BioAgilytix

EBF training day: Bioanalytical Strategies for Cell & Gene Therapies

Case Studies: The use of ELISpot in CGT

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2019 WRIB recommendations

Reminder
Piccoli *et al.*, (2019)

Challenges

- ELISpot assays require a more complex workflow from sample collection to testing
- Sample collection procedures should be developed.
- Multiple pre-dose samples can be collected in order to generate a more robust baseline value.
- Results can be normalized relative to pre-study values in order to partially mitigate inter-site differences in sample collection and handling.
- The potential sources of variability make the need for standardized approaches even more important.

Recommendations

- ELISpot may be used to monitor for cellular immunity, if used it should be developed using a risk-based approach factoring in the route of administration;
- ELISpot results can be normalized (intra- and inter-subject), special accommodation should be made for particular sites taking into account known variabilities in the assay. Not every site may be required to conduct every analysis. Multiple baselines can be used;
- ELISpot harmonization consortium White Paper which outlines assay expectations and performance criteria may be helpful to develop ELISpot assays;

2019 White Paper on Recent Issues in Bioanalysis: FDA Immunogenicity Guidance, Gene Therapy, Critical Reagents, Biomarkers and Flow Cytometry Validation (Part 3 – Recommendations on 2019 FDA Immunogenicity Guidance, Gene Therapy Bioanalytical Challenges, Strategies for Critical Reagent Management, Biomarker Assay Validation, Flow Cytometry Validation & CLSI H62)

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Outline

- Challenges addressed
  - Sample logistics and processing:
    - Sample quality
    - Mitigate differences due to sample collection and handling
  - Lack of positive controls
  - Variability of response to various antigens
  - Standardization
  - Validation parameter

- Evolving trends: items for further discussions

Flow of the Immune Response

Sample quality – isolation technique

Step 1: Isolation of Peripheral Blood Mononuclear Cells (PBMC) from whole blood: which technique?

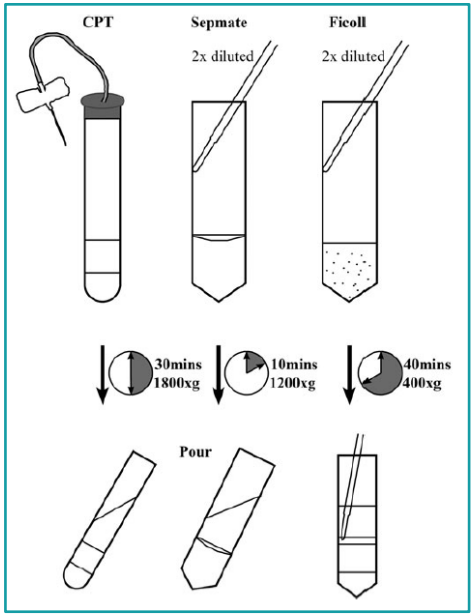


TABLE 1. SUMMARY OF FINDINGS

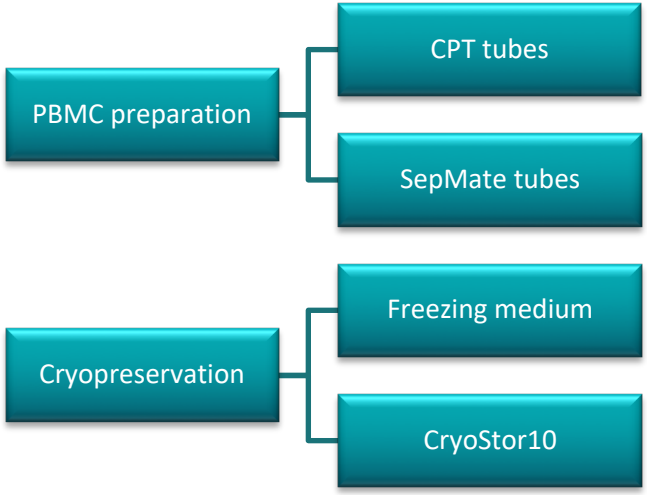
Item	*Comparison of techniques*
Cell recovery	CPT, SepMate>Ficoll
Cell viability	No major differences between techniques
PBMC population composition	No major differences between techniques
Functionality; SEB-induced cytokines	CPT, SepMate>Ficoll
Functionality; spontaneous cytokines	CPT, SepMate>Ficoll
Functionality; cytotoxicity/cell stress	No major differences between techniques

