NRL EQAS 2021 ANNUAL REPORTS

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INTRODUCTION

This is a collection of NRL EQAS annual reports for programs provided in 2021.

As part of the 2021 NRL EQAS reformulation, we made some improvements to the EQAS report format. We added customized comments and statistical graphs in the OASYS-generated performance reports, whilst simplifying the written reports for each Test Event.

In addition to that, we have introduced annual reports, which provides an in-depth analysis across all three Test Events of 2021, reviewing key information including, but not limited to, the overall panel design, target analytes, observed issues and participants' performance on various test kits.

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ACKNOWLEDGEMENT

NRL EQAS team would like to thank all our participants for their support and cooperation, and also apologise for the delayed release of annual reports. As a new attempt of developing annual reports, the level of data analysis and investigation exceeded our initial schedule. Page 3 listed the current completed annual reports. More annual reports will be release in the coming months.

If you have any comments or queries about the annual reports, please do not hesitate to email the EQAS team on qa@nrlquality.org.au.

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2021 ANNUAL REPORT Multimarker Blood Screening Serology (MMBS4310)

INTRODUCTION

NRL EQAS Multimarker Blood Screening Serology Program (MMBS) was designed as a comprehensive EQA program for laboratories that perform serology testing for blood and tissue screening. The MMBS scheme is the only ISO accredited infectious disease blood screening serology program that offers samples representative of those normally tested in routine blood and tissue screening facilities.

In 2021, three unique panels were provided by NRL, one panel for each of the three Test Events (TEs).

After each TE, the assay interpretations reported by participants were compared to the reference results. Additional statistical analyses of participants' assay interpretations and measurable analyte values compared to their peer group were presented in tabular and graphical displays.

In addition to the OASYS generated performance reports, including NRL's comments, this annual report reviews the overall performance of various test kits from participant data across all three TEs of 2021.

Aim

The aims of this report were to:

- Review the program and panel design;
- Investigate the overall performance of various test kits;
- Discuss potential issues that were observed.

METHODS

Panel Samples

In 2021, NRL provided panels for three TEs. Each panel contained ten samples, which were fully characterised for all the analytes included in the program using NRL's validated testing algorithms. Each panel vial contained 1.8 mL of sample. All panels for a given TE were produced together and shipped at ambient temperature prior to the opening of the TE. All panels were required to be stored at 2-8°C or according to the assay manufacturer's Instructions for Use until tested.

The majority of the 30 samples in MMBS 2021 were only provided once during the year. The HIV-1 p24 positive sample was used three times throughout 2021 as samples TE1-B, TE2-E and TE3-I. This sample was manufactured by spiking 8E5 cell culture supernatant into pooled normal human plasma that was confirmed negative for all target analytes, in order to mimic HIV early infection. The bulk sample was stored frozen, and aliquoted for each TE.

Panel Composition

Multiple analytes for multiple organisms can be tested and reported in MMBS, including anti-HBc and HBsAg for HBV, anti-HCV and HCV Ag for HCV, anti-HIV and HIV-1 p24 Ag for HIV, anti-HTLV for HTLV, and Treponemal antibodies (*anti-T. pallidum*) for Syphilis *(Treponema pallidum*):

- Each reactive/positive panel sample provided in 2021 was reactive/positive for a single organism (HBV, HCV, HIV, HTLV or Syphilis);
- Samples that were positive for HIV were either positive for anti-HIV or HIV-1 p24 Ag, not both;
- Samples that were positive for HBV, were reactive for both HBsAg and Anti-HBc;
- Samples that were positive for HCV, were reactive for both anti-HCV and HCV Ag;
- Samples that were positive for Syphilis, were reactive for anti-*T. pallidum*. The Non-treponemal antibodies analyte, such as RPR, was not included in this program;
- HTLV was included in the MMBS program for the first time in 2021, as anti-HTLV testing is required for blood and tissue screening in some counties.

Figure 1 presents the frequency of reactive/positive samples for HBV, HCV, HIV, HTLV, Syphilis and Negative samples that were included in MMBS 2021. Each of the organisms were included in a similar frequency.

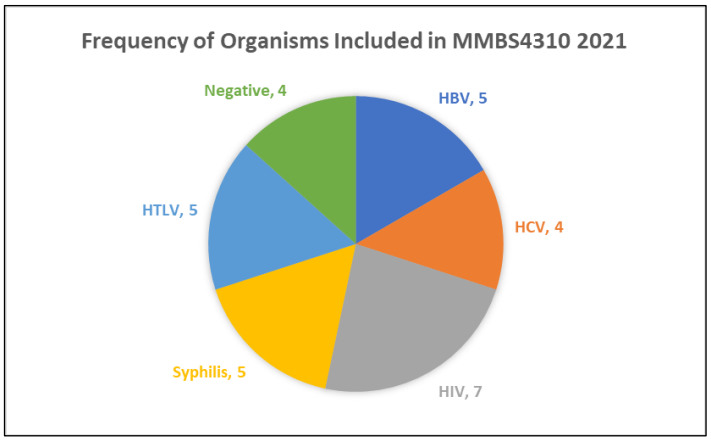

Figure 1. Frequency of organisms included in MMBS4310 panels in 2021.

Figure 2 presents the frequently of reactive/positive analytes and Negative analytes included in MMBS 2021.

