Duke Virology Quality Assurance

Executive Summary

Version 1.0

Year 1 VQAAB Annual Review Meeting

October 15 & 16, 2020

Contract # 75N93019C00015

Finalized Date: _____

Thomas Denny,
VQA PI

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(Date)

Bill Meyer,
Chair, VQAAB

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(Date)

National Institute of Allergy and Infectious Diseases

Overall Summary

The Duke Virology Quality Assurance (VQA) Advisory Board (VQAAB) Annual Review (VAR) meeting for Year 1 was held virtually on October 15 & 16, 2020. **Table 1** summarizes all of the attendees external to Duke including the VQAAB members (voting and non-voting), NIH representatives, collaborators (Vitalant Research Institute, VRI), and guests that were in attendance for one or both days of the meeting.

Table 1: VQAAB Annual Review Meeting YR 1 Attendees

VQAAB Voting Members		
Bill Meyer (Chair)	Robert Coombs (ACTG/HVTN)	Nicole Tobin (IMPAACT)
Joan Dragavon (ACTG/HVTN)	Urvi Parikh (MTN)	Jessica Fogel (HPTN)
Belinda Yen-Lieberman (ACTG)	Grace Aldrovandi (ACTG/IMPAACT)	Marco Schito
VQAAB Non-voting Members		
Ron Bosch (SDAC)	Diane Costello (IMPAACT/ACTG)	
HANC		
Tyler Brown		
NIH Representatives		
Joe Fitzgibbon (NIAID/DAIDS)	Keith Crawford (NIAID/DAIDS)	Lori Merril (NICHD)
Collaborators		
Mike Busch (VRI)	Sonia Bakkour (VRI)	Mars Stone (VRI)
Additional Guests		
Neil Parkin (WHO consultant)	Jonathan Li (ACTG VSL/ U of Pitt)	

During the meeting, the VQA provided a summary of Year 1 activities and proposed milestones for Year 2. Before the meeting, the VQA provided a Meeting Booklet that contained updated and edited versions of the VQA Participation Requirements and Scoring Procedures for the Quantitative HIV-1 RNA, Qualitative HIV Total Nucleic Acid, and HIV-1 Drug Resistance Proficiency Testing (PT) Programs. Summaries of the updates, and edits made for each PT program, were presented to the VQAAB for their approval.

In July 2020, NIAID executed an option for SARS-CoV-2 work including:

- Sequencing of SARS-CoV-2 collected from patient samples
- Develop a method for determining SARS-CoV-2 viral load by quantitative RT-PCR (or other means).
- Develop validation panels for quantitative SARS-CoV-2 assays and distribute the panels at the direction of the COR.
- Collect, blood, serum, and PBMCs from convalescent patients to be donated to the BEI repository
- Develop a concordance survey for evaluating SARS-CoV-2 neutralizing antibodies used in clinical vaccine and therapeutic trials

Members of the Duke VQA team and collaborators (M. Meng, T, DeMarco, and Dr. D. Montefiori) provided updates on SARS-CoV-2 work. Lastly, our collaborators from VRI gave a presentation on the HIV Reservoir Assay Validation and Evaluation Network (RAVEN) Program, which was followed by a discussion to determine the need for quality control materials (QCM) for reservoir testing.

Overall Recommendations

Overall VQAAB recommendations for the VQA Program include:

- Survey the VQA participating labs to identify concerns or areas of improvement. The survey should be five questions or less.
- Notify networks of exception and on-hold requests as soon as they are requested.
- The Duke VQA should finalize a list of rules regarding requests from laboratories to be on-hold or granted an exception or extension for a PT. The rules should be approved by the VQAAB, clinical trial network leadership, and NIAID prior to distribution. Once distributed laboratories should receive a 2-month grace period to comply with the rules. After that time, penalties will be enforced.
    - VQAAB approved the VQA's suggestion to limit exemption requests to once every four PTs for all PT programs.
- The Duke VQA and NIAID will review the labs currently participating in the VQA PT programs and create a list of labs they recommend for removal from the VQA program. The Labs will not be removed from the VQA program if they performed testing for NIH funded clinical studies within the past two years. Laboratories will not be removed from the VQA program until:
    - The Clinical Trials Networks recompetition is complete
    - The list has been reviewed by the VQA Advisory Board

GCLP-Compliance and ISO/IEC 17043 Accreditation

Thomas Denny presented an overview of the GCLP and ISO/IEC 17043 activities for the first year of the VQA Contract. The Quality Assurance for Duke Vaccine Immunogenicity Programs (QADVIP), led by Marcella Sarzotti-Kelsoe, oversees all GCLP and ISO/IEC 17043 compliance. Duke has received ISO/IEC 17043 accreditation as a PT provider for the Quantitative HIV-1 RNA, Qualitative HIV-1 NAT, and HIV-1 Drug Resistance PT programs.