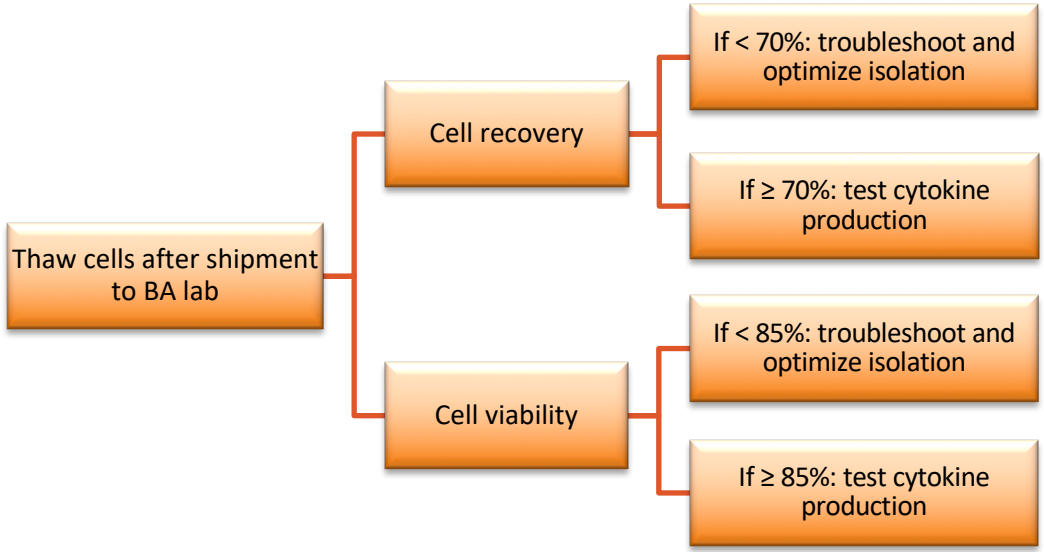
CPT, cell preparation tube; PBMC, peripheral blood mononuclear cell; SEB, staphylococcal enterotoxin B.

Grievink et al., (2016) Comparison of Three Isolation Techniques for Human Peripheral Blood Mononuclear Cells: Cell Recovery and Viability, Population Composition, and Cell Functionality

