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# Validation of Treg, Th17 and Plasma Cell Assays

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## PURPOSE

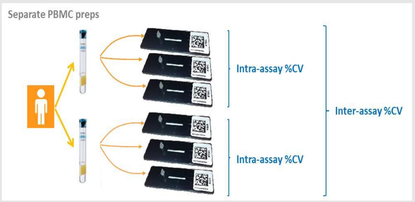
Cytometry is a powerful analytical tool for the analysis of multiple biological parameters of individual immune cells within heterogeneous cell populations [1,2]. The implementation of standardized cytometry-based biomarker assays in clinical trial support remains a challenge due to inter-instrument variations and the limited stability of clinical specimens.

Chipcytometry is an image cytometry platform enabling long-term sample storage. Here we present validation data (precision, stability) on three assays (regulatory T-cells (Treg), T-helper 17 cells (Th17) and plasma cell assays), following industry guidelines using an automated Chipcytometry system (CYTOBOT™).

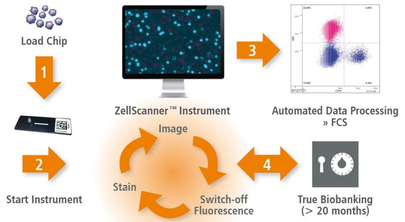
## METHODS

- Three assay panels were developed using commercially available, directly labelled antibodies against 7 markers (see table below).
- Human PBMC samples from 5 healthy donors were loaded in microfluidic cassettes (ZellSafe™) for precision and stability assessments.
- Assays were validated for Treg, Th17 and plasma cells, according to industry guidelines [3,4].
- Data processing of images was performed using an automated gating algorithm (ZellScanner™ software) and the statistical programming language R [5].

| Cell Type | Markers |
|---|---|
| Plasma Cells | CD19/CD27/CD38 |
| Th17 Cells | CD3/CD4/CD161 |
| Treg Cells | CD3/CD4/Foxp3 |




## PRINCIPLE




## INSTRUMENT

## RESULTS - PRECISION

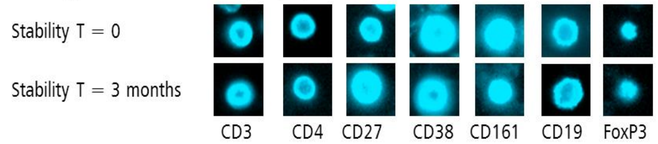
Determination of the intra-assay precision showed a mean CV of 14.6% (plasma cell assay), 16.2% (Th17 assay) and 19.1% (Treg assay). The mean inter-assay CV, which comprises both within- and between-batch variability was 19.3% (plasma cell assay), 29.6% (Th17 assay) and 22.5% (Treg assay).

| Parameter | Assay | Donor | | | | |
|---|---|---|---|---|---|---|
| | | A | B | C | D | E |
| **Intra-Assay Precision** | | | | | | |
| Plasma Cell Assay | 1 | 15.7 | 18.3 | 12.0 | 9.8 | 14.2 |
| | 2 | 18.3 | 18.3 | 10.0 | ND | ND |
| Th17 Assay | 1 | 12.8 | 13.3 | 15.5 | 10.2 | 8.2 |
| | 2 | 24.0 | 29.6 | 15.9 | ND | ND |
| Treg Assay | 1 | 26.7 | 32.3 | 19.5 | 13.2 | 12.6 |
| | 2 | 39.6 | 3.9 | 4.9 | ND | ND |
| **Inter-Assay Precision** | | | | | | |
| Plasma Cell Assay | | 25.1 | 22.6 | 10.1 | ND | ND |
| Th17 Assay | | 34.5 | 27.8 | 26.6 | ND | ND |
| Treg Assay | | 30.2 | 20.7 | 16.6 | ND | ND |

## RESULTS - STABILITY

Stability analysis of 5 PBMC samples from 5 donors, stored in ZellSafe™ cassettes at 4° C for 3 months, revealed that 5/5 samples (Treg assay) and 4/5 samples (plasma cell assay and Th17 assay) were within the 25% range from baseline level, which was within predefined acceptance criteria.

**Staining on PBMCs from a single preparation (baseline vs. 3 months)**




## CONCLUSIONS

- Three Chipcytometry assays for quantification of Treg, Th17 and plasma cells in human PBMC samples were deemed fit-for-purpose after validation.
- Chipcytometry is an alternative cytometric platform, which allows banking of PBMC samples, permitting retrospective multi-parameter batch analysis for novel biomarkers that may not have been envisioned at the beginning of a clinical trial.
- Further characterization of this method will determine its value for immunomonitoring purposes in the clinical development of immune-based therapeutics.

## REFERENCES

[1] Hennig et al. Cytometry A. 2009: A versatile platform for comprehensive chip-based explorative cytometry.

[2] Happle et al. Science Translational Medicine. 2014: Pulmonary transplantation of macrophage progenitors as effective and longlasting therapy for hereditary pulmonary alveolar proteinosis.

[3] O'Hara et al. Journal of Immunological Methods (2010): Recommendations for the validation of flow cytometric testing during drug development: II assays.

[4] Wood et al. Clinical Cytometry, Part B 2013: Validation of Cell-based Fluorescence Assays: Practice Guidelines from the ICSH and ICCS.

[5] R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna,Austria. URL https://www.R-project.org/.