ELISpot Assay

Orientation: ELISpot EQA Program
Overview: EQAPOL Web-based application

Updated 1/24/2019
Introduction to ELISpot Program

• The EQAPOL ELISpot EQA Program will assess the following measures:
  
  • the range of responses detected by the lab;
  • the precision of the measurements (i.e., replicates);
  • the comparison of the results to what is observed among labs;
  • comparison with central reading;
  • comparison with a reference kit.

• Sites are graded according to criteria have been developed over the course of several EPs with input by the Scientific Advisory Board, ELISpot Advisory Committee, and NIH/NIAID/DAIDS.
EQAPOL ELISpot Cytometry EQA Approach

**EQAPOL Assay**
- Cryopreserved PBMCs
  - Blinded
- EQAPOL Assay Reagents
  - Blinded Peptide Mixes
  - NovaRed, Strep, Ab
  - 2 pre-coated plates
- Protocol/Documents
  - Protocol
  - Data Template
  - Post-Assay Questionnaire
- Site runs EP
  - Results returned using web-system
- Analysis (Site and plate reading by EOL)
- Grading
- Reports
- No Grading

**In-House Assay**
- Cryopreserved PBMCs
  - Blinded
- In-House Assay
  - Blinded Peptide Mixes
- Protocol/Documents
  - Data Template
  - Post-Assay Questionnaire
- Site runs EP
  - Results returned using web-system
- Analysis (Site and plate reading by EOL)
- Grading
- Reports
ELISpot Data

- **In-house Assay**
- EQAPOL Peptides and PBMCs

**Read by Site**

**Grading**

**No Grading**

- To identify:
  - Problems with Reader

**Read by EOL**

**No Grading**

- To identify:
  - Problems with In-house Assay
  - Technician Problems

- **EQAPOL Assay**
- EQAPOL Peptides and PBMCs

**Read by Site**

**No Grading**

- To identify:
  - Problems with Reader

**Read by EOL**

**No Grading**

- To identify:
  - Problems with In-house Assay
  - Technician Problems
What your site will receive for

- Two shipments with packing manifests will be sent to your site
  - Wet ice shipment containing the substrates, plates, antibodies, etc.
    - Store materials at 2-8°C
  - Cryoshipper containing the PBMCs and peptides
    - Store PBMCs in LN$_2$
    - Store peptides at -80°C

- Data Logger
  - Upon receipt deactivate the data logger according to the protocol and return to EQAPOL using the provided shipping envelope and waybill

- Assay Protocol
  - Thoroughly familiarize yourself with all aspects of the EP specific protocol, as this assay may vary slightly from your current ELISpot procedures
  - Please do not deviate from the provided protocol for the EQAPOL plates
  - Submit data to EQAPOL within 4 weeks of kit receipt

- Return LN$_2$ Shipper immediately upon receipt
Summary of Differences

- **In-House (Graded)**
  - PBMCs (EQAPOL)
  - Peptides (EQAPOL)
  - Protocol (Site-specific)
  - Plates (Site-specific)
  - Key Reagents (Site-specific)
  - Other Reagents (Site-specific)

- **EQA**
  - PBMCs (EQAPOL)
  - Peptides (EQAPOL)
  - Protocol (EQAPOL)
  - Plates (EQAPOL)
  - Key Reagents (EQAPOL)
    - Streptavidin-HRP
    - Detection Ab
    - Nova Red Substrate
  - Other Reagents (Site-specific)
## Required EP Plate 1 Layout

