EQAPOL Luminex Program
Orientation

Overview:
EQAPOL Web-Based Application

Updated: 2/1/2019
Presentation Outline

• Overview of EQAPOL

• Overview of EQAPOL Luminex EQA

• General EQA Process

• Participation in a Luminex-based EP
  • What you will receive
  • Web-based System
  • Reports and Remediation
External Quality Assurance Program Oversight Laboratory (EQAPOL)

- NIAID/DAIDS-sponsored 7-year contract
- Eight components to support HIV/AIDS research and vaccine trials
- Goal of EQAPOL: help sites improve
EQAPOL Luminex: Overall Goals

1. Assess Luminex cytokine assay proficiency in domestic and international clinical research sites twice a year

2. Identify and reduce key variables that negatively impact Luminex cytokine assay performance within/between sites

3. Promote proficiency improvement through testing, remediation and training
Luminex EP Site Choice Track (optional)

Offered every other send-out (once per year)
Spring 2015: Launched a Two-Track EP model for EQAPOL Luminex

<table>
<thead>
<tr>
<th>EP Track</th>
<th>Site Participation</th>
<th>Assay Kit</th>
<th>Sample Panel</th>
<th>Graded</th>
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<tbody>
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<td>Mandatory</td>
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<td>Site-chosen Site-purchased</td>
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Benefit to Participants:
An unbiased comparison of their site-selected Luminex assay performance
- Quantifying pg/mL of five cytokine analytes
- Panel of standardized EQAPOL-created human serum samples
- Centralized assessment of accuracy to consensus and precision
EQAPOL Luminex Multiple Tech Proficiency (optional)

- Offered every other send-out (once per year)
- Pilot EP12
- Sites requested that we extend our program to enable labs to have more than one technician complete the EP.
- We proposed that sites could opt-in to receive up to one additional EQAPOL Common kit and sample set to have a second technician run the EP
- The site will receive a separate report for each kit
- Program to be continued every other EP
Assay Items supplied by EQAPOL

- **EQAPOL Assay:**
  - 21 vials containing 200 µL of cytokine spiked Human AB serum
  - One Human Cytokine 5-plex assay kit
  - One 96-well flat bottomed plate
  - Protocol and Data template

- **Site Choice Assay:**
  - 15 vials containing 200 µL of human cytokine spiked Human AB serum
  - Data template
EQAPOL Luminex: Approach

- 2 Scored EPs per year – common kit and sample test panel

- Currently monitoring 25 active sites (US, Canada, Europe, Africa, Australia and Asia)
Initiating an EP

- EPs are scheduled twice per year about six months apart

- EQAPOL will alert sites of a send-out date AT LEAST three weeks in advance
  - Sites may request a delay in shipment due to holidays, etc.

- Import permits for international sites

- Sites will be alerted when their EP ships.

- Results are due within 4 weeks of EP receipt.
EP Shipment

- **Two shipments with packing manifests**
  - **Wet ice shipment** containing one Human Cytokine 5-plex assay kit.
    - Store materials at 2-8°C
  - **Dry ice shipment** containing 21 vials of 200 µL of cytokine spiked Human AB serum
    - All vials should be stored at -80°C to -20°C upon receipt.

- **Data Logger**
  - Upon receipt deactivate the data loggers 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 in advance of performing the assay. This is necessary as the assay may vary slightly from your current Luminex procedures and from previous EPs.
Requesting a New Shipment

• If your shipment had an error, please contact EQAPOL to receive a new kit.
  • The site timeline will be reset based on the new shipment.

• If your site had a technical error that you believe will impact your results, EQAPOL may be able to replace your kit.
  • The site timeline will not be reset based on the new shipment at the discretion of the PI.

• Please contact us if you need to extend your deadline for extenuating circumstances.
Assay Procedure

- Prepare standard curve
- Prepare antibody bead cocktail
- Bead wash – magnetic method
- Analyte capture
- Analyte detection
- Assay reading
- Data analysis
- Data transfer
Required plate layout

All standards, blanks, and test samples are to be loaded in triplicate.

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* site control (optional)
Reporting Template (.xlsx file) from EQAPOL Web System

Enter values of the final observed pg/mL

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<th>IL10 pg/mL</th>
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</table>
EQAPOL Web-based System: Log In
https://eqapolapp.dhvi.duke.edu
Select “Luminex” from the Menu

** Some options will not be available for your site
Select “EP” from Luminex Program Page

Welcome to the EQAPOL Luminex Program

On behalf of EQAPOL and the CIC/NIAID Luminex Proficiency Steering Committee, we want to thank you for participating in the EQAPOL and CIC/NIAID Luminex Proficiency Panel (LPP) program. This page will enable your site to access both active and completed External Proficiency (EP) rounds by selecting your site listed under the EP of interest. Please note that documents in the “Completed EPs” are available for viewing/download only; they cannot be edited.