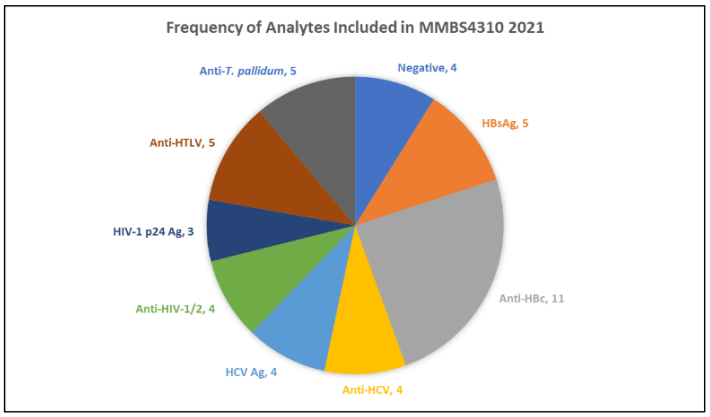

Figure 2. Frequency of analytes included in MMBS4310 panels in 2021.

RESULTS

Participants

Overall, 110 participants from 28 countries reported results in the MMBS program in 2021 during the TE opening frames, and four participants submitted results after TEs closed due to shipping difficulties. Not all participants reported results for each TE.

The participants were from different regions of the world, mainly from the Western Pacific and Southeast Asian regions (Figure 3).

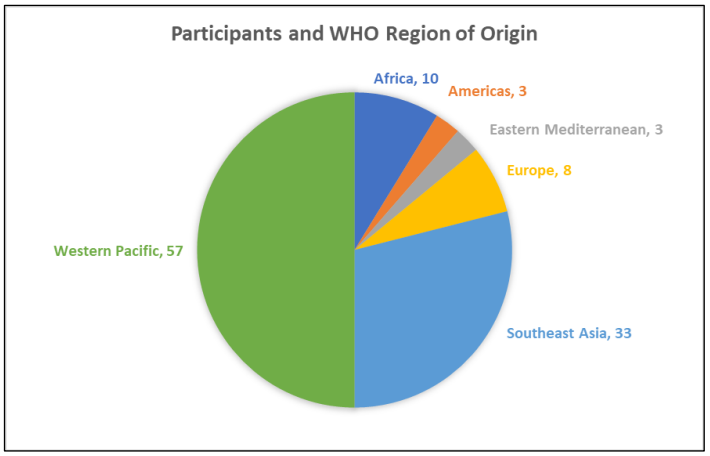

Figure 3. World Health Organization (WHO) region of origin of participants for MMBS4310, 2021.

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Testing Profile

The participants tested and reported results for some or all analytes included in the MMBS4310 program. There were 18 groupings of analytes tested by participants (Table 1). Half of participants (49.12%) tested the panel samples for HBsAg, anti-HCV, anti-HIV, HIV-1 p24 Ag and Syphilis.

Table 1. Testing profiles of participants in MMBS4310, 2021.

Participant Number	HBsAg	Anti-HBc	Anti-HCV	HCV Ag	Anti-HIV	HIV Ag	Anti-*T. pallidum*	Anti-HTLV
49	Y	N	Y	N	Y	Y	Y	N
14	Y	N	Y	N	Y	Y	N	N
13	Y	N	Y	N	Y	Y	Y	Y
7	Y	Y	Y	N	Y	Y	Y	N
6	Y	Y	Y	N	Y	Y	Y	Y
4	N	N	Y	N	Y	Y	N	N
2	Y	Y	Y	N	Y	Y	N	N
2	Y	N	Y	N	Y	N	Y	Y
2	Y	Y	Y	N	Y	Y	Y	N
1	N	N	Y	N	Y	Y	Y	Y
1	Y	N	Y	Y	Y	Y	N	N
1	N	N	N	N	N	N	N	Y
1	Y	Y	Y	Y	Y	Y	N	N
7	Y	N	Y	Y	Y	Y	Y	N
1	Y	Y	Y	N	Y	Y	N	Y
1	Y	Y	Y	N	Y	N	N	N
1	Y	N	Y	N	Y	N	N	N
1	Y	N	Y	N	Y	N	Y	N

Note: Y=Tested, N=Not Tested

Different assay types were used by participants for testing MMBS4310 panel samples. The assay types included, but were not limited to:

- Closed system immunoassays, which can only be used in a dedicated instrument;
- Open system immunoassays, which can either be performed manually or using an EIA processor;
- Immunoblot;
- Rapid test devices.

a. HBV

For HBV, two analytes (HBsAg and anti-HBc) were included in MMBS4310.

➢ *HBsAg*

HBsAg results were received from 108 (94.7%) participants.

Table 2 and Figure 4 present the different assay types and assays that were used by participants to detect HBsAg in the 2021 MMBS4310 samples. Similar numbers of closed and open system immunoassays were used by participants. A few participants also used neutralisation assays to confirm the HBsAg reactivity that was detected in the screening assays.

Table 2. HBsAg Assay Types and Assays used by Participants in MMBS4310, 2021.