### In-house or EQAPOL Plate 1

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Sample 1 Reagent A</td>
<td>Sample 1 Reagent A</td>
<td>Sample 1 Reagent A</td>
<td>Sample 1 Reagent A</td>
<td>Sample 1 Reagent A</td>
<td>Sample 1 Reagent A</td>
<td>Sample 1 Reagent A</td>
<td>Sample 1 Reagent A</td>
<td>Sample 1 Reagent A</td>
<td>Sample 1 Reagent A</td>
<td>MEDIUM NO CELLS</td>
</tr>
<tr>
<td>PBS</td>
<td>Sample 1 Reagent B</td>
<td>Sample 1 Reagent B</td>
<td>Sample 1 Reagent B</td>
<td>Sample 1 Reagent B</td>
<td>Sample 1 Reagent B</td>
<td>Sample 1 Reagent B</td>
<td>Sample 1 Reagent B</td>
<td>Sample 1 Reagent B</td>
<td>Sample 1 Reagent B</td>
<td>Sample 1 Reagent B</td>
<td>MEDIUM NO CELLS</td>
</tr>
<tr>
<td>PBS</td>
<td>Sample 1 Reagent C</td>
<td>Sample 1 Reagent C</td>
<td>Sample 1 Reagent C</td>
<td>Sample 1 Reagent C</td>
<td>Sample 1 Reagent C</td>
<td>Sample 1 Reagent C</td>
<td>Sample 1 Reagent C</td>
<td>Sample 1 Reagent C</td>
<td>Sample 1 Reagent C</td>
<td>Sample 1 Reagent C</td>
<td>MEDIUM NO CELLS</td>
</tr>
<tr>
<td>PBS</td>
<td>Sample 2 Reagent A</td>
<td>Sample 2 Reagent A</td>
<td>Sample 2 Reagent A</td>
<td>Sample 2 Reagent A</td>
<td>Sample 2 Reagent A</td>
<td>Sample 2 Reagent A</td>
<td>Sample 2 Reagent A</td>
<td>Sample 2 Reagent A</td>
<td>Sample 2 Reagent A</td>
<td>Sample 2 Reagent A</td>
<td>MEDIUM NO CELLS</td>
</tr>
<tr>
<td>PBS</td>
<td>Sample 2 Reagent B</td>
<td>Sample 2 Reagent B</td>
<td>Sample 2 Reagent B</td>
<td>Sample 2 Reagent B</td>
<td>Sample 2 Reagent B</td>
<td>Sample 2 Reagent B</td>
<td>Sample 2 Reagent B</td>
<td>Sample 2 Reagent B</td>
<td>Sample 2 Reagent B</td>
<td>Sample 2 Reagent B</td>
<td>MEDIUM NO CELLS</td>
</tr>
<tr>
<td>PBS</td>
<td>Sample 2 Reagent C</td>
<td>Sample 2 Reagent C</td>
<td>Sample 2 Reagent C</td>
<td>Sample 2 Reagent C</td>
<td>Sample 2 Reagent C</td>
<td>Sample 2 Reagent C</td>
<td>Sample 2 Reagent C</td>
<td>Sample 2 Reagent C</td>
<td>Sample 2 Reagent C</td>
<td>Sample 2 Reagent C</td>
<td>MEDIUM NO CELLS</td>
</tr>
</tbody>
</table>
## Required EP Plate 2 Layout

### In-house or EQAPOL Plate 2

<table>
<thead>
<tr>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Sample 3 Reagent A</td>
<td>Sample 3 Reagent A</td>
<td>Sample 3 Reagent A</td>
<td>Sample 3 Reagent A</td>
<td>Sample 3 Reagent A</td>
<td>Sample 3 Reagent A</td>
<td>Sample 3 Reagent A</td>
<td>Sample 3 Reagent A</td>
<td>Sample 3 Reagent A</td>
<td>MEDIUM NO CELLS</td>
</tr>
<tr>
<td>PBS</td>
<td>Sample 3 Reagent B</td>
<td>Sample 3 Reagent B</td>
<td>Sample 3 Reagent B</td>
<td>Sample 3 Reagent B</td>
<td>Sample 3 Reagent B</td>
<td>Sample 3 Reagent B</td>
<td>Sample 3 Reagent B</td>
<td>Sample 3 Reagent B</td>
<td>Sample 3 Reagent B</td>
<td>MEDIUM NO CELLS</td>
</tr>
<tr>
<td>PBS</td>
<td>Sample 3 Reagent C</td>
<td>Sample 3 Reagent C</td>
<td>Sample 3 Reagent C</td>
<td>Sample 3 Reagent C</td>
<td>Sample 3 Reagent C</td>
<td>Sample 3 Reagent C</td>
<td>Sample 3 Reagent C</td>
<td>Sample 3 Reagent C</td>
<td>Sample 3 Reagent C</td>
<td>MEDIUM NO CELLS</td>
</tr>
<tr>
<td>PBS</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>PBS</td>
</tr>
<tr>
<td>PBS</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>PBS</td>
</tr>
<tr>
<td>PBS</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>In-house Control</td>
<td>PBS</td>
</tr>
</tbody>
</table>

