



# EQAPOL Luminex Program Orientation

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Overview:  
EQAPOL Web-Based  
Application

*Updated: 2/1/2019*

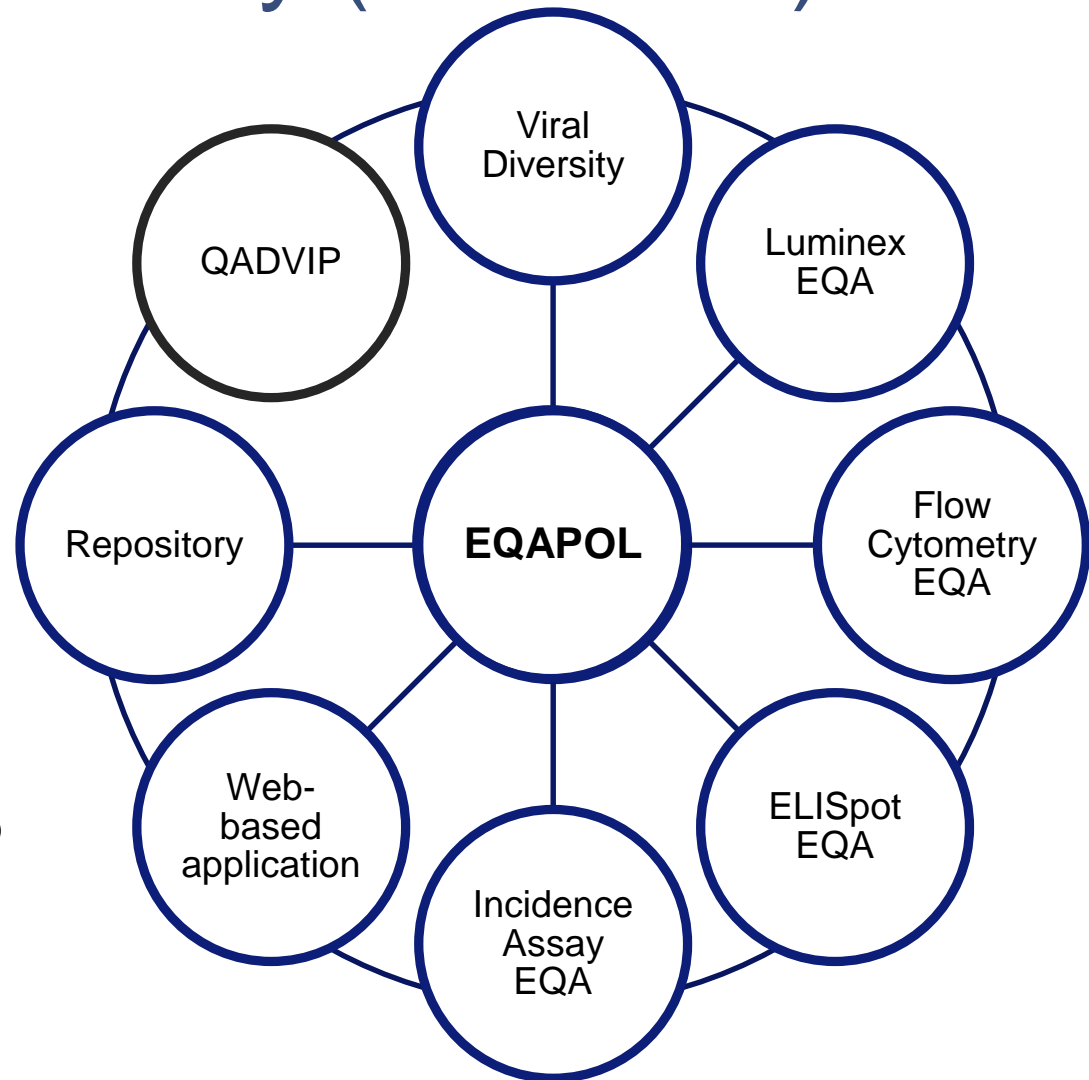


# Presentation Outline

- 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

EP Track	Site Participation	Assay Kit	Sample Panel	Graded
EPX.EQAPOL	Mandatory	EQAPOL-provided	Full EQAPOL Panel	✓
EPX.Choice	Optional	Site-chosen Site-purchased	15 sample subset	X

