Limited Antigen Avidity (LAg) Incidence Assay EQA Program

Orientation:  EQA Send-out Process
Overview:  EQAPOL Web-based application
Presentation Outline

- Overview of EQAPOL
- Overview of EQAPOL LAg EQA
- General EQA Process
- Participation in a LAg-based EP
  - What you will receive
  - Web-based System
  - Reports and Remediation
- New EQAPOL document of recognition
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
Introduction to LAg Incidence Program

- The EQAPOL LAg Program is modeled after the CDC BED Proficiency Testing Program
  - EQAPOL collaboration with VRI and CDC

- Program will initially only include Sedia and Maxim LAg assays

- The EQAPOL LAg 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;
  - the ability to properly classify recent vs. long-term infections as well as ability to properly run serology assays for HIV status

- Site grading first implemented in EP3. The purpose of grading is to examine differences in assay results across multiple sites
Pilot LAg Program

- A pilot study was conducted in December 2014 with EQAPOL, BSRI, the CDC, and two select sites to explore the feasibility of launching a proficiency testing program using the LAg Avidity Assays.

- As a result of the study, EQAPOL was approved to launch a formal program in 2015.

- EQAPOL used the participant list from the BED Program as a guide for enrolling sites.
Send-out Workflow

EQAPOL/BSRI Ships Kits → Sites Return Data Logger to EQAPOL → Sites Complete Initial Testing on LAg Assay → Sites Complete Confirmatory Testing per their Protocol

Sites Complete Serology (no VL) per their Protocol, as needed → Sites Return Raw Data, Analyzed Results and Complete Questionnaire → EQAPOL Analyzes Results → EQAPOL Releases Reports
What your site will receive

• One shipment with packing manifests will be sent to your site
  • **BOX 1:** Dry ice shipment containing 26 test samples – *store at -80°C*
  • **Please note that your site must supply the assay kit (Sedia and/or Maxim)**

• **Data Logger**
  • Temperature monitor that is a USB drive in the shipment box. It tracks the temperature of the box/samples to ensure that they maintain -80°C.
  • Upon receipt deactivate the data logger according to the protocol and submit the data in the web portal.

• **Assay Protocol**
  • Thoroughly familiarize yourself with all aspects of the EP-specific protocol
  • Be sure your plate reader is fully functional and QC’d prior to thawing any kit components
  • Please do not deviate from the provided protocol
  • Submit data to EQAPOL within 4 weeks of kit receipt

• **Files on Web-based System**
  • Protocol
  • Excel Reporting Template/Data Management Tool
  • Questionnaire
Required EP Plate Layout

Please run your initial testing using the layout provided in the protocol. Confirmatory testing plate layout will be dependent upon results from the initial test.

<p>| | | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
<td>G</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg</td>
<td>Neg</td>
<td>CAL</td>
<td>CAL</td>
<td>CAL</td>
<td>LPC</td>
<td>LPC</td>
<td>LPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPC</td>
<td>HPC</td>
<td>HPC</td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 3</td>
<td>Sample 4</td>
<td>Sample 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 6</td>
<td>Sample 7</td>
<td>Sample 8</td>
<td>Sample 9</td>
<td>Sample 10</td>
<td>Sample 11</td>
<td>Sample 12</td>
<td>Sample 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Excel Data Management Tool for LAg Testing Results

• File provided is essentially identical to CDC data management tool for LAg assays
• When saving the file, please use the following naming convention:
  • EPX_LAg Data Management File Maxim_SiteXXX
  • Simply replace the XXX with your site number
• There are multiple tabs within the file for the various steps in the assay process
• This file will be uploaded to the EQAPOL Web System
What to do with your EP data

• **Determine ODn of Test Samples**
  • Using your lab-specific method, your site will determine the following:
    • ODn values for all 13 human plasma samples; 3 of these are near cutoff that are not graded
    • Prepare Excel Data Reporting Template with ODn values and classifications

• **Data Reporting**
  • Sites will upload XLS File
  • Sites will Report ODn values, serology results and sample classifications.
  • Sites will complete post-assay questionnaire
  • All EP data reporting will take place via the EQAPOL web-based application:
    • Please make sure you have access to the web system and have completed registration
    • Upload results using the provided Excel Template and post ODn values and classifications using the web-based Application

https://eqapolapp.dhvi.duke.edu
Overview: EQAPOL Web-based application
EQAPOL Web-based System: Log In

https://eqapolapp.dhvi.duke.edu
Select Active “EP” from the LAg Program Page

On behalf of EQAPOL, we want to thank you for participating in the EQAPOL Limiting Antigen (LAG) Incidence Assay Proficiency Panel 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 Incidence Assay Team

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

Only sites for which you are a part of will display on this page
Sites running two kits will see their site listed twice as above
We appreciate your participation in the Limiting Antigen (LAg) Incidence Assay External Proficiency Program developed and managed by EQAPOL.