PBMC storage and shipment

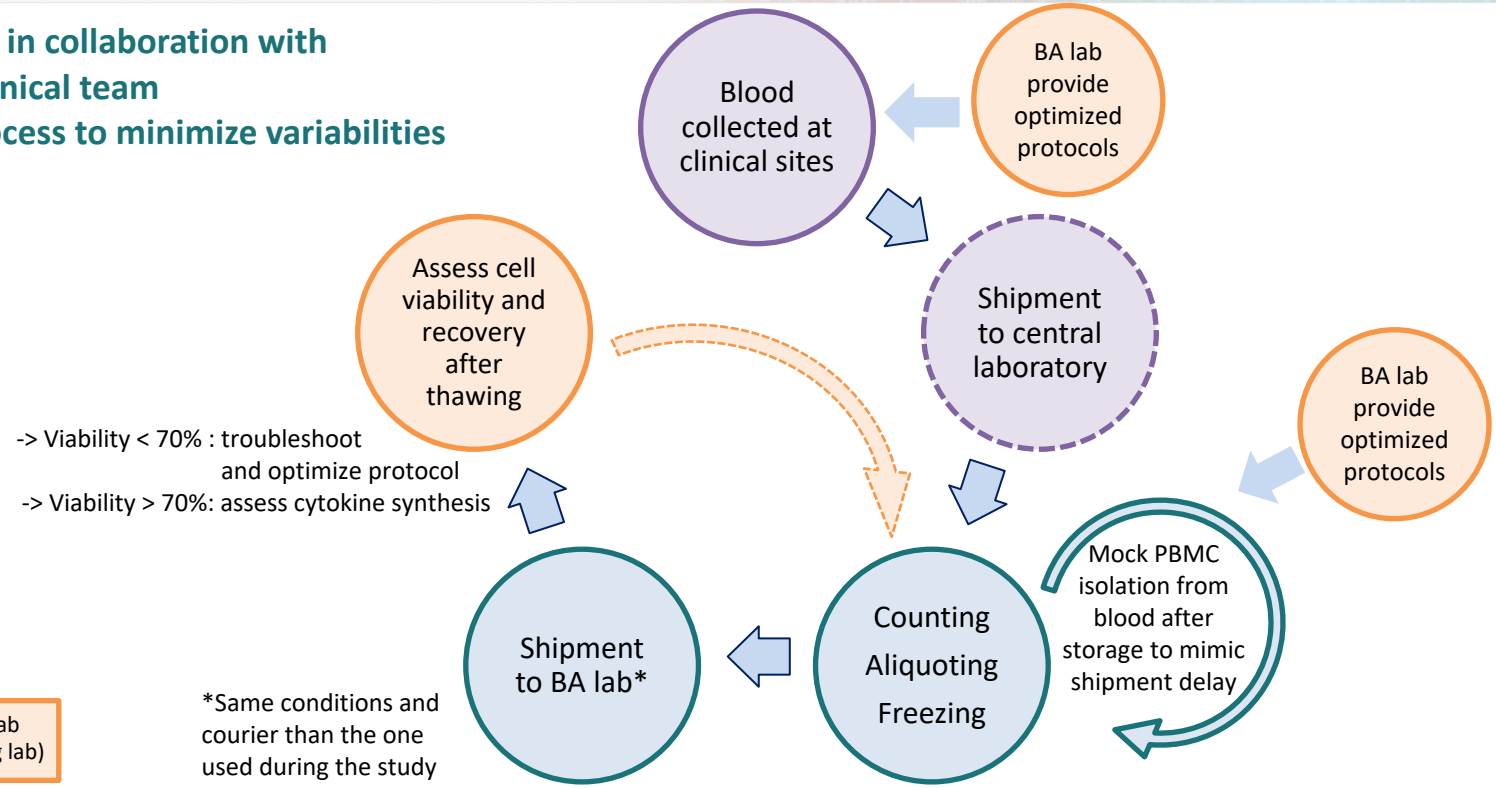
PBMC isolation: % recovery and cytokine synthesis testing as an integral part of ELISpot assay development





Mitigate inter-site differences

Plan PBMC collection in collaboration with the BA lab and the clinical team
=> recommended process to minimize variabilities



Case Study: Validation of an ELISpot assay

Validation of ELISpot assay for the qualitative assessment of cellular immune responses against virus-based gene therapy

- Multiple clinical sites and one central laboratory
- Human Peripheral Blood Mononuclear Cells (PBMCs) from total blood
- AAV vector: three peptide pools; Transgene: two peptide pools
- Positive controls: CPI/CEF; αCD3; pokeweed
- Read-out: Interferon-gamma colorimetric ELISpot (HRP)

Validated Parameters

Specificity
Negative cut-off determination for vector and transgene peptide pools