---

PBS PBS PBS PBS PBS PBS PBS PBS PBS PBS PBS PBS
What to do with your EP data

• **Determine Spot Counts of Test Samples**
  • Using your lab-specific method, your site will determine the following:
    • Prepare Excel Data Reporting Template with spot counts from 4 Test Plates

• **Data Reporting**
  • All EP data reporting will take place via the EQAPOL web-based application:
    • Upload provided Excel Template with your spot counts
      [https://eqapolapp.dhvi.duke.edu](https://eqapolapp.dhvi.duke.edu)

• **Returning Plates to EQAPOL**
  • Wrap plates in foil and return in padded envelope with datalogger (Domestic)
  • Return in dryshipper mushroom if international site using World Courier
Reporting Template

- There are six tabs for reporting data (must follow layout)

![Example Layout](Image)

![Paste In-house Plate 1](Image)

![Paste In-house Plate 2](Image)

![Paste EQA Plate 1](Image)

![Paste EQA Plate 1](Image)

![Type Cell Information](Image)

![Excel Spreadsheet](Image)
## Grading ELISpot Performance

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Target</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timeliness</strong></td>
<td>On time EP valid data and questionnaire upload</td>
<td>Per due date set by EQAPOL</td>
<td>• 10 points total</td>
</tr>
<tr>
<td><strong>PBMC Processing</strong></td>
<td>Viability: Pass/Fail per each donor sample</td>
<td>Donor must be &gt;80% for D1</td>
<td>• 9 points total</td>
</tr>
<tr>
<td></td>
<td>Recovery: Pass/Fail per each donor sample</td>
<td>Donor must be between 70-120% for D1</td>
<td>• 9 points total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 1.5 points available per donor for viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 1.5 points available per donor for recovery</td>
</tr>
<tr>
<td><strong>Background</strong></td>
<td>• Pass/Fail per each donor sample</td>
<td>Average for each donor must be &lt;10</td>
<td>• 9 total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 3 points available per donor</td>
</tr>
<tr>
<td><strong>Accuracy to the consensus</strong></td>
<td>• Assessed for each donor sample for each stimulation condition (CMV and CEF)</td>
<td>Site’s average for 9 wells must not be significantly different from consensus average</td>
<td>• 54 points total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 9 point deduction for each donor by stim condition significantly different from consensus average</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td>• Assessed for each donor sample for each stimulation condition (CMV and CEF)</td>
<td>The dispersion must be less than 3.3</td>
<td>• 18 points total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 3 points deducted for each donor/stimulation outside of range</td>
</tr>
</tbody>
</table>
## Grading ELISpot Performance

<table>
<thead>
<tr>
<th>EQAPOL Performance Ranges</th>
<th>Overall Performance Score</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>91-100</td>
<td>Excellent</td>
</tr>
<tr>
<td></td>
<td>75-90</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>66-74</td>
<td>Fair</td>
</tr>
<tr>
<td></td>
<td>0-65</td>
<td>Poor</td>
</tr>
</tbody>
</table>

Site performing “Fair” or “Poor” contacted for remediation
Overview: EQAPOL Web-based application
EQAPOL Web-based System: Log In

https://eqapolapp.dhvi.duke.edu
Select “ELISpot” from the Menu

** Some options will not be available for your site **
Select Active EP from ELISpot Program Page

** Some options will not be available for your site **
Select your Site under the Current EP

ELISpot EP 8 (Active)