The VQAAB did not make any additional recommendations.

The Quality Assurance milestones for YR2 of the VQA program are as follows:

- **Milestone 1:** Perform internal audits for all VQA PT programs.
- **Milestone 2:** Maintain ISO/IEC 17043 accreditation Quantitative HIV-1 RNA Program

Sal Scianna and Wes Rountree provided an overview of the Quantitative HIV RNA program for Year 1. Topics that were covered included:

- Frequency of PTs (decreased to 4 PTs/Year in 2021)
- Production and quality control testing of panels and controls
    - Virus stock for production of controls and PT was obtained from the External Quality Assurance Program Oversight Laboratory (EQAPOL)
    - Panel is tested using the Roche Taqman and Abbott m2000, but the VQA plans to include GeneXpert in the future. VQA is working on obtaining additional instruments to test panel.
        - Approximately 15 aliquots per concentration are tested prior to shipment
- Summary of the data analyzed by the Duke VQA Biostatisticians and scoring procedure
- Updates/edits to the VQA participation requirements and scoring procedures
    - Wes Rountree provided data showing that using an overall median for scoring is comparable to using kit specific median

The VQAAB did not make any additional recommendations for Year 2. However, there was discussion about the quality control steps in place for the PT panels and controls and the VQAAB suggested including an analysis of precision from the panel replicates tested. Data is to be presented at a future VQAAB meeting.

The milestones for the next year include:

Milestone 1: Ship the VQA RNA2021 panels to be tested as part of the four Quantitative HIV-1 RNA PTs in 2021

Milestone 2: Complete PTs RNA2020_12, RNA2021_02, RNA2021_05, and RNA2021_08

Milestone 3: Finalize revisions to the PT Participation Requirements and Scoring procedure.

Qualitative HIV-1 NAT

Miranda Carper provided an overview of the Qualitative HIV-1 NAT program for Year 1. Topics that were covered included:

- Frequency of PTs
- Composition, production, and shipment of panels
- Summary of the data analyzed by the Duke VQA and scoring procedures
- Updates/edits to the VQA participation requirements and scoring procedures
    - PT shipments occur 2x a year (instead of 4)
    - Each PT is composed of 5 samples (instead of 8)
    - Updated entire scoring section to be relevant to assays currently used
        - The previous scoring document was updated in 2006; VQA used the Quant RNA score procedures as a guide

The VQAAB approved of the changes and updates to the VQA participation requirements and scoring procedures, and did not make any additional recommendations for the program.

Based on the discussions during the meeting, the milestones for the next year are as follows:

Milestone 1: Complete NAT2021_03

Milestone 2: Complete NAT2021_09

Milestone 3: Deploy the automatic report generation function in VQA Web-application

Milestone 4: Finalize revisions to the PT Participation Requirements and Scoring procedure

HIV-1 Drug Resistance

Sal Scianna and Bhavna Hora provided an overview of the HIV-1 Drug Resistance program for Year 1. Topics that were covered included:

- Summary of laboratories enrolled in the program
    - VQA, WHO, WHO + VQA
- Frequency of PT
- Composition, production, quality control testing, and shipment of panels
    - Provided a diagram of the Homogeneity and Stability workflow
- Summary of the data analyzed by the Duke VQA and scoring procedures
- Overview of the HIV-1 Drug Resistance Virus Source Material
    - EQAPOL Viral Diversity Repository
    - Acquired IMCs deposited in the AIDS reagent program with known DRMs

  - Develop molecular clones with random mutations
    - Introduce mutations into subtype A1, B, C, D, and URF_A1D IMCs
  - Internal Collaborations in Duke Health system
  - Samples from Drs. Zabrina and Charles Brumme (Canada)
  - Collaboration with Kilimanjaro Christian Medical Centre (Tanzania)
- Updates/edits to the VQA participation requirements and scoring procedures
  - Edited the language for the consensus sequence. One consensus sequence/sample is used for scoring regardless of the kit used for testing.
  - Updated the watch region in accordance to IAS 2019 guidelines
    - PR aa 9 to 94 (originally 9 to 91)
    - INT aa 50 to 264 (originally 50 to 200)

The VQAAB made the following recommendations:

- To prevent discordance in reporting between labs that used Sanger vs Next Generation Sequencing (NGS) platforms, the VQA should recommend that laboratories participating in the program using NGS assays should set the threshold to 20%
- Continue to review and compare NGS and Sanger data from QC testing to see if there are any differences between mutation calls
- Perform phylogenetic tree analysis as an additional check to catch contamination or other issues that may not be detected by the normal scoring procedures.
- Track RNA extraction methods used
- Hold release of alignment reports till after all labs (WHO) have performed repeat testing
- Review scoring for PR/RT; ensure that a lab that submits data with an indel (insertion or deletion) will not receive a passing score

