GENERATION OF BLOOD-DERIVED REFERENCE SAMPLES WITH ENDOGENOUS CYTOKINES

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ENSURING BIOMARKER DATA ARE RELIABLE

- **Biomarkers are an integral part of drug development and decision-making.**
- Robust and performant methods needed for measuring immune mediators, commonly used to gain insights into pathological processes & monitoring therapeutic intervention in **IBD**.

Context

1. Is the calibrator material suitable for the quantification of the endogenous analyte : is the sample-dilution response curve parallel to calibration curve?
2. Is the endogenous analyte selectively measured in the milieu of complex matrix components at the selected MRD?
3. How long will the biomarker be stable in the biological matrix under the storage conditions of the clinical study?

Objectives

Generating reference samples with sufficient endogenous levels of cytokines / chemokines closer than samples spiked with recombinant material to clinical samples to enable evaluation of :

- Parallelism & Selectivity of the method
- Stability of the biomarker in the biological fluid

1. Production & characterization of MASTER ACT QC

I. Generation of Cytokine Concentrates

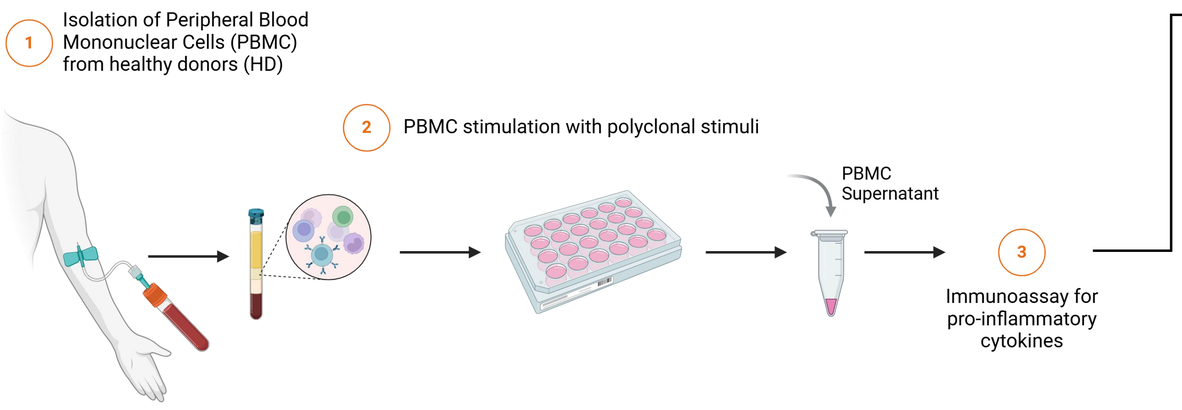

Figure 1 – From PBMC collection to pro-inflammatory quantification

II. Generation of plasma & serum with different levels of pro-inflammatory cytokines