We appreciate your participation in EQAPOL ELISpot External Proficiency 8 (EP8). Please follow the instructions below to complete EP8:

1. Download the provided instructions below and use them to perform the ELISpot assay using the EQAPOL-provided kit and samples. The EQAPOL samples should also be tested using your in-house kit. Please do not deviate from the protocol.
2. Once you have completed the assay, please navigate to the “Results” tab and complete the post-assay questionnaire and upload your data. Complete the questionnaire by selecting “Fill out the Questionnaire.”
3. For data upload: Browse for and upload your completed Excel data template as a “Results Spreadsheet.” There is no need to upload results as “Additional Files.” Only the completed Excel workbook is required for EP8.
4. Once the workbook is uploaded, you must save and then submit your results. Note: Pressing “Save” will only save a copy of the file to the system, it will not be recorded as final until you press “Submit.” Shortcut: pressing “Submit” will both save and submit the file as final in one step.
5. Once your file and post-assay questionnaire are submitted you will not be able to add or edit files unless you contact us at EQAPOL@duke.edu.

Should you have any questions about EP8 or need assistance with the web-based system, please do not hesitate to contact us.

Kind Regards.

The EQAPOL ELISpot Team

Snapshot of EP status

- Only sites for which you are a part of will display on this page
We appreciate your participation in EQAPOL ELISpot External Proficiency 16 (EP16). Please follow the instructions below to complete EP16:

1. Download the provided instructions below and use them to perform the ELISpot assay using the EQAPOL-provided kit and samples. The EQAPOL samples should also be tested using your In-house kit. Please do not deviate from the protocol.

2. Once you have completed the assay, please navigate to the “Results” tab and complete the post-assay questionnaire and upload your data. Complete the questionnaire by selecting “Fill out the Questionnaire.”

3. For data upload: Browse for and upload your completed Excel data template as a “Results Spreadsheet.” There is no need to upload results as “Additional Files” Only the completed Excel workbook is required for EP16.

4. Once the workbook is uploaded, you must save and then submit your results. Note: Pressing “Save” will only save a copy of the file to the system. It will not be recorded until you press “Submit”. Shortcut: pressing “Submit” will both save and submit the file as final in one step.

5. Once your file and post-assay questionnaire are submitted, you will not be able to add or edit files unless you contact us.

Should you have any questions about EP16 or need assistance with the web-based system, please do not hesitate to contact us at EQAPOL@duke.edu.

Kind Regards,

The EQAPOL ELISpot Team

Due 09/25/2018
ELISpotEP16 Site Instructions.pdf
EQAPOLELISpot_EP16Workbook.xlsx

Discussion between Site and EQAPOL

Upload Results (XLS template) and Take Survey

Download Protocol and Template

Shipment Information
# Acknowledge Receipt of Shipments

Check “Received at” when your shipment arrives

<table>
<thead>
<tr>
<th>Item</th>
<th>Label Number</th>
<th>Quantity</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>09252014</td>
<td>1</td>
<td>200.0 μL</td>
</tr>
<tr>
<td>Reagent B</td>
<td>09252014</td>
<td>1</td>
<td>200.0 μL</td>
</tr>
<tr>
<td>Reagent C</td>
<td>09252014</td>
<td>1</td>
<td>200.0 μL</td>
</tr>
<tr>
<td>Sample 1</td>
<td>J990898N-36</td>
<td>1</td>
<td>2000000.0 CEL</td>
</tr>
<tr>
<td>Sample 1</td>
<td>J990898N-38</td>
<td>1</td>
<td>2000000.0 CEL</td>
</tr>
<tr>
<td>Sample 1</td>
<td>J990898N-39</td>
<td>1</td>
<td>2000000.0 CEL</td>
</tr>
<tr>
<td>Sample 2</td>
<td>E9907975-20</td>
<td>1</td>
<td>2000000.0 CEL</td>
</tr>
<tr>
<td>Sample 2</td>
<td>E9907975-21</td>
<td>1</td>
<td>2000000.0 CEL</td>
</tr>
<tr>
<td>Sample 2</td>
<td>E9907975-22</td>
<td>1</td>
<td>2000000.0 CEL</td>
</tr>
<tr>
<td>Sample 3</td>
<td>F990809L-11</td>
<td>1</td>
<td>2000000.0 CEL</td>
</tr>
<tr>
<td>Sample 3</td>
<td>F990809L-12</td>
<td>1</td>
<td>2000000.0 CEL</td>
</tr>
<tr>
<td>Sample 3</td>
<td>F990809L-13</td>
<td>1</td>
<td>2000000.0 CEL</td>
</tr>
</tbody>
</table>