Based on the discussions during the meeting, the milestones for the following year include:

Milestone 1: Complete analysis of GEN2020_05

Milestone 2: Complete GEN2020_11

Milestone 3: Complete GEN2021_05

Milestone 4: Finalize revisions to the PT Participation Requirements and Scoring procedure

Milestone 5: Expand available source material of viruses with drug resistance mutations for use in future PTs with an emphasis on samples with integrase mutations

VQA Advisory Board October Bi-Monthly Meeting

The Duke VQA (M. Carper and S. Scianna) provided the VQAAB with a summary of the data analyzed for DNA2020_03 (secondary analysis) and DNA2020_09 (primary analysis) in a blinded manner. The VQAAB reviewed the data and provided suggestions for the laboratories that may be having issues. Additionally, J. Fitzgibbon led a discussion about removing laboratories from the VQA program that are not part of a clinical trial network or performing testing for NIH funded studies. The VQAAB provided recommendations regarding NIAID's proposal (see overall recommendations).

NIAID will also review the list of WHO laboratories participating in the GEN Drug resistance program. Currently, some laboratories are classified as part of the VQA and WHO Programs. These labs receive two PT panels per year. However, not all of the VQA + WHO laboratories perform testing for NIH funded studies. NIAID may reclassify some of these laboratories as WHO only and they will receive one plasma panel per year instead of two.

SARS-CoV-2

On July 6, 2020, NIAID executed a VQA option for SARS-CoV-2 related activities. Duke VQA and collaborators provided an update on the following SARS-CoV-2 activities:

- Sequence characterization of SARS-CoV-2 isolates
  - Manfred Meng presented a summary of the Sanger sequencing of the SARS-CoV-2 S gene and NGS sequencing of the whole SARS-CoV-2 genome from samples collected from patients diagnosed with COVI-19 in NC.
    - D614G was the predominant mutation found in the samples. Additional mutations observed were:
      - Q474* (causing a premature termination)
      - D134H and E554D observed but were not located at the receptor-binding site
    - Our study indicated that the samples belong to G, GH, and GR clades and were infected from the following sources:
      - US (GH), and Europe (G and GR)
- Development of a quantitative SARS-CoV-2 assay
  - Todd DeMarco presented an update on the development of a quantitative SARS-CoV-2 laboratory-developed test (LDT) that was developed in the Denny Laboratory (IVQAC).
    - Single target TaqMan RT qPCR assay based on the workflow of our validated NHPCVL SIV viral load assay using WHO-E primer/probe set.
    - Assay was tested using a 10 member validation panel (N = 70) that ranged from 5 x 10^6 to 15 RNA copies/mL. The quantitative SARS-CoV-2 LDT assay is currently used for campus surveillance at Duke University.
- Multi-site SARS-CoV-2 Neutralization Antibody Concordance Study (SNACS).
  - David Montefiori summarized the findings of the concordance survey. The study was considered a pilot survey of assay concordance across a large number of labs and assay types. The survey aimed to assess specificity, accuracy and precision of SARS-CoV-2 live virus and pseudovirus neutralization assays.
  - The program is a collaborative effort between the VQA and EQAPOL programs. Samples for study were provided by collaborators at VRI.
  - 57 assay results were submitted from 46 sites for the Neutralization Assay. There were additional participants that submitted data from the ACE2 and Binding assays.
  - For the Neutralization Assays there was a higher variability for laboratory ID50 values compared to ID80 values and potentially greater accuracy at ID80. Higher variability was seen in laboratories that had less experience running their assay.

The VQAAB made the following recommendations:

- The VQA program should develop QCMs for SARS-CoV-2 sequencing to assess NGS used in clinical testing.
- Assess if future SARS-CoV-2 neutralization antibody concordance surveys are needed in the future and create panels for survey as needed.

HIV Reservoir Assay Validation and Evaluation Network (RAVEN)

Sonia Bakkour from VRI provided an overview of the RAVEN program including a description of the objectives, key resources, sample collection and repository, assays evaluated, and the transition and prospective use of the RAVEN samples for the VQA.

The presentation was followed by a discussion, in which the VQAAB made the following recommendations:

- Develop cell-associated quality control materials
- Develop a PT program for reservoir assay testing

RAVEN milestones for the following year are:

Milestone 1: Complete transition of Raven Repository into the VQA

Milestone 2: Develop plans for quality control material based on the needs reservoir assay controls