4 Spiking of PBMC supernatants at different ratios (≤5%) in plasma and/or serum from HD

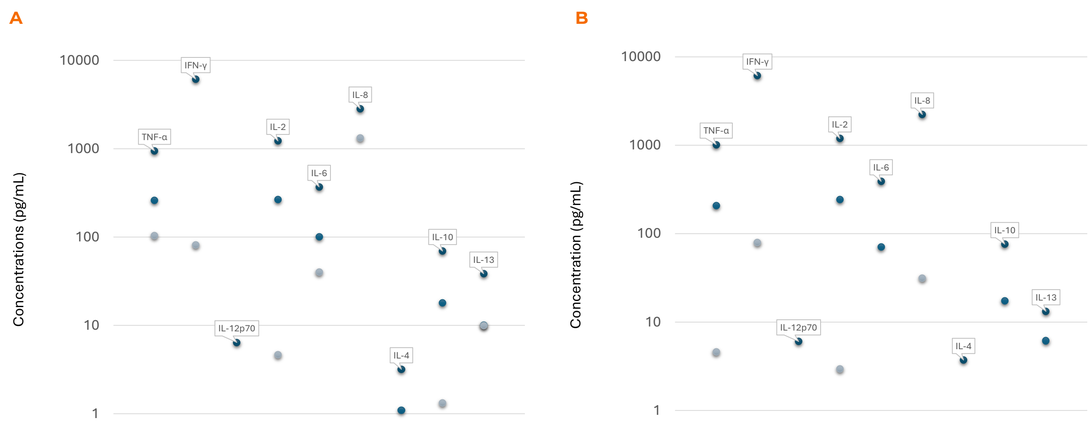

Figure 2 – Cytokine concentrations after spiking of PBMC supernatatants in serum (A) or plasma (B) to get reference samples with one (IL-12p70), 2 (IFN-γ, IL-4, IL-8 & IL-13) or 3 different levels (TNF-α , IL-2, IL-6 & IL-10) of the proinflammatory cytokines. Levels of cytokines were measured in duplicate with the MSD V-plex Human pro-inflammatory Panel 1 kit at the minimal required dilution (MRD) recommended by MSD.

2. Selectivity in plasma and serum

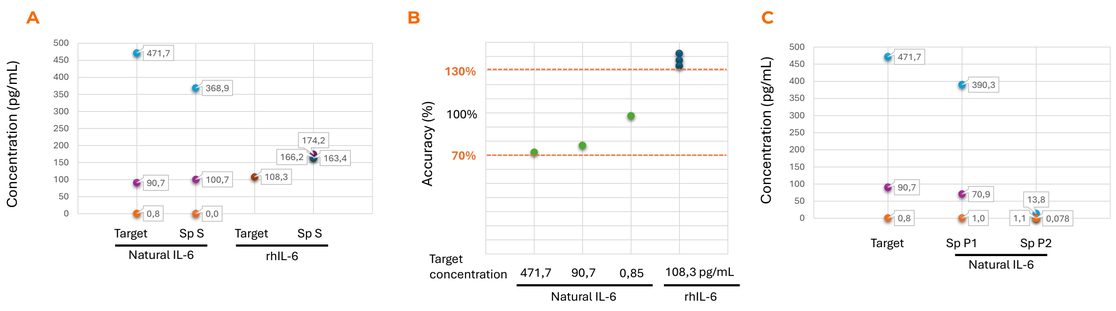

Figure 3 - Selectivity of ELLA method for quantification of natural and recombinant human IL-6 in human serum (S) or plasma (P1-2). Cytokine concentrations values (data labels) in pg/mL were measured in duplicate with the ELLA Simple plex at MRD in spiked serum (Sp S, A) or plasma (Sp P, C). Accuracy of IL-6 quantitation for natural (green symbols) or rhIL-6 (dark blue symbol) spiked in serum is shown (B). Acceptance boundaries (70-130%) and target concentration values for natural & rhIL-6 are indicated.

- **Natural cytokines are sometimes more accurately quantified than recombinant** Bias in the quantitation of rhIL-6 (B, blue symbols) compared to natural IL-6 (green symbols) with the ELLA Simple plex method
- **Major interference detected in 1 of 2 human plasma samples** (C, P2) likely due to the presence of soluble IL-6 receptor