## 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)

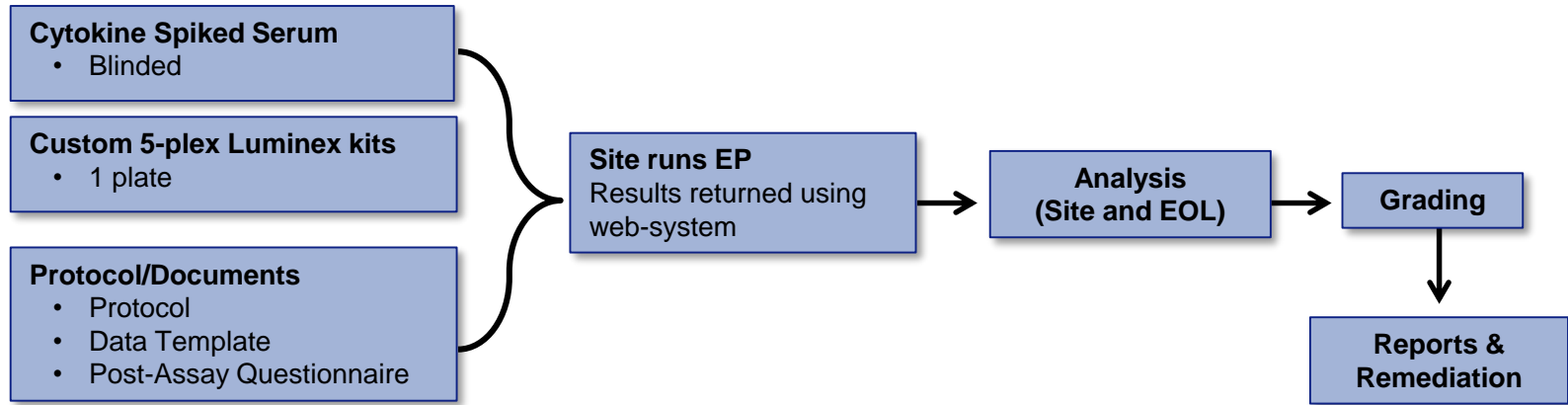
- Offered every other send-out (once per year)
- Pilot EP12
- Sites requested that we extend our program to enable labs to have more than one technician complete the EP.
- We proposed that sites could opt-in to receive up to one additional EQAPOL Common kit and sample set to have a second technician 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

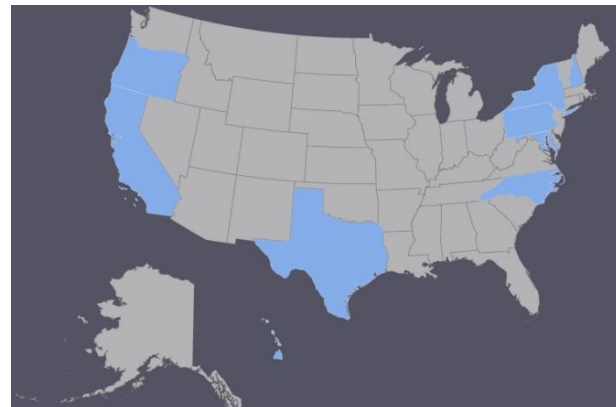
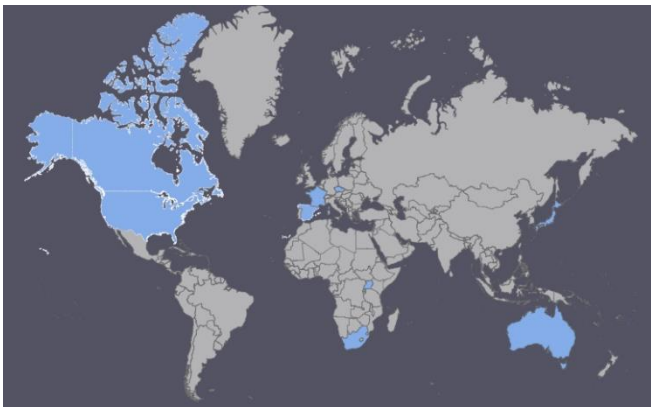
- EQAPOL Assay:
  - 21 vials containing 200  $\mu$ L of cytokine spiked Human AB serum
  - One Human Cytokine 5-plex assay kit
  - One 96-well flat bottomed plate
  - Protocol and Data template
- Site Choice Assay:
  - 15 vials containing 200  $\mu$ L of human cytokine spiked Human AB serum
  - Data 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