Analytical sensitivity

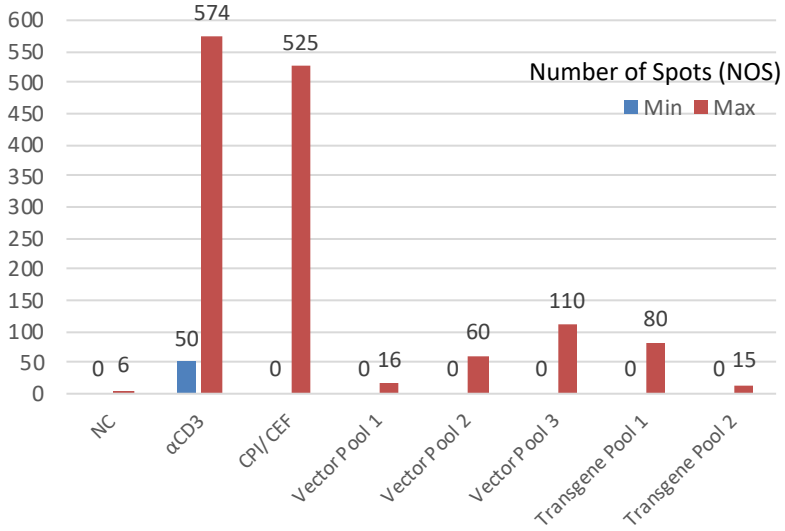
Dose-dependent reactivity

Precision and repeatability

Robustness

Stability
-> Mimic sample handling during study

Assay and reference range



Assay Specificity

Assay specificity: ability to detect a positive reaction to a specific stimulation and to differentiate this reaction from background (*i.e* to correctly reject negative samples)

Ideal case: availability of PBMCs from donors reactive to vector and transgene; usually impractical as not commercially available.

=> Determination of a negative cut-off based on the assay's background

Cut-off for each peptide pool

Option 1:

establishment of an overall negative cut-off:

CON: depending on the peptide pool, variability in background spot counts between individuals and even within samples from the same individual

Option 2:

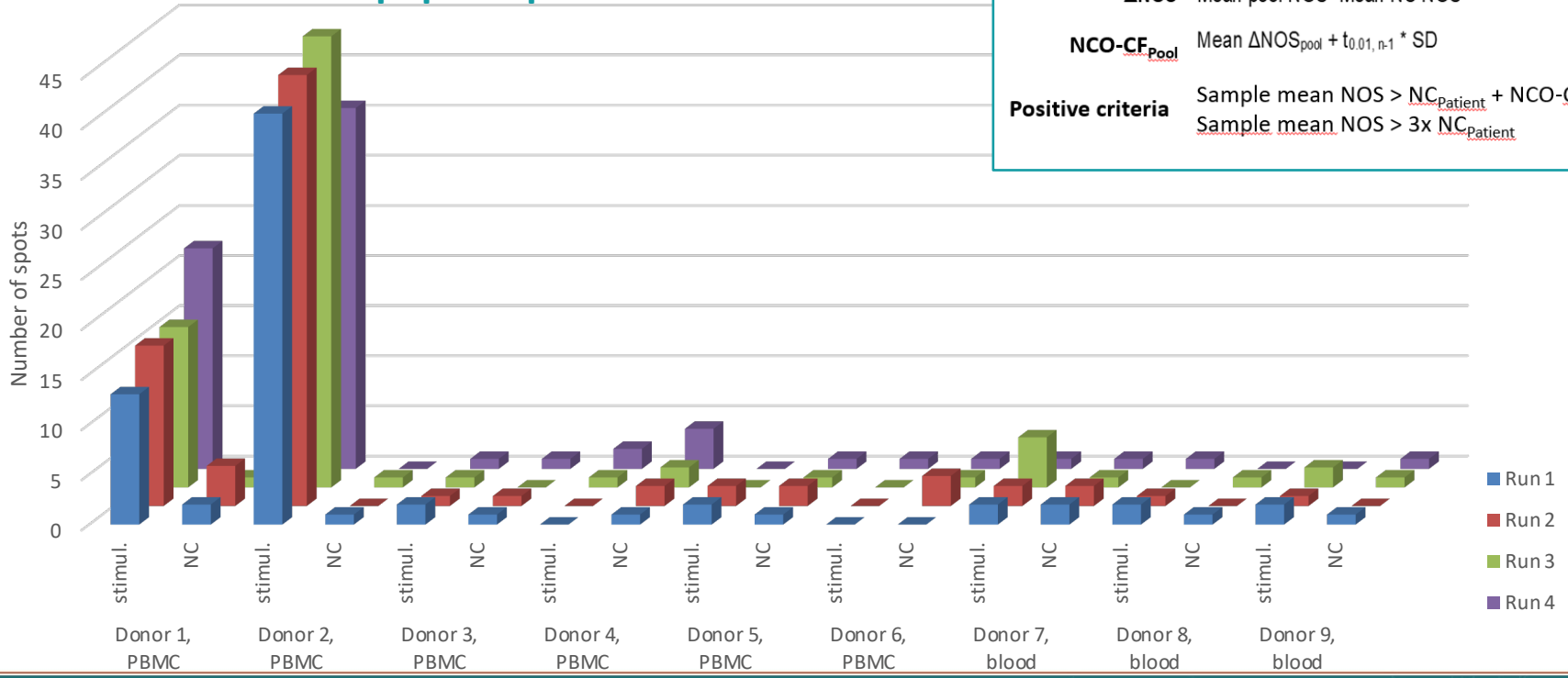
Establishment of a correction factor (CF) specific to each peptide pool