3. Endogenous cytokines dilute similarly to calibrator, except for IL-13

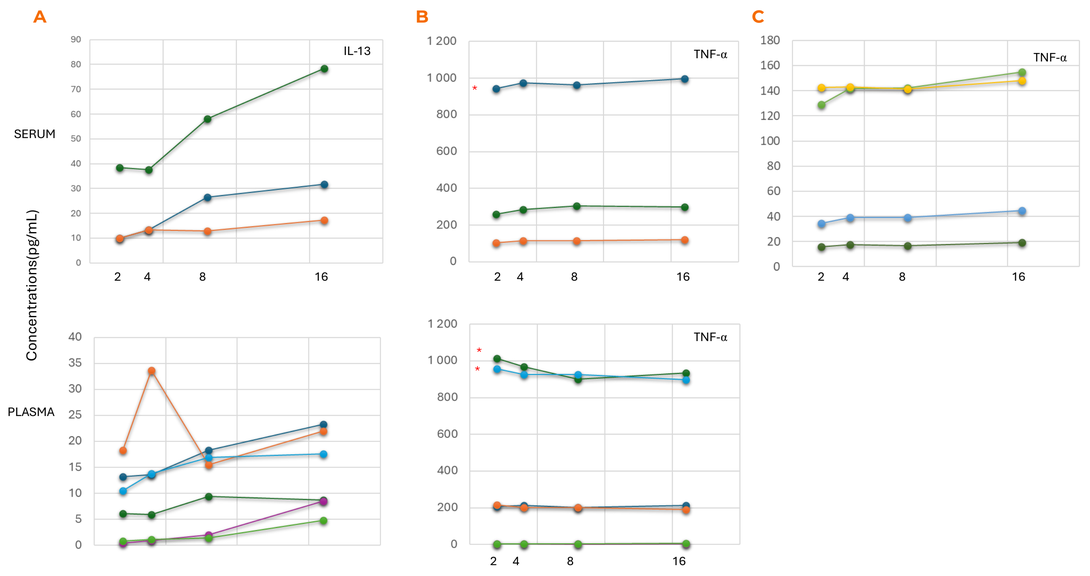

Figure 4 - Parallelism of IL-13 and TNF-α in blood matrices. Serum (n=1) or plasma (n=2) from healthy volunteers were spiked neat with 3 levels of IL-13 (A) or TNF- α (B-C). Cytokine concentrations were measured in duplicate with the MSD V-plex Human pro-inflammatory panel 1 kit (A-B) or the ELLA Simple plex human 7-plex cytokine kit (C) from 1/2 (MRD) to 1/16 dilution. Concentrations, adjusted for dilution, are shown.
* Above LOQ

4. Long term stability at -80°C

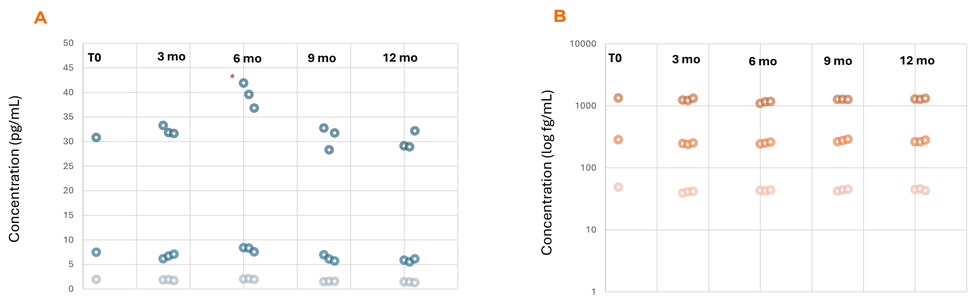

Figure 5 - Long term stability of IL-1β (A) and IL-4 (B) in serum. Serum was spiked at T0 with different concentrations of PBMC supernatants, aliquoted and frozen at -80°C before cytokine quantitation. Concentrations of IL-1β in pg/mL (A) and IL-4 in fg/mL (B, log scale) were measured in duplicate with respectively the ELLA Simple plex human 7-plex cytokine kit or the MSD S-plex human IL-4 kit at T0, and after 3, 6, 9 and 12 months (mo) storage at -80°C. * One out of 3 values above the accuracy criteria (Bias <30%)

Stability of all cytokines in MASTER ACT'QC for 12 months at -80°C

5. Conclusion

Reference samples with endogenous cytokines/chemokines :

- can be produced from primary immune cells
- are more representative of clinical samples than samples spiked with recombinant proteins
- can be used to assess parallelism, long-term stability but also inter-lot consistency when sufficient volume of matrices with high enough concentrations are not available
- but still a long journey for using them as QC for precision & accuracy runs in method validation (Broader & higher levels of immune mediators, deeper characterization of cytokine cocktail,...)

Reach out for more information