Assay Type	HBsAg Assay	Participant Numbers
Closed System Immunoassay	Abbott Alinity i HBsAg Qualitative II CMIA	20
	Abbott Alinity s HBsAg CMIA	16
	Abbott ARCHITECT HBsAg Qualitative CMIA	1
	Abbott ARCHITECT HBsAg Qualitative II CMIA	18
	Abbott PRISM HBsAg ChLIA	1
	Autobio HBsAg CLIA Microparticles	1
	Beckman Coulter Access HBsAg ChLIA	1
	bioMerieux VIDAS HBsAg Ultra ELFA (Long Protocol)	1
	Mindray HBsAg CLIA	1
	Ortho VITROS HBsAg Assay	1
	Ortho VITROS HBsAg ES assay	1
	Roche Elecsys HBsAg II ECLIA	12
	Roche Elecsys HBsAg II ECLIA (cobas e 801)	4
	Suzhou Bacme Diagnostic Kit for HBsAg (CLIA)(SMART)	1
Open System Immunoassay	ACON Incontrol HBsAg EIA Test Kit	1
	Bio-Rad MONOLISA HBsAg ULTRA EIA	14
	DiaSorin Murex HBsAg Version 3 EIA	17
	InTec ADVANCED Diagnostic Kit for HBsAg (ELISA)	12
	InTec ADVANCED HBsAg ELISA Test	2
	J. Mitra Hepalisa HBsAg	1
	Kehua HBsAg ELISA	6
	Livzon/LiZhu Diagnostic Kit for HBsAg (ELISA)	2
	Meril Merilisa HBsAg	1
	Wantai AiD HBsAg ELISA	1
	Wantai Screening HBsAg ELISA	15
Neutralisation Assay	Abbott Alinity s HBsAg Confirmatory CMIA	5
	Abbott ARCHITECT HBsAg Qualitative II Confirmatory CMIA	4
	DiaSorin Murex HBsAg Confirmatory Version 3	1
Rapid Assay	J. Mitra Hepacard HBsAg	1

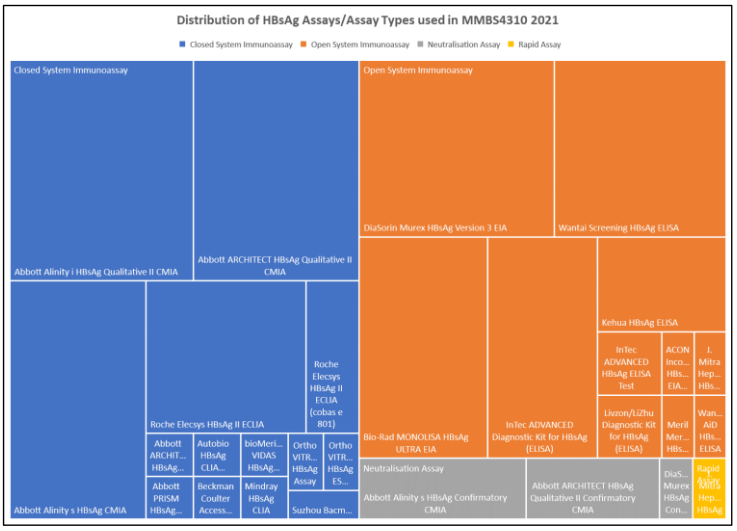

Figure 4. Distribution of HBsAg Assays/Assay Types used in MMBS4310, 2021.

➢ *Anti-HBc*

Anti-HBc results were received from 20 (17.5%) participants.

Table 3 and Figure 5 presents the different assay types and assays that were used by participants to detect anti-HBc in the 2021 MMBS4310 samples. There were more closed system immunoassays used than open system immunoassays.

Table 3. Anti-HBc Assay Types and Assays used by Participants in MMBS4310, 2021.

Assay Type	Anti-HBc Assay	Participant Numbers
Closed System Immunoassay	Abbott Alinity i Anti-HBc II CMIA	1
	Abbott Alinity s Anti-HBc CMIA	3
	Abbott ARCHITECT Anti-HBc II CMIA	7
	DiaSorin Murex anti-HBc (total) EIA	1
	Roche Elecsys Anti-HBc ECLIA	2
	Roche Elecsys Anti-HBc II ECLIA	2
	Roche Elecsys Anti-HBc II ECLIA (cobas e 801)	1
Open System Immunoassay	Autobio Anti-HBc CLIA Microparticles	1
	Wantai Anti-HBc ELISA	3

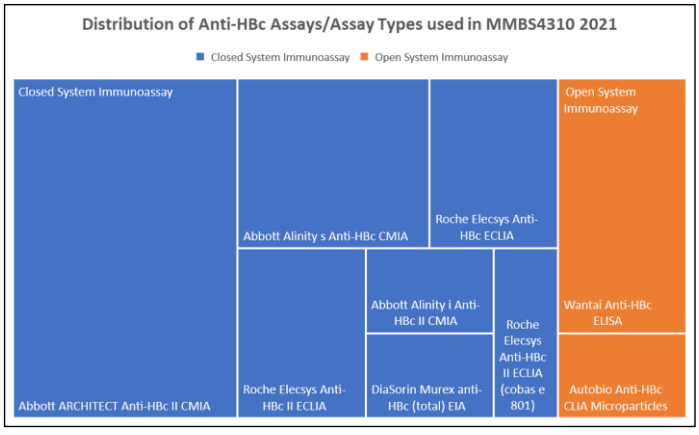

Figure 5. Distribution of Anti-HBc Assays/Assay Types used in MMBS4310, 2021.

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b. HCV

For HCV, two analytes (anti-HCV and HCV Ag) were included in MMBS4310. The majority of participants used assays that detected anti-HCV only. Nine participants used HCV Ab-Ag combo assays, but no participant used an HCV Ag only assay (Figure 6).