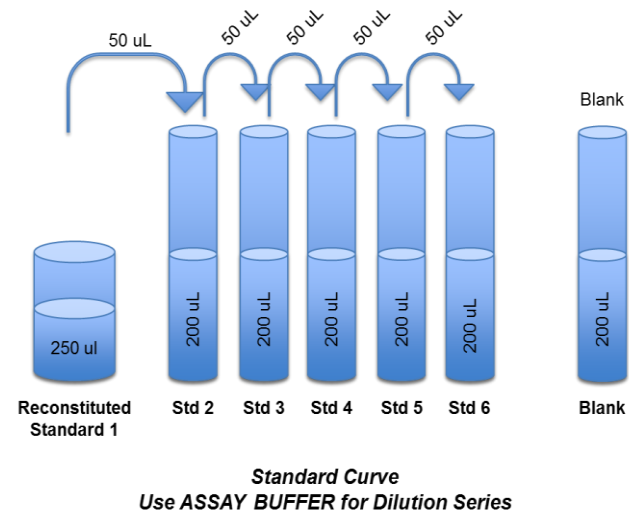
- **Two shipments with packing manifests**
  - **Wet ice shipment** containing one Human Cytokine 5-plex assay kit .
    - Store materials at 2-8°C
  - **Dry ice shipment** containing 21 vials of 200 µL of cytokine spiked Human AB serum
    - All vials should be stored at -80°C to -20°C upon receipt.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

EP12 Plan	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1			P5D6			W5V9			U5D6		
B	Std2			H7P8			U2E5			E8M7		
C	Std3			C2Q3			R4D3			Z6Z6		
D	Std4			T4Z5			N9H0			A9C0		
E	Std5			L8D0			S7J6			N7D5		
F	Std6			D8X7			O3V4					
G	BLANK			Z2K7			W2J4					
H				H4P3			G6B4			*Site control (optional)		

# Reporting Template (.xlsx file) from EQAPOL Web System

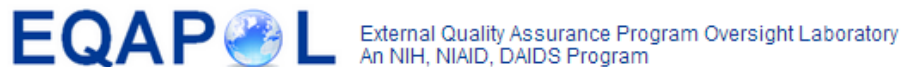
Don't Edit/change

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Well_Numbe	WELLID	IL10_pg/mL	IL6_pg/mL	IFNg_pg/mL	TNFa_pg/mL	IL2_pg/mL	IL10_MFI	IL6_MFI	IFNg_MFI	TNFa_MFI	IL2_MFI	Sample_ID_Code	Well_Type
2	1	A1											Standard1	S1
3	2	A2											Standard1	S1
4	3	A3											Standard1	S1
5	4	A4											P5D6	X1
6	5	A5											P5D6	X1
7	6	A6											P5D6	X1
8	7	A7											W5V9	X9
9	8	A8											W5V9	X9
10	9	A9											W5V9	X9
11	10	A10											U5D6	X17
12	11	A11											U5D6	X17
13	12	A12											U5D6	X17
14	13	B1											Standard2	S2
15	14	B2											Standard2	S2
16	15	B3											Standard2	S2
17	16	B4											H7P8	X2
18	17	B5											H7P8	X2
19	18	B6											H7P8	X2
20	19	B7											U2E5	X10
21	20	B8											U2E5	X10
22	21	B9											U2E5	X10
23	22	B10											E8M7	X18
24	23	B11											E8M7	X18
25	24	B12											F8M7	X18

Enter values of the  
final observed pg/mL

# EQAPOL Web-based System: Log In

<https://eqapolapp.dhvi.duke.edu>



## Sign In

Email

Password

[Forgot/Reset your password](#)

[Sign in with DHE credentials](#)

