



ELISpot Assay

Orientation: ELISpot EQA Program

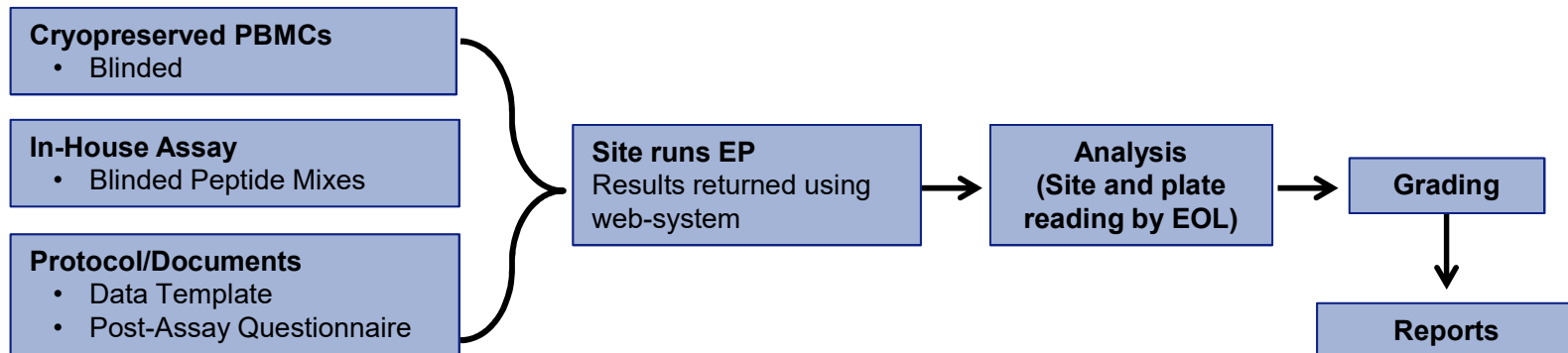
Overview: EQAPOL Web-based application

Introduction to ELISpot Program

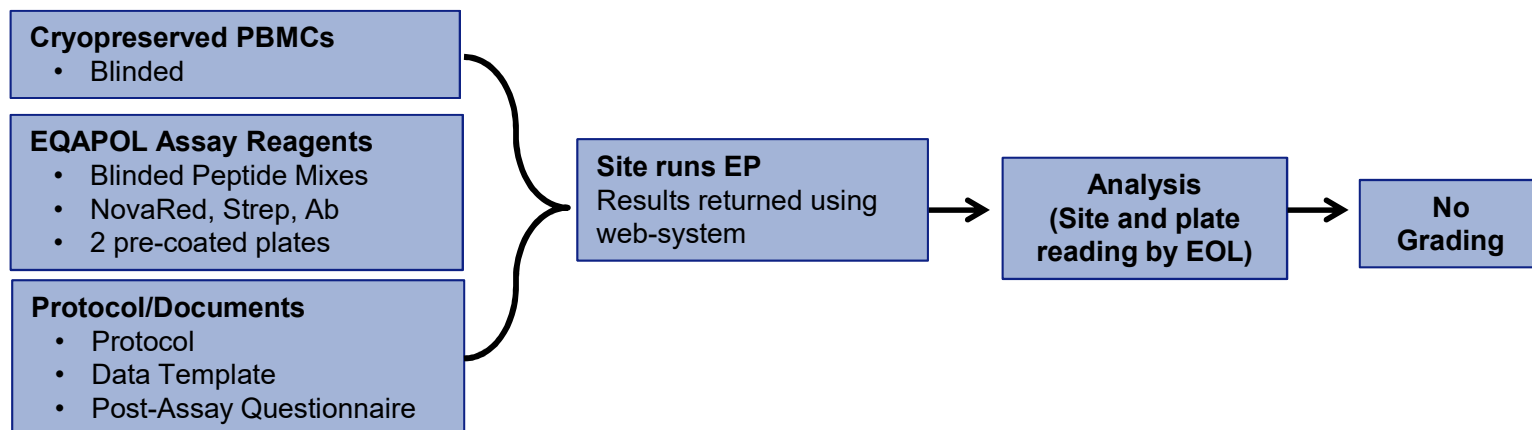
- The EQAPOL ELISpot EQA Program will assess the following measures:
 - the range of responses detected by the lab;
 - the precision of the measurements (i.e., replicates);
 - the comparison of the results to what is observed among labs;
 - comparison with central reading;
 - comparison with a reference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

