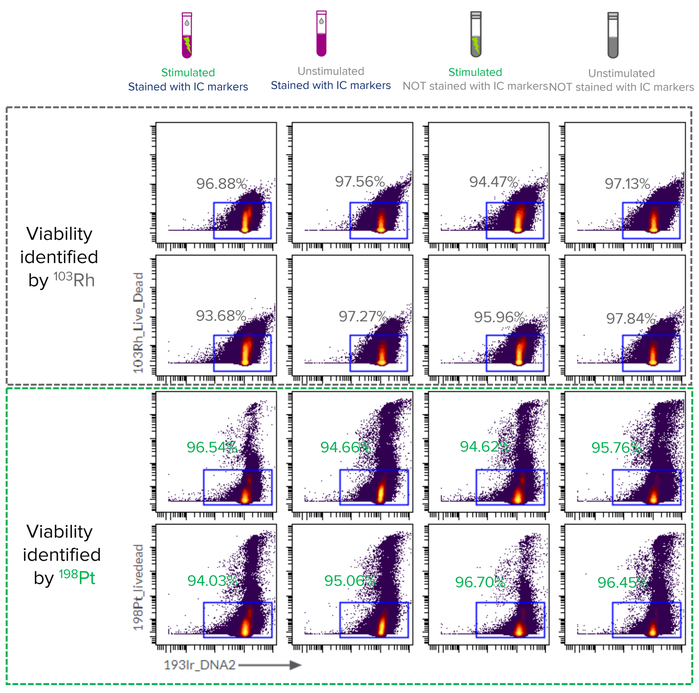

Figure 4. Percentages of live cells identified by Cell-ID Intercalator-Rh are comparable to those identified by Cell-ID Cisplatin-^{198}Pt in intracellular cytoplasmic staining of whole blood samples. Biaxial plots are presented to show ^{103}Rh negative (red dashed boxes) or ^{198}Pt negative (green dashed boxes) populations from 2 whole blood donors. Data were gated on all cellular events cleaned up by the Gaussian parameters.[4] The applicability of Cell-ID Intercalator-^{103}Rh in intracellular cytoplasmic staining was demonstrated by: 1) measured cell viabilities in samples that underwent both surface staining and intracellular cytoplasmic staining were comparable to samples that were only surface-stained by the Maxpar Direct Assay; 2) cell viabilities measured by Cell-ID Intercalator-^{103}Rh were comparable to those measured by Cell-ID Cisplatin-^{198}Pt in samples that underwent both surface staining and intracellular cytoplasmic staining.

Results

^{114}Cd-TNFα

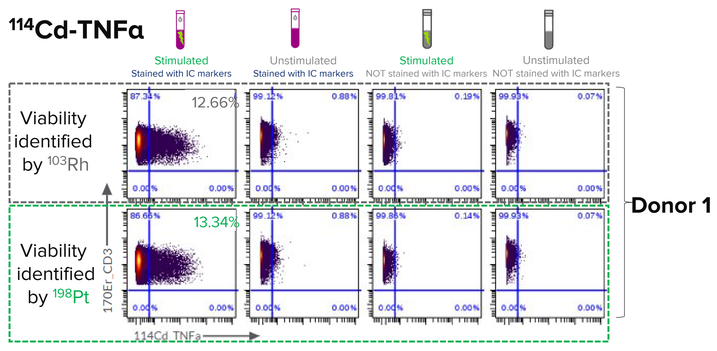

Figure 5. Intracellular cytoplasmic markers show comparable functional performance in live cells gated by Cell-ID Intercalator-^{103}Rh or Cell-ID Cisplatin-^{198}Pt. Results from Donor 1 are shown. Biaxial plots show TNFa and CD3 expression on CD3+ T cells (top panel) and IFNy and CD3 expression on CD8+ T cells (bottom panel) in stimulated and IC stained whole blood samples. Both IC markers showed comparable functional gating when the live cells were identified by Cell-ID Intercalator-Rh or Cell-ID Cisplatin-^{198}Pt Minimal background staining was observed with both intracellular markers in unstimulated samples

Cell-ID Intercalator-^{103}Rh is compatible with cytoplasmic staining of PBMC samples

