EQAPOL 4C ICS Sendout #2 Data

FlowCAP 3 (29 Nov 2012)

Washington DC
External Quality Assurance Program Oversight Laboratory (EQAPOL)

• NIH/NIAID/DAIDS contract to develop external quality assurance (EQA) programs
• Monitor laboratories involved in HIV/AIDS research and vaccine trials
• Components of EQAPOL:
  – Peripheral Blood Mononuclear Cell (PBMC) Cell Bank and Reagent Repository
  – Unique Clade-Specific HIV Virus Panels
  – EQA Program for Enzyme-linked Immunosorbent Spot (ELISpot) Assay
  – EQA Program for Luminex Assay
  – EQA Program for A3R5 Neutralization Assay
  – EQA Program for Flow Cytometry-based Assays
• Participation
  – At the discretion of DAIDS
  – Reserved for laboratories performing assays for DAIDS-sponsored clinical trial studies.
• EQAPOL website at: http://eqapol.dhvi.duke.edu
EQAPOL Flow Cytometry Program

• External proficiency (EP) evaluation of laboratories and remediation if necessary on specific flow cytometry protocols
• Current focus on detection of low frequency antigen-specific T cells via intracellular staining (ICS) assays
• 4-color ICS (EP1, EP2)
  – CD3, CD4, CD8, Interferon-γ (IFN-γ) and IL-2
  – Mono-functional read-out is measured as a single fluorophore (multiplexed)
• 7-color ICS (EP3)
  – CD3, CD4, CD8, Interferon-γ (IFN-γ), IL-2, and TNF-a
  – Poly-functional readout is measured as 3 separate fluorophores
• 8-color ICS (upcoming EP4 & EP5)
  – CD3, CD4, CD8, Interferon-γ (IFN-γ), IL-2, and TNF-a, CD107a simultaneously
  – Poly-functional readout is measured as 4 separate fluorophores
4-color ICS Assay

Thaw → Stimulate → Permeabilize → IC Stain → Acquire and analyze

- Brefeldin
- Pepmixes
- 6 hrs
- Wash
- Wash
- DMSO (Negative control)
- CEF
- CMV pp65

Cartoon by Holden Maecker
**EQAPOL Flow Cytometry EQA**
Kit sent to participating lab

- PBMCs selected based on pre-screening of multiple donors for cytokine responses
- Stimulation LyoPlates (lyophilized peptides)
- Staining LyoPlates (lyophilized antibodies)
- Permeabilization reagents
- Instrument set-up and QC assessment beads
- Protocol (including gating strategy)
- Reporting templates
- Assay Questionnaire
4-Color ICS Kit Detail

- **Shipment #1: Dry Shipper**
  - 1 vial of cryopreserved PBMCs from three donors randomized as Sample 1,2 or 3

- **Shipment #2: Wet Ice Shipment**
  - 1 Stim LyoPlate containing CMV & CEF peptides plus DMSO Control wells in triplicate
  - 1 Stain LyoPlate containing CD4 FITC, IFN-g + IL2 PE, CD8 PerCP-Cy5.5, and CD3 APC along with negative and fluorophore-labeled beads for compensation
  - Staining Reagents: EDTA solution, Fix/Perm, and Perm Wash
  - Instrument Qualification Beads: 8-peak & CS&T

- **Documents**
  - Site Instructions
  - 4-Color ICS Protocol for EQAPOL EP1 including gating instructions – provided as hard copy in shipment and as PDF via email
  - Assay questionnaire - provided as a PDF form via email
  - Excel and PowerPoint templates for data reporting via email

- **BioTrue access for Questionnaire with analyzed and raw data**
- **Envelope and Fed-Ex label to return data logger to EQAPOL**
Context information

- Lab ID
- Donor ID (3)
- Stimulation condition (3)
  - CEF
  - CMV
  - DMSO
- Replicate (3)
- Data files → 27 tubes per laboratory
- Analysis → 54 antigen-specific frequencies (CD4 and CD8) per laboratory
4-color ICS Manual Gating Strategy

Positive: ≥0.05% and 2xbg
Very Low: 0.05 – 0.09 %
Low: 0.1 – 0.49 %
Medium: 0.5 – 0.99 %
High: ≥ 1.00 %
% are percent of parent gate
Dot plots from / Donor 1 / CD4 Gated / Stainings 1-9

- **Brefeldin A**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A01.fcs

- **Brefeldin A + CMV**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A05.fcs

- **Brefeldin A + CEF**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A09.fcs

- **Brefeldin A**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A02.fcs

- **Brefeldin A + CMV**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A06.fcs

- **Brefeldin A + CEF**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A07.fcs

- **Brefeldin A**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A03.fcs

- **Brefeldin A + CMV**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A08.fcs

- **Brefeldin A + CEF**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A10.fcs

- **Brefeldin A**
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  - CD3 APC
  - 002_C6901J7C_02_A04.fcs

- **Brefeldin A + CMV**
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  - CD3 APC
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- **Brefeldin A + CEF**
  - IL-2+IFNγ
  - CD3 APC
  - 002_C6901J7C_02_A11.fcs

- **Brefeldin A**
  - IL-2+IFNγ
  - CD3 APC
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Dot plots from / Donor 1 / CD8 Gated / Stainings 1-9