Should you encounter any problems while working with the EQAPOL web-based system, please contact us at EQAPOL@duke.edu for assistance.

Best regards,

The EQAPOL Luminex Team

<table>
<thead>
<tr>
<th>Active EPs</th>
</tr>
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<tbody>
<tr>
<td>EP 13</td>
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<tr>
<td>EP 13 Site Choice</td>
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</table>

<table>
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<tr>
<th>Completed EPs</th>
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<tbody>
<tr>
<td>EP 1 Polystyrene</td>
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<tr>
<td>EP 2 Polystyrene</td>
</tr>
<tr>
<td>EP 3 Polystyrene</td>
</tr>
<tr>
<td>EP 4 Polystyrene</td>
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</tbody>
</table>

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

**Luminex EP 15 (Active)**

We appreciate your participation in the CIC/NIAID Luminex External Proficiency Program developed and managed by EQAPOL.

**Please follow the instructions below to complete EP15 EQAPOL:**

1. Upon receipt of your Luminex EP15 shipments, navigate to the “Send-out” tab and mark shipments as “received.” If any shipment issues were noted, please record them here.

2. Download the protocol provided below and use it to perform the Luminex assay using the EQAPOL-provided kit and samples. Please do not deviate from the protocol.

3. Download the Excel template below and complete it using the instructions provided in the protocol. Copy and paste the pg/mL for each analyte in each well onto the template. Copy and paste the raw MFI for each analyte in each well onto the template.

4. 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.”

5. 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 EP15.

6. 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.

7. Once your file is 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 EP15 or need assistance with the web-based system, please do not hesitate to contact us.

Thank you for your participation in EP15,

The EQAPOL Luminex Team

<table>
<thead>
<tr>
<th>Site</th>
<th>Assay Run</th>
<th>Send-Out</th>
<th>Results</th>
<th>Reports</th>
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</thead>
<tbody>
<tr>
<td>EQAPOL</td>
<td>1</td>
<td>Due date not yet set</td>
<td></td>
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</tbody>
</table>
Navigating Your Site’s Home Page

- **Shipment Information**
- **Report tab will appear after EP results are available**
- **Discussion between Site and EQAPOL**

**INSTRUCTIONS**

We appreciate your participation in the CIC/NAID Luminex External Proficiency Program developed and managed by EQAPOL.

Please follow the instructions below to complete EP15 EQAPOL:

1. **Download Protocol and Template**
2. **Upload Results (XLS template) and Take Survey**

3. Download the Excel template below and complete it using the instructions provided in the protocol. Copy and paste the pg/mL for each analyte in EACH well onto the template. Copy and paste the raw MFI for each analyte in EACH well onto the template.
Acknowledge Receipt of Shipments

Enter date in “Received at” when your shipment arrives then choose “Update Shipment” below.
Acknowledge Receipt of Shipments

- 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 Documents: Select “Results”

- **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.

Submit Results Excel Document
• “Remove” and “Save” will remove all files selected
• The results sheet will require the proper formatting in order to be successfully imported by EQAPOL
• Submitted documents are final and can only be viewed by sites
• To make any changes contact us (EQAPOL@duke.edu)
Assay Assessment

• EQAPOL analyzes data from sites provided via XLS template and questionnaire.

• Grading is based on consensus standards (i.e., not gold standard laboratory)

• Sites receive a score out of 100 per EP
# EQAPOL Luminex: Grading Scheme

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Target</th>
<th>Max. Points</th>
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<tr>
<td><strong>Timeliness</strong></td>
<td>On time upload of valid EP data and survey</td>
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<tr>
<td><strong>Protocol Adherence</strong></td>
<td>Followed prescribed instrument setup/QC, plate layout, assay procedure, and analysis instructions</td>
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<td>Acceptable fit probability of site-reported standard curve(s)</td>
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<tr>
<td><strong>Accuracy</strong></td>
<td>Site-reported results within 95% CI boundaries of EP consensus</td>
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<tr>
<td><strong>Precision</strong></td>
<td>Well to well variability (9 estimates per samples; %CV site-reported results)</td>
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**Overall Performance:**

<table>
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<th>Grade</th>
<th>Score Range</th>
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<td>Excellent</td>
<td>91-100</td>
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<td>Good</td>
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<td>Fair</td>
<td>66-74</td>
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<td>Poor</td>
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* Remediation Required
Reports

• Once the EP is closed and the Centralized Analysis is completed, site reports will be made available in the EQAPOL web system. Sites are alerted via email when reports have been uploaded.

• A new tab “Reports” will appear with the report file available for download.

• A Site Choice Report will be available to sites who participated in the site choice component, however the report does not include a numerical score or grade.

• 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

Luminex Oversight Laboratory Study Director
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Assistant Director, RBL Programs and Development
Heather Lynch: heather.lynch@duke.edu

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

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GHRB 1019
909 S. LaSalle St.
Durham, NC 27710 USA

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