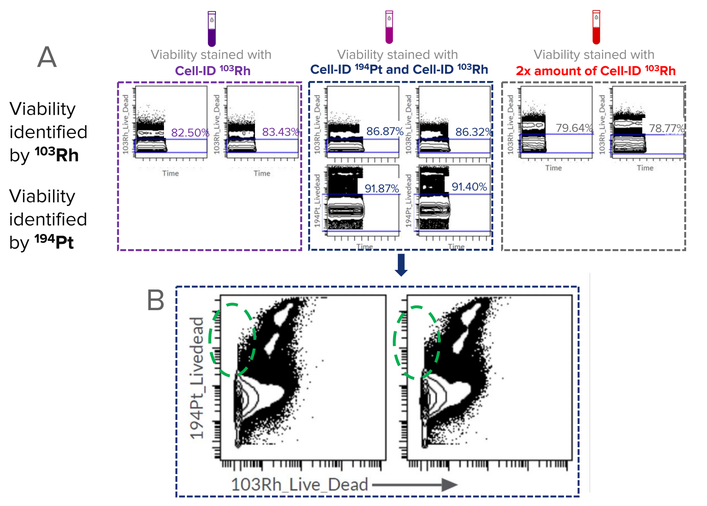

Figure 6. Cell-ID Intercalator-Rh demonstrates resilience in fixation and permeabilization processes during intracellular cytoplasmic staining. Biaxial plots show each staining condition was performed in duplicate.
A) Marginally higher percentages of live cells were observed in the samples that were stained with the Maxpar Direct Assay (contains **Cell-ID ^{103}Rh, purple dashed rectangle**) than those stained with the Maxpar Direct Assay with added ^{103}Rh (**2X amount of Cell-ID ^{103}Rh, red dashed rectangle**). The two sets of samples were stained following very similar workflow (Figure 3). However, the 2X amount of Cell-ID ^{103}Rh could result in more nonspecific staining as demonstrated by the wider ^{103}Rh negative gates in the biaxial plots in the **red dashed rectangle**. Small variation in the percentages of live cells was observed in samples that had both **Cell-ID ^{194}Pt and Cell-ID ^{103}Rh** (samples stained with Cell-ID ^{194}Pt followed by the Maxpar Direct Assay). This variation is likely a result of more cells dying during the additional step of staining by **Cell-ID ^{194}Pt**. **B)** Among the samples that had both **Cell-ID ^{194}Pt and Cell-ID ^{103}Rh**, biaxial plots show that there is no ^{194}Pt+ ^{103}Rh− population present. Such population would represent cells that were viability stained by both ^{194}Pt and ^{103}Rh and subsequently lost the intercalator ^{103}Rh, likely during the harsh condition of cell permeabilization. The absence of this ^{194}Pt+ ^{103}Rh− population demonstrates the applicability of using Cell-ID Intercalator-^{103}Rh as a viability indicator during intracellular cytoplasmic staining.

^{198}Pt-granzyme B

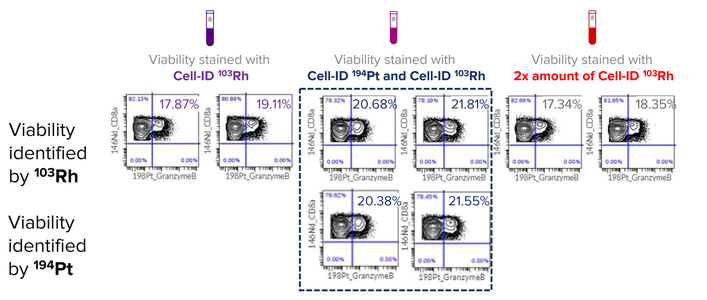

Figure 7. Intracellular cytoplasmic markers show comparable functional gating in live cells gated by Cell-ID Intercalator-^{103}Rh or Cell-ID Cisplatin-^{194}Pt. Results for ^{198}Pt-granzyme B are presented. Biaxial plots show granzyme B and CD8a expressions in CD8+ T cells in human PBMC samples. No significant difference was observed in the percentages of granzyme B+ CD8a+ populations among the samples that were stained with the Maxpar Direct Assay (contains **Cell-ID ^{103}Rh**) or the Maxpar Direct Assay with added ^{103}Rh (**2x amount of Cell-ID ^{103}Rh**). The two sets of samples were stained following very similar workflows (Figure 3). Small variation of granzyme B+ CD8a+ populations was observed in samples that had both **Cell-ID ^{194}Pt and Cell-ID ^{103}Rh** (samples stained with Cell-ID ^{194}Pt followed by the Maxpar Direct Assay) and is shown in the biaxial plots in **the dashed rectangle**. Among these samples, no significant difference in the percentages of granzyme B+ CD8a+ populations was observed when the population was gated on live cells identified by Cell-ID Intercalator-^{103}Rh or Cell-ID Cisplatin-^{194}Pt.

Conclusions

- Cell-ID Intercalator-^{103}Rh shows comparable functional gating as the viability indicator in intracellular cytoplasmic staining of human whole blood and PBMC samples versus Cell-ID Cisplatin-^{194}Pt or -^{198}Pt.
- Intracellular cytoplasmic markers used in this work show effective staining on human whole blood and PBMC samples following staining by the Maxpar Direct Immune Profiling Assay, when the live cells were gated using either Cell-ID Intercalator-^{103}Rh or Cell-ID cisplatin reagents.
- This work provides a foundational understanding to support expanding the applications of the Maxpar Direct Immune Profiling Assay to immune profiling involving intracellular cytoplasmic markers.

References

1. Beckmann, N. et al. “Downregulation of exhausted cytotoxic T cells in gene expression networks of multisystem inflammatory syndrome in children.” *Nature Communications* 12 (2021): 4854.
2. De Biasi, S. et al. “Endogenous control of inflammation characterizes pregnant women with asymptomatic or paucisymptomatic SARS-CoV-2 infection.” *Nature Communications* 12 (2021): 4677.
3. The Maxpar Direct Immune Profiling System (FLDM-400246).
4. Fluidigm Technical Note: Approach to Bivariate Analysis of Data Acquired Using the Maxpar Direct Immune Profiling Assay (FLDM-400248).

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Acknowledgments and ethics statement
We are grateful to the Canadian Blood Services and the blood donors who made this research possible. Donors provided IRB approved consent. The views expressed herein do not necessarily represent the views of Canadian Blood Services or the federal, provincial or territorial governments of Canada.