**Shipment #11740**

- **Shipped:** 12/10/2014 05:12 PM
- **Courier:** FedEx
- **Tracking #:** 772159719208
- **Documents:** receipt, label, etc. (watch 1237 for details)

**Received at:**

**Shipment issues:** None

Please describe the issues with your shipment:

- [Update Shipment]
## Acknowledge Receipt of Shipments

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description</th>
<th>Quantity</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>V7R2Y</td>
<td></td>
<td>1</td>
<td>80.0 µL</td>
</tr>
<tr>
<td>C2A3T</td>
<td></td>
<td>1</td>
<td>80.0 µL</td>
</tr>
<tr>
<td>A4M18</td>
<td></td>
<td>1</td>
<td>80.0 µL</td>
</tr>
<tr>
<td>B1U2L</td>
<td></td>
<td>1</td>
<td>80.0 µL</td>
</tr>
<tr>
<td>N5K2Q</td>
<td></td>
<td>1</td>
<td>80.0 µL</td>
</tr>
<tr>
<td>F5E4U</td>
<td></td>
<td>1</td>
<td>80.0 µL</td>
</tr>
<tr>
<td>S4Q6M</td>
<td></td>
<td>1</td>
<td>80.0 µL</td>
</tr>
<tr>
<td>X2G5R</td>
<td></td>
<td>1</td>
<td>80.0 µL</td>
</tr>
</tbody>
</table>

**Change “Shipment Issues” to “Yes” to note any shipping issues and select “Received”**
Completing Questionnaire

All answers will be lost unless you press the submit button. You cannot change your answers after they are submitted unless you contact us (EQAPOL@duke.edu).
Upload Completed XLS Template: Select “Results”

<table>
<thead>
<tr>
<th><strong>INSTRUCTIONS</strong></th>
<th><strong>SEND-OUT</strong></th>
<th><strong>RESULTS</strong></th>
<th><strong>CENTRALIZED ANALYSIS</strong></th>
<th><strong>REPORTS</strong></th>
<th><strong>COMMUNICATION/FEEDBACK</strong></th>
</tr>
</thead>
</table>

**Questionnaire**  
[Select “Results”]

**Results spreadsheet**  
[Browse] No file selected.

**Additional files**  
[Browse] No file selected.

**Comments**  
There are no comments

- **Save** will enable you to delete and upload new documents if needed. Results will not be final.
- **Submit** will indicate you are done with the EP. Documents can no longer be uploaded without contacting EQAPOL.

No additional files are being requested for the current EP
Reports

• Once the EP is closed and the Centralized Analysis is completed, reports will be made available in the EQAPOL web system.
• A new tab “Reports” will appear with the report file available for download.
• The reports will summarize the results and provide your site with a numerical score and grade category
• Remediation calls are held with sites that receive a Fair or Poor score to help troubleshoot potential issues
Troubleshooting, Support, Questions

Email: EQAPOL@duke.edu
Phone: 919-660-0905

EQAPOL Program Management
Andrea Pappas: andrea.pappas@duke.edu
Cassie Porth: cassandra.porth@duke.edu

EQAPOL
Duke Human Vaccine Institute
Duke University Medical Center
GSRB II, 210 Research Drive
Room 4006C
Durham, NC 27710 USA

This project has been funded in whole or in part with Federal funds from the Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract No. HHSN272201700061C, entitled External Quality Assurance Program Oversight Laboratory (EQAPOL).