Please follow the instructions below to complete EP1:

1. Upon receipt of your LAg EP1 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 LAg assay using the EQAPOL-provided 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 values into the appropriate cells of the template.
4. Once you have completed the assay, please navigate to the “Results” tab and complete the post-assay questionnaire and upload your Excel file. 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 EP1.
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.
8. Once your file is submitted, you will navigate to the “Direct Entry Results” tab to manually enter your results.

Should you have any questions about EP1 or need assistance with the web-based system, please do not hesitate to contact us.

Thank you for your participation in EP1.

The EQAPOL LAg Incidence Assay Team
Acknowledge Receipt of Shipments

Enter date when your shipment arrives and state whether there were issues with the samples
Acknowledge Receipt of Shipments

Change “Shipment Issues” to “Yes” to note any shipping issues and select “Update Shipment”
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 “Raw 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.

Upload data logger PDF under “additional files”
### Direct Entry

**Select Sedia/Maxim**

<table>
<thead>
<tr>
<th>Kit Type</th>
<th>Calibrator Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Test QC</td>
</tr>
<tr>
<td></td>
<td>Neg a</td>
</tr>
<tr>
<td></td>
<td>Neg b</td>
</tr>
<tr>
<td></td>
<td>CAL a</td>
</tr>
<tr>
<td></td>
<td>CAL b</td>
</tr>
<tr>
<td></td>
<td>CAL c</td>
</tr>
<tr>
<td></td>
<td>LPC a</td>
</tr>
<tr>
<td></td>
<td>LPC b</td>
</tr>
<tr>
<td></td>
<td>LPC c</td>
</tr>
<tr>
<td></td>
<td>HPC a</td>
</tr>
<tr>
<td></td>
<td>HPC b</td>
</tr>
<tr>
<td></td>
<td>HPC c</td>
</tr>
</tbody>
</table>

Paste Spreadsheet Results here to auto-fill columns:
- Paste Results

Paste Spreadsheet Results here to auto-fill columns:
- Paste Results
## Calibrator Results

Copy these cells from Excel File (Initial Results and Confirmatory Results)

<table>
<thead>
<tr>
<th>Spec ID</th>
<th>OD</th>
<th>Mediat</th>
<th>ODn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg 1a</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>Neg 1b</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>CAL 1a</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>CAL 1b</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>CAL 1c</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>LPC 1a</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>LPC 1b</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>LPC 1c</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>HPC 1a</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>HPC 1b</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
<tr>
<td>HPC 1c</td>
<td>0.0</td>
<td>0.000</td>
<td>#DIV/0!</td>
</tr>
</tbody>
</table>
Direct Entry

Paste data here

<table>
<thead>
<tr>
<th>Kit Type</th>
</tr>
</thead>
</table>

**Calibrator Data**

<table>
<thead>
<tr>
<th>Initial Test QC</th>
<th>Confirmatory Test QC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg a</td>
<td>Neg a</td>
</tr>
<tr>
<td>Neg b</td>
<td>Neg b</td>
</tr>
<tr>
<td>CAL a</td>
<td>CAL a</td>
</tr>
<tr>
<td>CAL b</td>
<td>CAL b</td>
</tr>
<tr>
<td>CAL c</td>
<td>CAL c</td>
</tr>
<tr>
<td>LPC a</td>
<td>LPC a</td>
</tr>
<tr>
<td>LPC b</td>
<td>LPC b</td>
</tr>
<tr>
<td>LPC c</td>
<td>LPC c</td>
</tr>
<tr>
<td>HPC a</td>
<td>HPC a</td>
</tr>
<tr>
<td>HPC b</td>
<td>HPC b</td>
</tr>
<tr>
<td>HPC c</td>
<td>HPC c</td>
</tr>
</tbody>
</table>

Paste Spreadsheet Results here to auto-fill columns:

Paste Results
Direct Entry of Results

Manually enter ODn values in the text boxes and click the appropriate radio button for final classifications. Click save and/or submit at bottom of screen when you are ready. You must click submit for results to be viewed at EQAPOL.
## EQAPOL LAg: Grading Scheme

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Target</th>
<th>Max. Points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timeliness</strong></td>
<td>On time EP valid data and questionnaire upload</td>
<td>10</td>
</tr>
<tr>
<td><strong>Protocol Adherence</strong></td>
<td>Following protocol instructions and algorithm</td>
<td>24</td>
</tr>
<tr>
<td><strong>Classification</strong></td>
<td>Correct classification of recency status</td>
<td>48</td>
</tr>
<tr>
<td><strong>Accuracy/Precision</strong></td>
<td>Statistically different from consensus average</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>The within site variability must be included in 95% CI boundary</td>
<td></td>
</tr>
</tbody>
</table>

### Overall Performance:

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

* Remediation Required
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

LAg Oversight Laboratory Study Director
Mike Busch: mbusch@bloodsystems.org

Assistant Director
Mars Stone: mstone@bloodsystems.org

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

EQAPOL Address:
EQAPOL
Duke Human Vaccine Institute
Duke University Medical Center
MSRB II, 2 Genome Court
Room 4002
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).