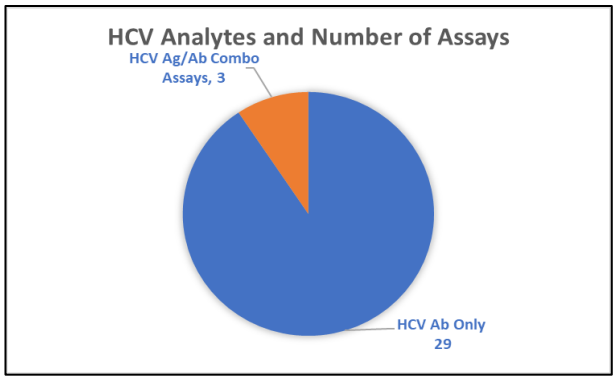

Figure 6. Number of assays used for different HCV analytes in MMBS4310, 2021.

For all HCV analytes, results were received from 113 participants (99.1%). Table 4 and Figure 7 present the different assay types and assays that were used by participants to detect HCV in the 2021 MMBS4310 samples. The majority of participants used closed system immunoassays and open system immunoassays.

Table 4. HCV Assay Types and Assays used by Participants in MMBS4310, 2021.

Assay Type	HCV Assay	Participant Numbers
Closed System Immunoassay	Abbott Alinity i Anti-HCV CMIA	22
	Abbott Alinity s Anti-HCV CMIA	16
	Abbott ARCHITECT Anti-HCV CMIA	21
	Abbott PRISM HCV ChLIA	1
	Autobio Anti-HCV CLIA Microparticles	1
	bioMerieux VIDAS Anti-HCV ELFA	2
	Bio-Rad Access HCV Ab V3 ChLIA	2
	Ortho VITROS Anti-HCV Assay	2
	Roche Elecsys Anti-HCV II ECLIA	12
	Roche Elecsys Anti-HCV II ECLIA (cobas e 801)	6
	Suzhou Bacme Diagnostic Kit for Antibody to HCV (CLIA)(SMART)	1
Open System Immunoassay	ACON Incontrol HCV Antibody EIA Test Kit	1
	BIO-RAD Monolisa Anti-HCV PLUS Version 3 EIA Kit	1
	Bio-Rad MONOLISA HCV Ag-Ab ULTRA EIA	1
	Bio-Rad Monolisa HCV Ag-Ab ULTRA V2 EIA	2
	DiaSorin Murex anti-HCV (version 4.0) EIA	11
	DiaSorin Murex HCV Ag/Ab Combination EIA	4
	Kehua Anti-HCV ELISA	5
	Livzon/LiZhu Diagnostic Kit for Antibody to HCV (ELISA)(Sandwich Method)	5
	Livzon/LiZhu Diagnostic Kit for Antibody to HCV ELISA	2
	Meril Merilisa HCV	1
	Ortho HCV 3.0 ELISA with Enhanced SAVe (STANDARD INC)	9
	Siemens Enzygnost Anti-HCV 4.0 EIA	1
	SIIC HCV EIA	3
	Wantai AiD anti-HCV ELISA	1
	Wantai Diagnostic Kit for Antibody to HCV (ELISA) (Sandwich Method)	11
	Wantai Screening anti-HCV ELISA	5
Immunoblot Assay	Fujirebio INNO-LIA HCV Score	3
	MP Diagnostics HCV BLOT 3.0 WB	4
Rapid Assay	J. Mitra Tridot HCV Spot	1

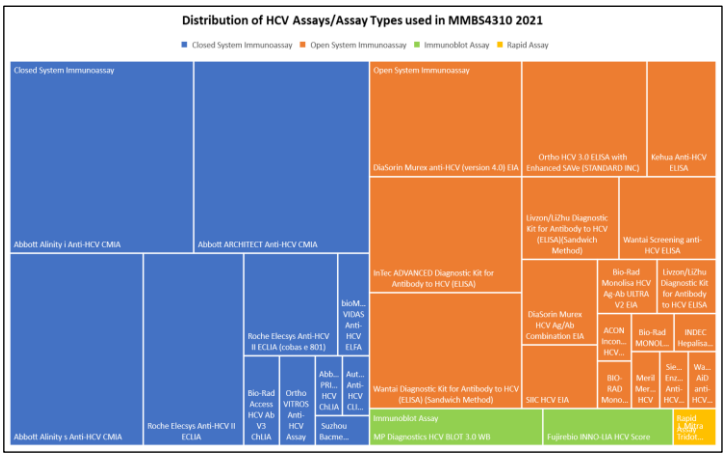

Figure 7. Distribution of HCV Assays/Assay Types used in MMBS4310, 2021.

c. HIV

For HIV, two analytes (anti-HIV and HIV p24 Ag) were included in MMBS4310. Approximately half of the assays detected anti-HIV only and half of the assays detected both anti-HIV and HIV-1 p24 Ag. No participant used an HIV-1 p24 Ag only assay (Figure 8). However, the majority of participants used HIV Ag/Ab combo assays (for detecting both HIV-1 p24 Ag and anti-HIV).

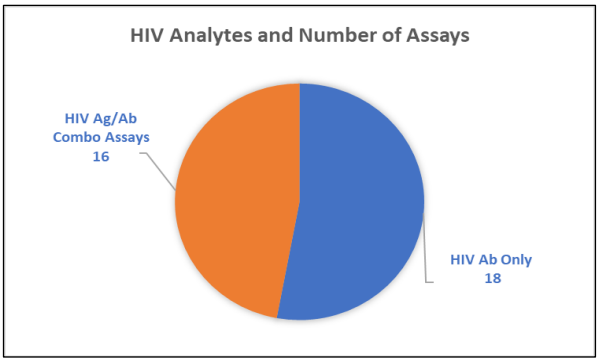

Figure 8. Number of assays used for different HIV analytes in MMBS4310, 2021.