Brefeldin A

IL-2+IFNγ

CD3 APC

002_C6901J7C_02_A01.fcs

0.0258

002_C6901J7C_02_A02.fcs

0.0319

002_C6901J7C_02_A03.fcs

0.0277

Brefeldin A + CMV

IL-2+IFNγ

CD3 APC

002_C6901J7C_02_A05.fcs

0.219

002_C6901J7C_02_A06.fcs

0.175

002_C6901J7C_02_A07.fcs

0.206

Brefeldin A + CEF

IL-2+IFNγ

CD3 APC

002_C6901J7C_02_A09.fcs

0.546

002_C6901J7C_02_A10.fcs

0.541

002_C6901J7C_02_A11.fcs

0.605
Dot plots from / Donor 2/ CD4 Gated / Stainings 1-9

Brefeldin A

Brefeldin A + CMV

Brefeldin A + CEF
Dot plots from / Donor 2 / CD8 Gated / Stainings 1-9

Brefeldin A

IL-2+IFNγ

CD3 APC

Brefeldin A + CMV

IL-2+IFNγ

CD3 APC

Brefeldin A + CEF

IL-2+IFNγ

CD3 APC
Dot plots from / Donor 3/ CD4 Gated / Stainings 1-9

Brefeldin A

IL-2+IFNγ

CD3 APC

002_E6905M6Z_02_E01.fcs

0.023

002_E6905M6Z_02_E02.fcs

0.0284

002_E6905M6Z_02_E03.fcs

0.0186

Brefeldin A + CMV

IL-2+IFNγ

CD3 APC

002_E6905M6Z_02_E05.fcs

0.0141

002_E6905M6Z_02_E06.fcs

0.0282

002_E6905M6Z_02_E07.fcs

0.0185

Brefeldin A + CEF

IL-2+IFNγ

CD3 APC

002_E6905M6Z_02_E09.fcs

0.112

002_E6905M67_07_E010.fcs

0.0949

002_E6905M67_02_E011.fcs

0.0744
Dot plots from / Donor 3/ CD8 Gated / Stainings 1-9

Brefeldin A

IL-2+IFNγ

CD3 APC

002_E6905M6Z_02_E01.fcs

0.0133

002_E6905M6Z_02_E02.fcs

0.0132

002_E6905M6Z_02_E03.fcs

0.0154

Brefeldin A

IL-2+IFNγ

CD3 APC

002_E6905M6Z_02_E05.fcs

0.0198

002_E6905M6Z_02_E06.fcs

8.82e-3

002_E6905M6Z_02_E07.fcs

6.58e-3

Brefeldin A

IL-2+IFNγ

CD3 APC

002_E6905M6Z_02_E09.fcs

0.239

002_E6905M6Z_02_E10.fcs

0.209

002_E6905M6Z_02_E11.fcs

0.286

+ CMV

+ CEF
Variation across laboratories
Instrument setup issues

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<tr>
<td>Max</td>
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Use of target regions for MFI

**EP1**

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**Range**

- Minimum: 32,320
- Maximum: 216,000

**EP2**

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**Range**

- Minimum: 126,000
- Maximum: 134,000
Compensation Issues

* Samples provided for FlowCAP 3 have been correctly compensated by central laboratory
Gating issues

SA

EOLm

Rep 1  Rep 2  Rep 3

012_26Jul11_C05.fcs 012_26Jul11_C06.fcs 012_26Jul11_C07.fcs

False negative
Flow Cytometry EP2 Reports – basic subsets results

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<th>High</th>
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<td>79.1</td>
<td>99.7</td>
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<td>58.7</td>
<td>64.2</td>
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<td>CD8+</td>
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**Inter-laboratory**

**Table 3: Site-reported Intra-laboratory %CD3, %Lymphs, %CD4+ and %CD8+ Subset Analysis**

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<th>Site</th>
<th>Sample</th>
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<td></td>
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<td>%CD4+</td>
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<td>%CD8+</td>
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<td>%CD8+</td>
<td>31.5</td>
<td>31.2</td>
<td>31.6</td>
<td>0.4</td>
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Automated analysis

• Can we fully automate the quantification of rare antigen-specific cells across multiple laboratories?

• Challenges
  – Methods for data alignment/registration/compensation
  – Methods for cell subset identification
  – Methods for rare event enrichment/quantification
  – Methods for cell subset annotation
  – Any other methods that improve performance
Current pipeline

- Data preprocessing (compensate/transform)
- Selective/biased sampling (none)
- Data alignment (minimize DKL)
- Clustering (hierarchical DPGMM)
- Cell subset identification (supervised learning)
- Cell subset annotation (user provided labels)
Sendout 2 Analysis: Basic subsets
Sendout 2 Analysis: ICS+ve subsets
Comparisons
Acknowledgements

Management
• Tom Denny
• Ambrosia Garcia
• Ana Sanchez

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• Kent Weinhold
• Janet Staats
• Jennifer Enzor
• Twan Weaver
• Jianling Shi
• Scott White
• Darongsae Kwon

Flow Cytometry Advisory Committee
• Mario Roederer
• Steve DeRosa
• Charles Rinaldo

BDB Sub-contract
• Skip Maino
• Maria Jaimes
• Laurel Nomura
• Amitabh Gaur
• Joshua Kotner

Central Laboratory
• Linda Walker
• Sara Brown
• Sarah Keinonen

QAU
• Marcella Sarzotti-Kelsoe
• Christopher Todd
• Dan Ozaki

Statistics
• Wes Roundtree

IT
• Paul Debien
• Shana Zaman
• Adam Stasio (Scimed)