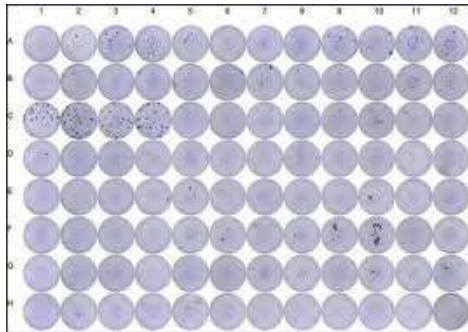
In-House Assay



EQAPOL Assay



ELISpot Data



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

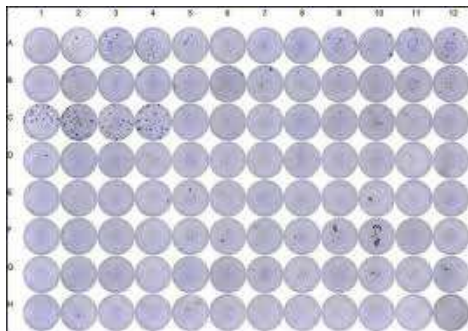
Read by Site

Grading

Read by EOL

No Grading-To identify:

- Problems with Reader



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

Read by Site

No Grading-To identify:

- Problems with In-house Assay
- Technician Problems

Read by EOL

No Grading-To identify:

- Problems with Reader

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 LN₂
 - 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 LN₂ 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

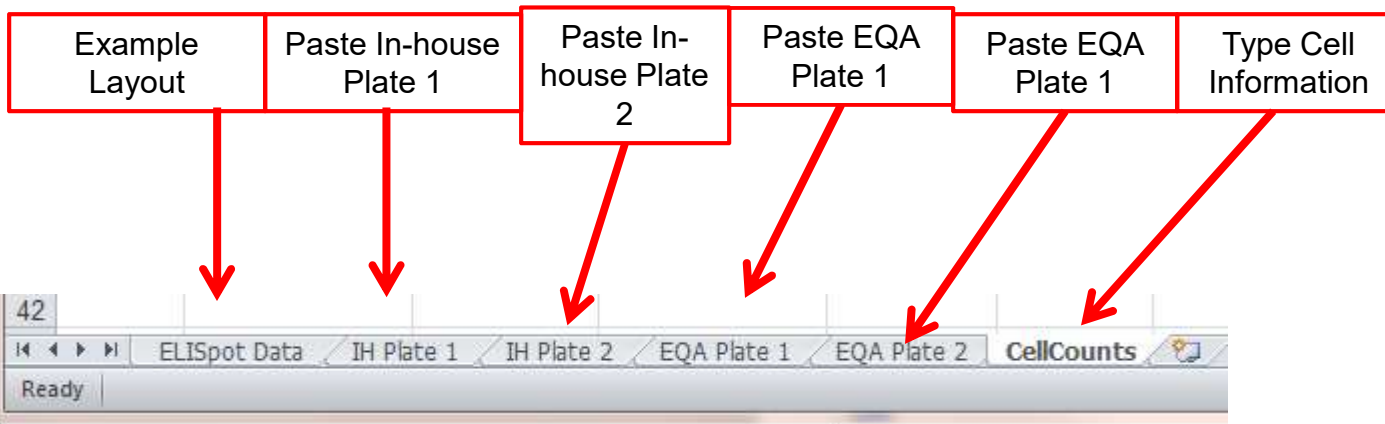
- PBMCs (EQAPOL)
- Peptides (EQAPOL)
- Protocol (EQAPOL)
- Plates (EQAPOL)
- Key Reagents (EQAPOL)
 - Streptavidin-HRP
 - Detection Ab
 - Nova Red Substrate
- Other Reagents (Site-specific)

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>
- **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)



Well	1	2	3
D			
E			
F			
G			
H			

This column should contain these characters in this order.

These columns (1-12) should contain a number. If a well was not done then "-1" should be recorded. If a well had confluent spots then "-2" should be recorded.

No decimal points, commas or percentage signs are allowed. The lowest number allowed is "-2". The highest number allowed is 9999. No letters are allowed.