For all HIV analytes, results were received from 113 participants (99.1%). Table 5 and Figure 9 present the different assay types and assays that were used by participants to detect HIV in the 2021 MMBS4310 samples. The majority of participants used closed system immunoassays and open system immunoassays.

Table 5. HIV Assay Types and Assays used by Participants in MMBS4310, 2021.

Assay Type	HIV Assay	Participant Numbers
Closed System Immunoassay	Abbott Alinity i HIV Ag/Ab Combo CMIA	22
	Abbott Alinity s HIV Ag/Ab Combo CMIA	16
	Abbott ARCHITECT HIV Ag/Ab Combo CMIA	22
	Abbott PRISM HIV O Plus ChLIA	1
	bioMerieux VIDAS HIV DUO ELFA	1
	bioMerieux VIDAS HIV DUO Ultra ELFA	2
	Bio-Rad Access HIV Combo ChLIA	2
	Mindray HIV CLIA	1
	Ortho VITROS HIV Combo Assay	2
	Roche Elecsys HIV combi PT ECLIA	9
	Roche Elecsys HIV Duo ECLIA (cobas e 801)	5
	Suzhou Bacme Diagnostic Kit for Antibody and P24 Antigen to HIV (CLIA)(SMART)	1
Open System Immunoassay	ACON Foresight HIV 1/2/O Antibody EIA Test Kit	1
	Bio-Rad Genscreen ULTRA HIV Ag-Ab EIA	22
	DiaSorin Murex HIV Ag/Ab Combination EIA	10
	DiaSorin Murex HIV-1.2.O EIA	3
	InTec ADVANCED Diagnostic Kit for Antibody to HIV (ELISA)	10
	InTec ADVANCED HIV Ag/Ab ELISA	5
	Kehua Anti-HIV (1+2) ELISA	2
	Livzon/LiZhu Diagnostic Kit for Antibody to HIV (ELISA)	3
	Livzon/LiZhu HIV Ag/Ab ELISA	1
	Meril Merilisa HIV 1-2 Gen 3	1
	Siemens Enzygnost HIV Intergral 4 EIA	1
	Wantai AiD anti-HIV 1+2 ELISA	1
	Wantai Screening HIV (1+2) Ag&Ab ELISA	12
	Wantai Screening HIV 1+2 ELISA	3
Rapid Assay	Abbott Determine HIV-1/2 Rapid Test (S/P/WB)	2
	Alere Determine HIV-1/2 Rapid Test	12
	Bio-Rad Geenius HIV 1/2 Confirmatory Rapid Assay (Auto Int)	3
	J. Mitra HIV TRI-DOT	1
	Kehua HIV (1+2) Antibody (Colloidal Gold) Rapid Test	1
Immunoblot Assay	Fujirebio INNO-LIA HIV I/II Score	1
	MP Diagnostics HIV BLOT 2.2 WB (Overnight Assay)	1
	MP Diagnostics HIV BLOT 2.2 WB (Rapid Assay)	2

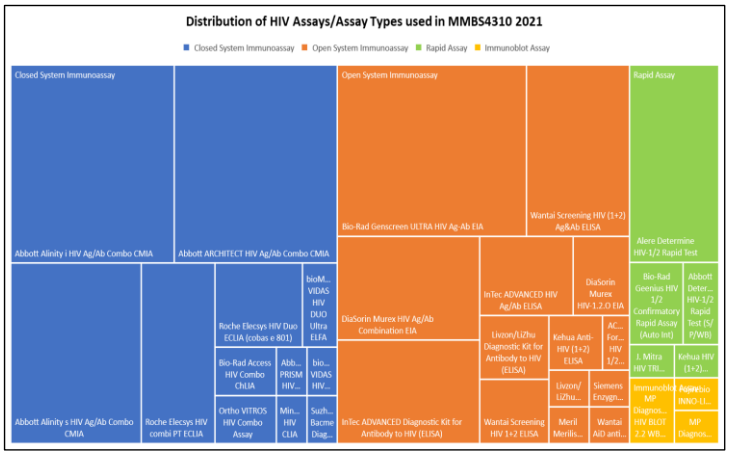

Figure 9. Distribution of HIV Assays/Assay Types used in MMBS4310, 2021.

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d. HTLV

For HTLV, one analyte (anti-HTLV) was included in MMBS4310. Table 6 and Figure 10 presents the five different assays that detect anti-HTLV which were used by 24 participants (21.1%) to test the MMBS4310 2021 panel samples. The most common assay type used was the closed system immunoassay.

Table 6. HTLV Assay Types and Assays used by Participants in MMBS4310, 2021.

Assay Type	HTLV Assay	Participant Numbers
Closed System Immunoassay	Abbott Alinity s HTLV I/II CMIA	7
	Abbott ARCHITECT rHTLV-I/II CMIA	5
	DiaSorin Murex HTLV I+II EIA	4
Open System Immunoassay	WanTai HTLV Antibody EIA	8
Immunoblot Assay	MP Diagnostics HTLV BLOT 2.4 WB	1

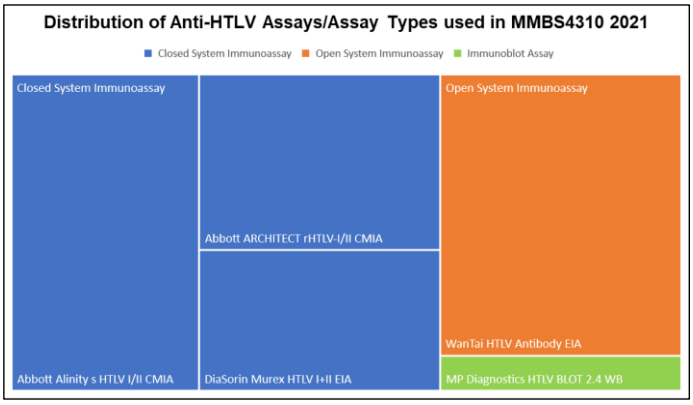

Figure 10. Distribution of HTLV Assays/Assay Types used in MMBS4310, 2021.