Deemed more appropriate

Negative cut-off per peptide pool

Raw data for vector peptide pool 3

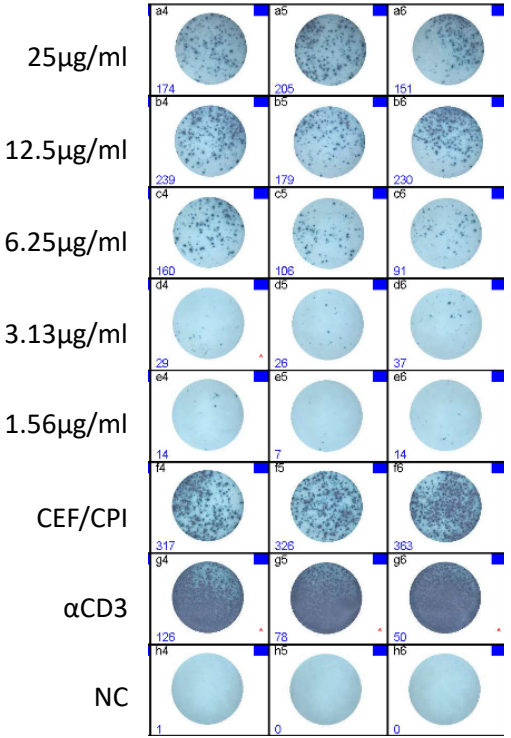
ΔNOS	Mean pool NOS -Mean NC NOS
NCO-CF_{Pool}	Mean ΔNOS_{pool} + $t_{0.01, n-1}$ * SD
Positive criteria	Sample mean NOS > $NC_{Patient}$ + NCO-CF_{Pool} Sample mean NOS > 3x $NC_{Patient}$



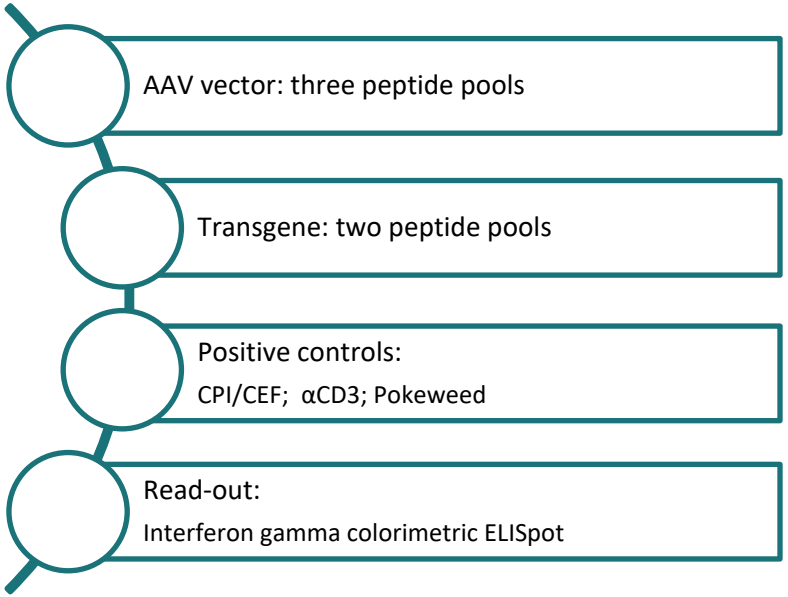
Dose-dependent reactivity

- Aim: determine the optimal concentration of antigen (i.e. peptide pool) to induce a specific response, without causing unspecific effects due to overdosing or stimulation by other additives in the formula, such as DMSO.
- Established as the lowest peptide pool concentration resulting in a consistent mean spot count in all donors.