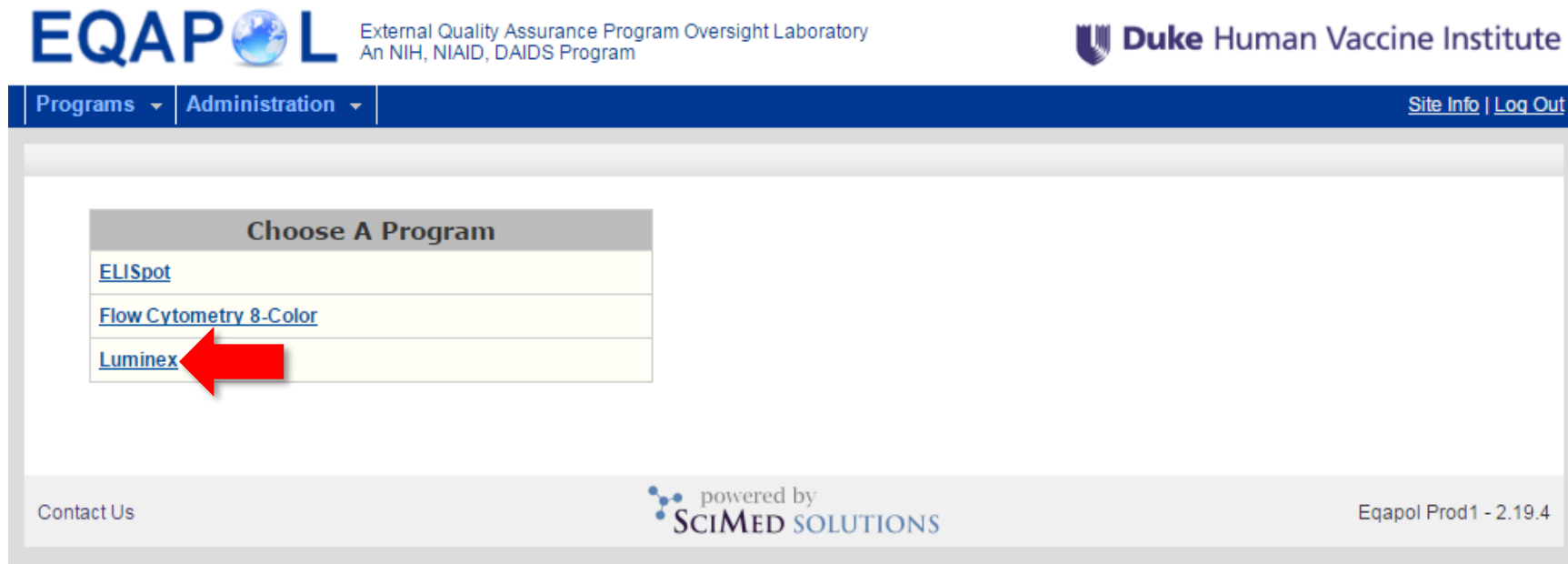
EQAPOL is a U.S. Government information System. Use of the system indicates consent to monitoring, recording, and auditing. Unauthorized use is prohibited and subject to criminal and civil penalties.

[Contact Us](#)

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# Select “Luminex” from the Menu



The screenshot displays the EQAPOL website interface. At the top left, the logo for EQAPOL (External Quality Assurance Program Oversight Laboratory, An NIH, NIAID, DAIDS Program) is shown. To the right is the Duke Human Vaccine Institute logo. Below the logos is a navigation bar with 'Programs' and 'Administration' dropdown menus, and links for 'Site Info' and 'Log Out'. The main content area features a 'Choose A Program' section with a table listing three options: 'ELISpot', 'Flow Cytometry 8-Color', and 'Luminex'. A red arrow points to the 'Luminex' option. The footer contains 'Contact Us', 'powered by SCIMED SOLUTIONS', and 'Eqapol Prod1 - 2.19.4'.

**EQAPOL** External Quality Assurance Program Oversight Laboratory  
An NIH, NIAID, DAIDS Program

**Duke** Human Vaccine Institute

Programs ▾ Administration ▾ [Site Info](#) | [Log Out](#)

Choose A Program
<a href="#">ELISpot</a>
<a href="#">Flow Cytometry 8-Color</a>
<a href="#">Luminex</a>

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*\*\* Some options will not be available for your site*



# Select “EP” from Luminex Program Page



External Quality Assurance Program Oversight Laboratory  
An NIH, NIAID, DAIDS Program



Programs ▾

Administration ▾

[Site Info](#) | [Log Out](#)

## Luminex

### 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](mailto:EQAPOL@duke.edu) for assistance.

Best regards,

The EQAPOL Luminex Team

#### Active EPs

[EP 13](#)

[EP 13 Site Choice](#)

#### Completed EPs

[EP 1 Polystyrene](#)

[EP 2 Polystyrene](#)

[EP 3 Polystyrene](#)

[EP 4 Polystyrene](#)

**\*\* 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](mailto: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

## Snapshot of EP status

Site	Assay Run	Send-Out	Results	Reports
<a href="#">EQAPOL</a>	1		Due date not yet set	