e. Syphilis

For Syphilis, one analyte anti-*Treponemal pallidum* was included in MMBS4310. Results were received from 88 participants (77.2%). Table 7 and Figure 11 present the different assay types participants used to detect anti-*T. pallidum* in the 2021 MMBS4310 samples and how many different assays were in each grouping. The largest groupings were for closed system immunoassays and open system immunoassays.

Table 7. Syphilis Assay Types and Assays used by Participants in MMBS4310, 2021.

Assay Type	Syphilis Assay	Participant Numbers
Closed System Immunoassay	Abbott Alinity i Syphilis TP CMIA	18
	Abbott Alinity s Syphilis CMIA	8
	Abbott ARCHITECT Syphilis TP CMIA	15
	DiaSorin LIAISON Treponema Screen CLIA	1
	Mindray Anti-TP CLIA	1
	Roche Elecsys Syphilis ECLIA	4
	Roche Elecsys Syphilis ECLIA (cobas e 801)	2
	Suzhou Bacme Diagnostic Kit for Antibody to Treponema Pallidum (CLIA)(SMART)	1
Open System Immunoassay	Athenese TRUSTwell Syphilis Ab ELISA	1
	BGI GBI Anti-TP Antibody ELISA kit (Double Antigen Sandwich)	2
	DiaSorin Murex ICE* Syphilis EIA	4
	EUROIMMUN Anti-Treponema pallidum Screen ELISA	1
	InTec ADVANCED Diagnostic Kit for Antibody to Treponema Pallidum (ELISA)	19
	Kehua Treponema pallidum ELISA	8
	Livzon/LiZhu Diagnostic Kit for Antibody to Treponema Pallidum (ELISA)	10
	Trinity Biotech Trep-Sure EIA	1
	Wantai Screening anti-TP ELISA	21
Agglutination Assay	Bio-Rad pk TPHA 2000	2
	BIOTEC TPHA Tests	3
	Fujirebio SERODIA-TPPA	3
	Fujirebio SERODIA-TPPA Auto PA	3
	Newmarket Biomedical newbio-pkTPHA	2
	OMEGA DIAGNOSTICS IMMUTREP TPHA	1
	Plasmatec TPHA Test Kit	1
	SpinReact TPHA	1
Rapid Assay	Abbott Determine Syphilis TP Rapid Test (S/P/WB)	4
	Alere Determine Syphilis TP Rapid Test	13

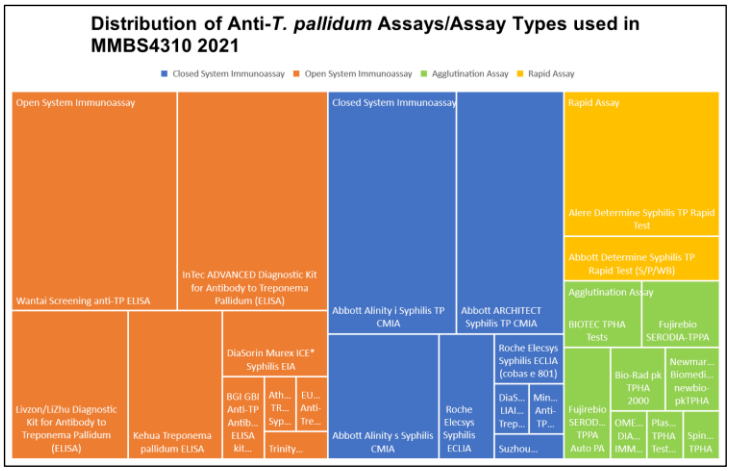

Figure 11. Distribution of Anti-*T. pallidum* Assays/Assay Types used in MMBS4310, 2021.

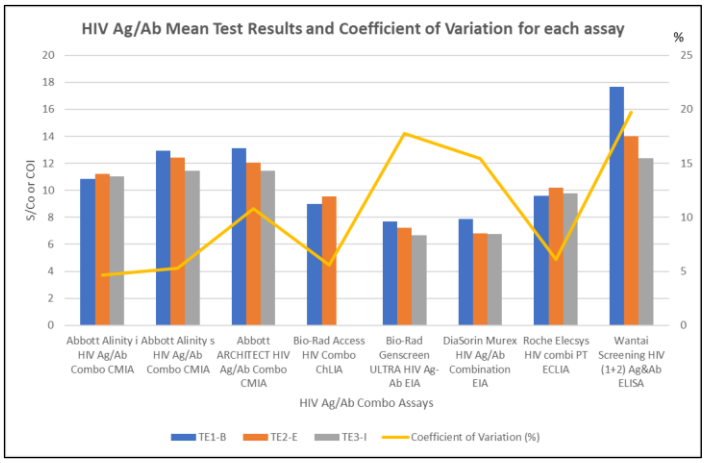

Figure 12. Mean HIV Ag/Ab test result for assays where peer group n≥5 across three TEs for MMBS4310, 2021.