Grading ELISpot Performance

Criteria	Description	Target	Points
Timeliness	On time EP valid data and questionnaire upload	Per due date set by EQAPOL	<ul style="list-style-type: none"> 10 points total
PBMC Processing	Viability: Pass/Fail per each donor sample	Donor must be >80% for D1	<ul style="list-style-type: none"> 9 points total 1.5 points available per donor for viability 1.5 points available per donor for recovery
	Recovery: Pass/Fail per each donor sample	Donor must be between 70-120% for D1	
Background	<ul style="list-style-type: none"> Pass/Fail per each donor sample 	Average for each donor must be <10	<ul style="list-style-type: none"> 9 total 3 points available per donor
Accuracy to the consensus	<ul style="list-style-type: none"> Assessed for each donor sample for each stimulation condition (CMV and CEF) 	Site's average for 9 wells must <u>not</u> be significantly different from consensus average	<ul style="list-style-type: none"> 54 points total 9 point deduction for each donor by stim condition significantly different from consensus average
Precision	<ul style="list-style-type: none"> Assessed for each donor sample for each stimulation condition (CMV and CEF) 	<u>The dispersion must be less than 3.3</u>	<ul style="list-style-type: none"> 18 points total 3 points deducted for each donor/stimulation outside of range

Grading ELISpot Performance

EQAPOL Performance Ranges	
Overall Performance Score	Performance
91-100	Excellent
75-90	Good
66-74	Fair
0-65	Poor

Site performing “Fair” or “Poor” contacted for remediation



Overview: EQAPOL Web-based application

Updated 1/24/2019

EQAPOL Web-based System: Log In

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

The screenshot displays the login interface for the EQAPOL system. At the top left, the EQAPOL logo is shown with the text "External Quality Assurance Program Oversight Laboratory An NIH, NIAID, DAIDS Program". To the right is the Duke Human Vaccine Institute logo. The main heading is "Sign In". Below this, there are two input fields: "Email" and "Password". A "Forgot/Reset your password" link is positioned to the right of the password field. A "Sign in" button is located below the password field. A link for "Sign in with DHE credentials" is at the bottom left of the form area. A disclaimer at the bottom right states: "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." The footer contains "Contact Us", the "powered by SCIMED SOLUTIONS" logo, and the version number "Eqapol Prod1 - 2.13.0".

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

Duke Human Vaccine Institute

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

powered by
SCIMED SOLUTIONS

Eqapol Prod1 - 2.13.0

Select “ELISpot” 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', 'Orders', and 'Administration' dropdown menus, and links for 'todch@gmail.com', 'Site Info', and 'Log Out'. The main content area features a 'Choose A Program' dropdown menu with two options: 'ELISpot' and 'Viral Diversity'. A red arrow points to the 'ELISpot' option. At the bottom, there is a footer with 'Contact Us', 'powered by SCIMED SOLUTIONS', and 'Eqapol Prod1 - 2.14.0'.

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

Select Active EP from ELISpot Program Page

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

Duke Human Vaccine Institute

Inventory ▾ Programs ▾ Orders ▾ Administration ▾ alp27 | Site Info | Log Out

ELISpot

Welcome to the EQAPOL ELISpot Program

On behalf of EQAPOL , we want to thank you for participating in the EQAPOL ELISpot 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 ELISpot Team

Active EPs

[EP 14](#)

Completed EPs

[EP 1](#)

[EP 2](#)

[EP 3](#)

[EP 4](#)

[EP 5](#)

[EP 6](#)

[EP 7](#)

[EP 8](#)

[EP 9](#)

[EP 10](#)

[EP 11](#)

[EP 12](#)

[EP 13](#)

**** 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

[eqapolelispot_ep8workbook.xlsx](#)
[elispotep8_siteinstructions_final.pdf](#)

Site	Assay Run	Send-Out	Questionnaire	Results	Reports
EQAPOL [REDACTED]	1	Received 11/11/2014, Received 11/11/2014, Shipped 12/10/2014, Shipped 12/10/2014	Submitted 12/09/2014	Due: 12/09/2014	

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

Download
Protocol and
Template

Shipment
Information

Upload Results
(XLS template)
and Take
Survey

Discussion between
Site and EQAPOL

ELISpot EP 16

Assay Run 1

INSTRUCTIONS

SEND-OUT

RESULTS

CENTRALIZED ANALYSIS

REPORTS

COMMUNICATION/FEEDBACK

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 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.