- 1.5x 10^5 cells/well from four different donors seeded in triplicates and stimulated:
  - with a 1:2 serial dilution of each peptide pool starting at 25µg/mL to limit the amount of DMSO to a maximum of 2% in the assay.
  - for ~36h and spot counts were evaluated for each peptide pool concentration.
  - with CEF/CPI and αCD3 as PC



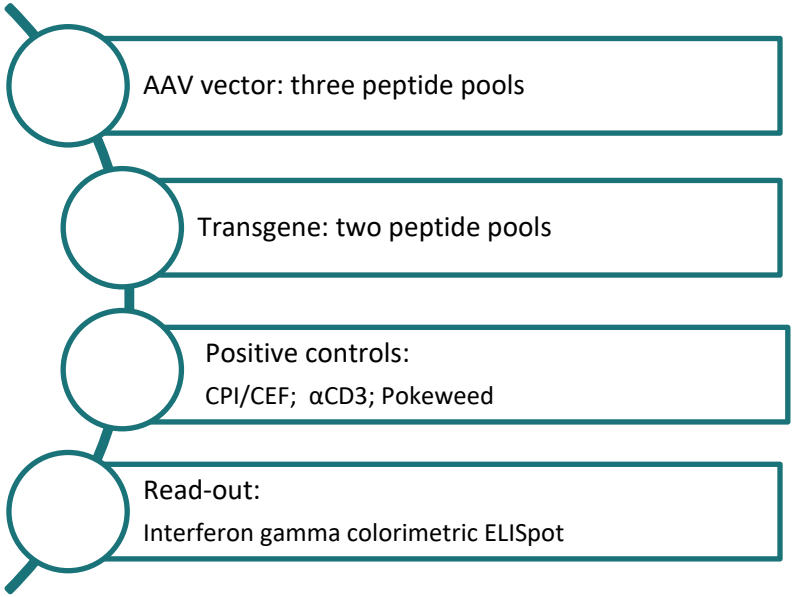
Summary of the Validated Parameters



Cell source	Frozen PBMC and PBMC isolated directly from blood
Analytical sensitivity	150,000 cells per well
PBMC viability	Commercial PBMC: 87.0% ± 6.5 Freshly isolated PBMC: 90.7% ± 2.1
PBMC recovery	Commercial PBMC: 137% ± 26
Peptide concentration per pool	Vectors pools 1 to 3: 12.5µg/ml Transgene pool 1: 3.13µg/ml Transgene pool 2: 6.25µg/ml
Incubation time PBMC/peptide	~43h, no restrictions (2 nights)
Intra-assay precision	≤ 10% CV
Repeatability	≤ 30% CV for spot counts < 300 ≤ 15% CV for spot counts ≥ 300
Assay range	0 – 574

Summary of the Validated Parameters

Validation of an ELISpot assay for the qualitative assessment of a cellular immune response against a virus-based gene therapy in human PBMCs



Positive criteria	Sample mean NOS > $NC_{Patient}$ + $NCO\text{-}CF_{Pool}$ Sample mean NOS > 3x $NC_{Patient}$
Stability	Cell viability and cytokine secretion PBMC: Weinberg et al (2009) Fresh blood < 24h 4°C before processing
Robustness	TIER 1 CONDITIONS: • Detection antibody incubation time • Dilutions of enzyme conjugated detection antibody • Standard culture period of cells TIER 2 CONDITIONS: • Kit lots (n=2) • Operators (n=3)

Evolving trends: items for discussion

- Duplicate or triplicate analysis?
- Is one read-out sufficient to assess T-cell responses?
- Qualification level?

Question & Answer

감사합니다

Thank You!

Danke

Merci

obrigado

Grazie

ありがとう

Спасибо

Gracias

Efharisto

Tack