DISCUSSION

General Observations

The majority of results submitted (>90%) were acceptable and concordant with the reference results in all three TEs for MMBS4310 2021. However, a few errors were observed. Some general reminders are listed below, to assist participants to avoid such errors:

- Participants should not perform further testing, such as an immunoblot, on samples that are negative in a screening assay;
- A second individual should double check the submitted data. Participants can edit their submitted data until a TE closes;
- Participants should properly train their staff to subjectively read assays and it is recommended that there always be a second reader and even a third reader should the first two readers disagree on the result. Those that subjectively read assays should have regular retraining;
- Participants should ensure that their instruments are maintained as recommended by the instrument manufacturers.

Sample Carry-over

It was observed that sample carry-over may have occurred on several occasions. For instance, a participant reported reactive HBsAg test results for two HBsAg negative samples that followed a HBsAg positive sample. Each of the HBsAg test results were lower than would have been anticipated had sample mix-up occurred. The HBsAg result for the sample that followed the HBsAg positive sample was lower than for the positive sample and the HBsAg test result for the second sample that followed the positive sample was again lower than the sample that preceded it. This pattern commonly indicates carry-over.

It is recommended that where sample carry-over is observed that the participant contact their instrument manufacturer for advice. It may be that, for example, the sample probe needs to be replaced.

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HIV-1 p24 Ag Positive Sample

A HIV-1 p24 positive manufactured sample was used throughout 2021 (as sample TE1-B, TE2-E and TE3-I). This bulk sample was stored at -20°C and aliquoted separately for each TE sample. Figure 12 presents the HIV Ag/Ab Mean S/Co or Mean COI (Roche Elecsys assay) for each of the HIV-1 p24 positive panel samples where peer group n ≥5. For a peer group, the mean test results for each TE were similar. This suggests consistency in assay performance and laboratory performance of the assay. For all of the assays presented, the mean test results reported by the participants were above the cut-off for the assays.

TE1 Sample G

TE1 sample G was a pooled sample which consisted of two different plasma donations from the same country of origin. The two individual plasma samples had the same serological profile. The pooled sample and the individual samples were confirmed positive for anti-HIV and negative for all other MMBS analytes according to the testing performed at NRL.

- Anti-HBc:

Analysis of participant data revealed that this sample had a tendency towards biological false reactivity in the Abbott ARCHITECT Anti-HBc II CMIA (100% results reported false reactive). Consequently, these anti-HBc Interpretations were identified as "Not Evaluated".

- Anti-*T. pallidum*:

Analysis of participant data revealed that 44% of anti-*T. pallidum* assay interpretations agreed with the reference results i.e. they reported "Negative", however, 55% reported "Reactive" and 1% reported "Inconclusive".

Further analysis of the assays that participants used did not reveal a pattern in the results obtained.

Both individual donations that made up sample G were further tested in the "Abbott ARCHITECT Syphilis TP CMIA" (70% of participants had reported "Reactive" in this assay). Both of the individual samples were "Reactive" just over the cut-off for the assay.

The anti-*T. pallidum* assay interpretations that were reported for this sample were not evaluated due to the wide distribution of the results.

Biological False Reactive (BFR) Samples

A few panel samples in MMBS4310 2021 appeared to have a tendency towards biological false reactivity in a few assays:

- TE2 Sample A for Anti-*T. pallidum* Interpretation tested on Kehua Treponema pallidum ELISA;
- TE3 Sample A for Anti-HIV-1/2 / p24 Ag Interpretation tested on Bio-Rad Genscreen ULTRA HIV Ag-Ab EIA.

It is possible for false reactive test results to occur in any assay. The assay interpretation was not evaluated for the whole peer group when biological false reactivity was observed.

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2021 ANNUAL REPORT Multimarker Blood Screening Molecular (NATA4310)

INTRODUCTION

NRL EQAS Multimarker Blood Screening Molecular Program (NATA) was designed as a comprehensive EQA program for blood and tissue screening laboratories that perform routine molecular testing for infectious diseases: HIV RNA, HBV DNA and HCV RNA. In 2021, three Test Events (TEs) were provided by NRL.

After each TE, the participants' results were evaluated against the reference results. Additional statistical analyses of participants' assay interpretations and measurable analytes (from 2021 TE3) compared to their peer groups were presented in tabular and graphical displays.

In addition to the OASYS generated performance reports, including NRL's comments, this annual report reviews the overall performance of various test kits from participants' data across all three TEs of 2021.

Aim

The aims of this report were to:

- Investigate the overall assay detection and LOD of HIV RNA, HBV DNA and HCV RNA across the three TEs;
- Investigate the reproducibility and repeatability of HIV RNA, HBV DNA and HCV RNA across the three TEs;
- Discuss potential issues that were observed.

METHODS

Panel Samples

In 2021, panels for three TEs were provided by NRL. Each panel contained ten samples. Each panel vial contained 4.4 mL of pooled plasma. All panels were produced concurrently and shipped on dry ice to participants in a single shipment prior to the first TE. Participants were requested to store panels below -20°C until tested.

Analytes

Three analytes of interest, HIV RNA, HBV DNA and HCV RNA, were included in this program. In the 2021 panels, each positive panel sample was reactive for a single analyte only. Each of the analytes were represented in the same proportion within the 2021 NATA4310 samples.

Figure 1 presents the frequency that reactive samples (by analyte) and Negative samples (that contained negative human plasma (NHP) only) were included in the 30 NATA4310 samples for 2021.