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](#)

Acknowledge Receipt of Shipments

Shipment #11740

Shipped: 12/10/2014 05:12 PM
Courier: Fedex
Tracking #: 772166719609
Documents: [eqapol_elispot_ep8_site_1267.xls](#) [batch_1267.xls](#)

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	20000000.0 CEL
Sample 3	F69080LX-12	1	20000000.0 CEL
Sample 3	F69080LX-13	1	20000000.0 CEL

Received at:

Shipment issues: None

Please describe the issues with your shipment:

Check "Received at" when your shipment arrives

Acknowledge Receipt of Shipments

V7R2Y	V7R2Y	1	80.0 µL
C2A3T	C2A3T	1	80.0 µL
A4M1S	A4M1S	1	80.0 µL
B1J2L	B1J2L	1	80.0 µL
N5K2Q	N5K2Q	1	80.0 µL
F5E4U	F5E4U	1	80.0 µL
S4Q6M	S4Q6M	1	80.0 µL
X2G5R	X2G5R	1	80.0 µL

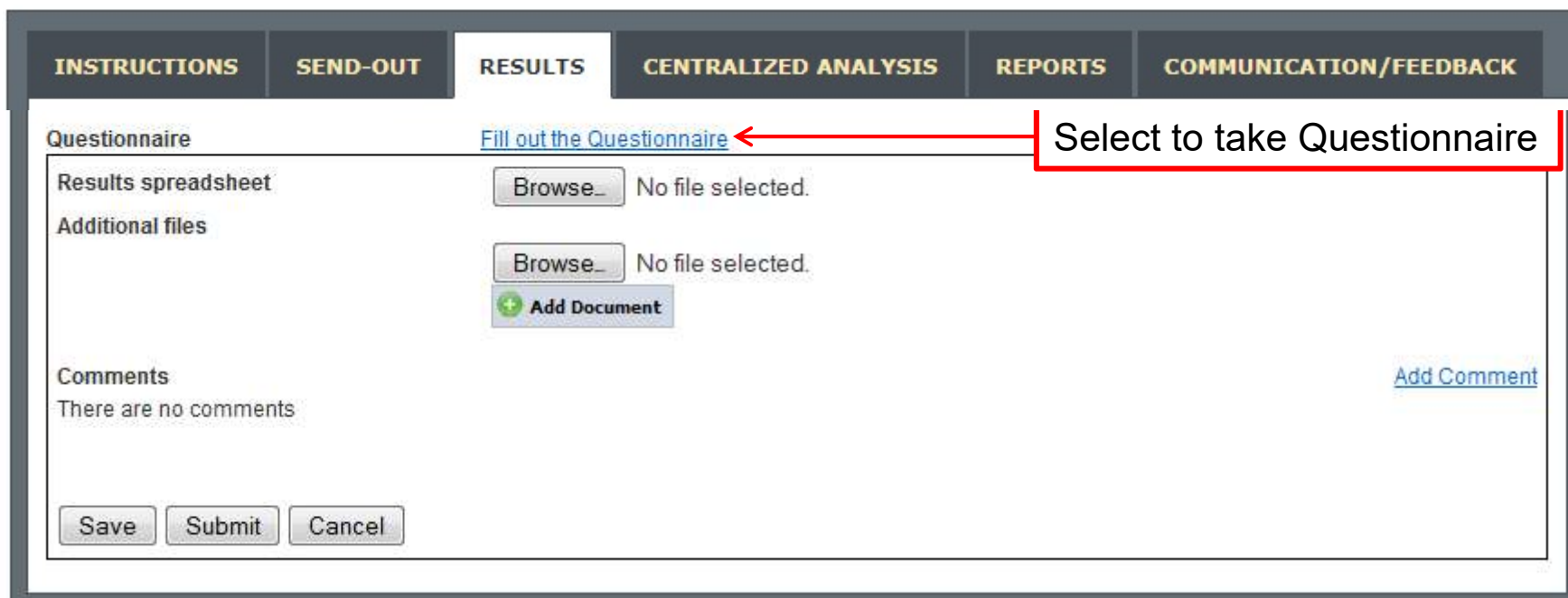
Received at:

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 navigation bar at the top containing the following tabs: INSTRUCTIONS, SEND-OUT, RESULTS, CENTRALIZED ANALYSIS, REPORTS, and COMMUNICATION/FEEDBACK. The 'RESULTS' tab is currently selected. Below the navigation bar, the main content area is titled 'Questionnaire'. It features a blue link labeled 'Fill out the Questionnaire' with a red arrow pointing to it from a red-bordered callout box on the right that contains the text 'Select to take Questionnaire'. Below this link are two file upload sections: 'Results spreadsheet' and 'Additional files'. Each section has a 'Browse...' button and the text 'No file selected.'. Under the 'Additional files' section, there is also a green '+ Add Document' button. At the bottom right of the main content area, there is a blue link labeled 'Add Comment'. At the bottom left, there are three buttons: 'Save', 'Submit', and 'Cancel'.

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”

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

Questionnaire [Fill out the Questionnaire](#)

Results spreadsheet No file selected.

Additional files No file selected.

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

No additional files are being requested for the current EP

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

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

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