Only sites for which you are a part of will display on this page

# Navigating Your Site's Home Page

The diagram illustrates the navigation flow on a website home page. It features a navigation bar with six tabs: INSTRUCTIONS, SEND-OUT, RESULTS, CENTRALIZED ANALYSIS, REPORTS, and COMMUNICATION/FEEDBACK. Below the navigation bar, there is a main content area with several paragraphs of text. Red arrows point from callout boxes to specific elements:

- Shipment Information** points to the **SEND-OUT** tab.
- Report tab will appear after EP results are available** points to the **REPORTS** tab.
- Discussion between Site and EQAPOL** points to the **COMMUNICATION/FEEDBACK** tab.
- Download Protocol and Template** points to the **INSTRUCTIONS** tab.
- Upload Results (XLS template) and Take Survey** points to the **RESULTS** tab.

The main content area contains the following text:

INSTRUCTIONS SEND-OUT RESULTS CENTRALIZED ANALYSIS REPORTS COMMUNICATION/FEEDBACK

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

Please follow the instructions below to complete EP15 EQAPOL:

EP15 "out" tab and mark shipments as "received." If any shipment issues were noted,

and below ex assay using the EQAPOL-provided kit and samples. *Please do not deviate from*

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

Shipment Information

Flow Cytometry 8-Color EP 9 - EQ

INSTRUCTIONS **SEND-OUT** RESULTS CENTRALIZED AN

We appreciate your participation in EQAPOL Flow Cytometry 8 EP9:

## Shipment #11740

Shipped: 12/10/2014 05:12 PM  
 Courier: Fedex  
 Tracking #: 772166719609  
 Documents

Item	Global Spec ID	Quantity	Volume
Reagent A	09252014	1	200.0 µL
Reagent B	09252014	1	200.0 µL
Reagent C	09252014	1	200.0 µL
Sample 1	J69059VN-36	1	20000000.0 CEL
Sample 1	J69059VN-37	1	20000000.0 CEL
Sample 1	J69059VN-38	1	20000000.0 CEL
Sample 2	E69027ST-20	1	20000000.0 CEL
Sample 2	E69027ST-21	1	20000000.0 CEL
Sample 2	E69027ST-22	1	20000000.0 CEL
Sample 3	F69080LX-11	1	
Sample 3	F69080LX-12	1	
Sample 3	F69080LX-13	1	

Received at:

Shipment issues:

Please describe the issues with your shipment:

Update Shipment

Enter date in "Received at" when your shipment arrives then choose "Update Shipment" below

# Acknowledge Receipt of Shipments

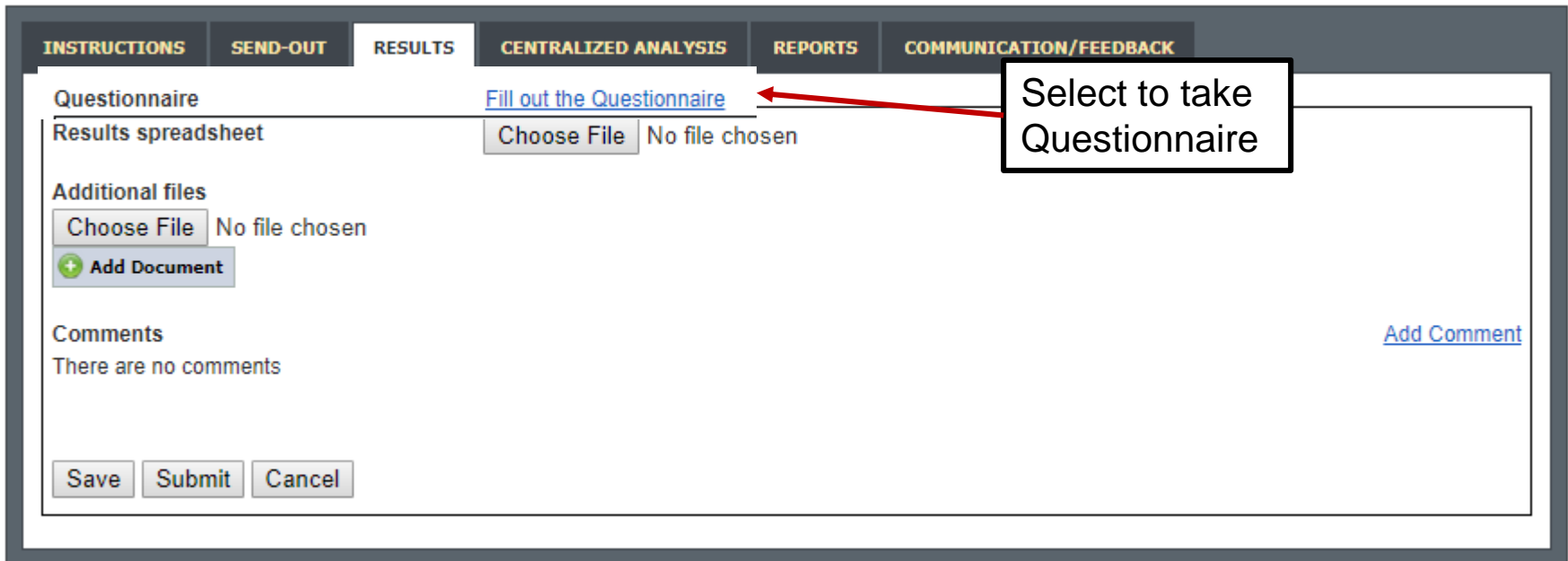
Received at:

Shipment issues:

Please describe the issues with your shipment:

Change "Shipment Issues" to "Yes" to note any shipping issues and select "Received"

# Completing Questionnaire



The screenshot shows a web interface with a dark grey header containing several tabs: INSTRUCTIONS, SEND-OUT, RESULTS, CENTRALIZED ANALYSIS, REPORTS, and COMMUNICATION/FEEDBACK. The 'RESULTS' tab is active. Below the tabs, the 'Questionnaire' section is highlighted, containing a blue link 'Fill out the Questionnaire'. A red arrow points from a callout box containing the text 'Select to take Questionnaire' to this link. Other sections include 'Results spreadsheet' with a 'Choose File' button and 'No file chosen' text; 'Additional files' with a 'Choose File' button, 'No file chosen' text, and a '+ Add Document' button; and 'Comments' with the text 'There are no comments' and an 'Add Comment' link. At the bottom, there are 'Save', 'Submit', and 'Cancel' buttons.

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”

INSTRUCTIONS SEND-OUT RESULTS CENTRALIZED ANALYSIS REPORTS COMMUNICATION/FEEDBACK

Questionnaire [Fill out the Questionnaire](#)

Results spreadsheet  No file chosen

Additional files  
 No file chosen

Comments  
 There are no comments [Add Comment](#)

**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

# Document “saved” Example

INSTRUCTIONS SEND-OUT RESULTS CENTRALIZED ANALYSIS REPORTS COMMUNICATION/FEEDBACK

Results spreadsheet [eqapolepDAA\\_000\\_8cics.xlsx](#) Remove

Additional files

Choose File No file chosen

+ Add Document

Comments

There are no comments [Add Comment](#)

Save Submit Cancel

Back to EP DAA Back to Flow Cytometry 8-Color

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- “Remove” and “Save” will remove all files selected
- The results sheet will require the proper formatting in order to be successfully imported by EQAPOL



# Document “Submitted” Example

**INSTRUCTIONS** **SEND-OUT** **RESULTS** **CENTRALIZED ANALYSIS** **REPORTS** **COMMUNICATION/FEEDBACK**

Submitted: 11/18/2016 03:52 PM by andrea.pappas@duke.edu.dev.null

Replace  No file chosen

Results spreadsheet [eqapolepDAA\\_000\\_8cics.xlsx](#)

Comments [Add Comment](#)

There are no comments

[Back to EP DAA](#) [Back to Flow Cytometry 8-Color](#)

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Eqapol Test - 2.19.3.rc-17

- 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

Criteria	Target	Max. Points
<b>Timeliness</b>	On time upload of valid EP data and survey	10
<b>Protocol Adherence</b>	Followed prescribed instrument setup/QC, plate layout, assay procedure, and analysis instructions	5
	Acceptable fit probability of site-reported standard curve(s)	5
<b>Accuracy</b>	Site-reported results within 95% CI boundaries of EP consensus	40
<b>Precision</b>	Well to well variability (9 estimates per samples; %CV site-reported results)	40

<b>Overall Performance:</b>	
Excellent	91-100
Good	75-90
Fair *	66-74
Poor *	0-65

\* 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](mailto:EQAPOL@duke.edu)

**EQAPOL Address:**

**Luminex Oversight Laboratory Study**

**Director**

Gregory Sempowski:

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Duke Human Vaccine Institute

GHRB 1019

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