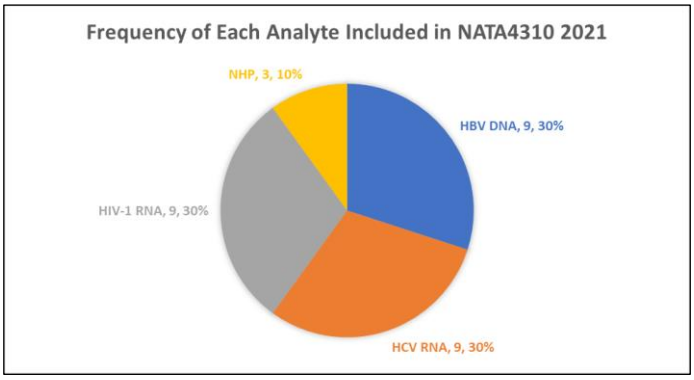

Figure 1. Frequency of each analyte included in NATA4310 2021.

Sample Concentrations

Each analyte of interest was included at three different concentrations (viral loads):

- For HBV: 40 IU/mL (HBV40), 100 IU/mL (HBV100) and 400 IU/mL (HBV400);
- For HCV: 40 IU/mL (HCV40), 100 IU/ml (HCV100) and 400 IU/mL (HCV400);
- For HIV: 100 IU/mL (HIV100), 250 IU/mL (HIV250) and 500 IU/mL (HIV500).

The three concentrations of each analyte represented the Low, Medium and High levels of the viral loads for the analyte. The three concentrations were included in NATA4310 2021 in the same proportion for each analyte. Figure 2 demonstrated the proportion of different concentrations. For each analyte, the Low concentration samples were included more frequently than the other two higher concentration samples.

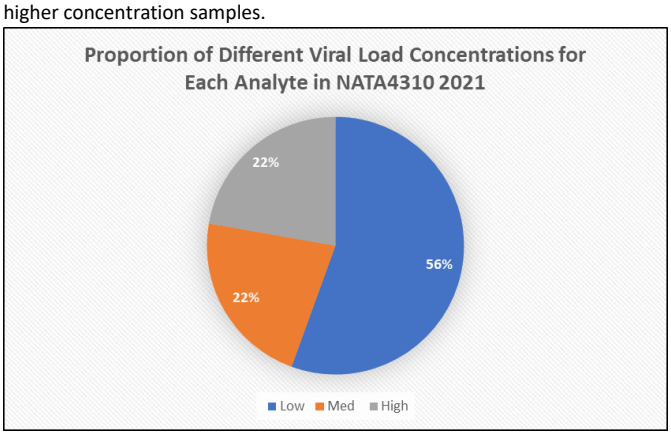

Figure 2. The proportion of different viral load concentrations for each analyte included in NATA4310 2021.

The stock materials for HIV RNA, HBV DNA and HCV RNA were calibrated against the WHO International Standards for HIV-1 (16/194), HBV (10/266) and HCV (18/184) (NIBSC, Potters Bar, UK). The diluent and the Negative sample were pooled Normal Human Plasma (NHP), which were tested and confirmed to be negative to HIV RNA, HBV DNA and HCV RNA.

Panel Composition

Each analyte concentration, along with the Negative sample, were aliquoted at the same time and used for multiple samples across the three TEs (Table 1).

Table 1. Panel composite of each sample.

Sample	Panel composite	Analyte	Target Concentration (IU/mL)
HIV100	TE1-F, TE1-H, TE2-E, TE2-H, TE3-C	HIV	100
HIV250	TE1-J, TE3-F	HIV	250
HIV500	TE1-D, TE3-I	HIV	500
HBV40	TE1-C, TE2-B, TE2-J, TE3-B, TE3-H	HBV	40
HBV100	TE1-E, TE3-D	HBV	100
HBV400	TE1-A, TE2-D	HBV	400
HCV40	TE1-B, TE1-G, TE2-C, TE2-F, TE3-E	HCV	40
HCV100	TE2-I, TE3-G	HCV	100
HCV400	TE1-I, TE3-A	HCV	400
Negative	TE2-A, TE3-G, TE3-J	N/A	Not Detected

RESULTS

Overall, one hundred and forty-four participants submitted results in 2021:

- one hundred and thirty-six participants submitted results for TE1;
- one hundred and twenty-nine participants submitted d results for TE2;
- one hundred and forty participants submitted results for TE3.

The participants were from different regions of the world, mainly from the Western Pacific region and Europe (Figure 3).

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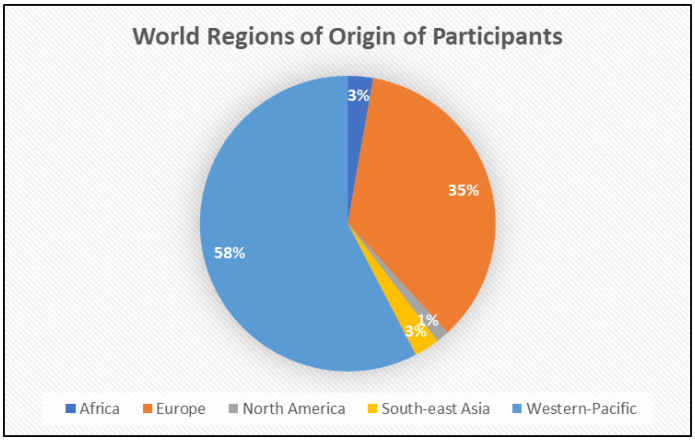

Figure 3. World regions of origin of participants that submitted results for NATA4310, 2021.

Assays Used By Participants

Eleven different commercial assay groups were used by participants in the program (Figure 4). Please note that multiplex assays were counted as one assay. Several participants in the program also used in-house assays.

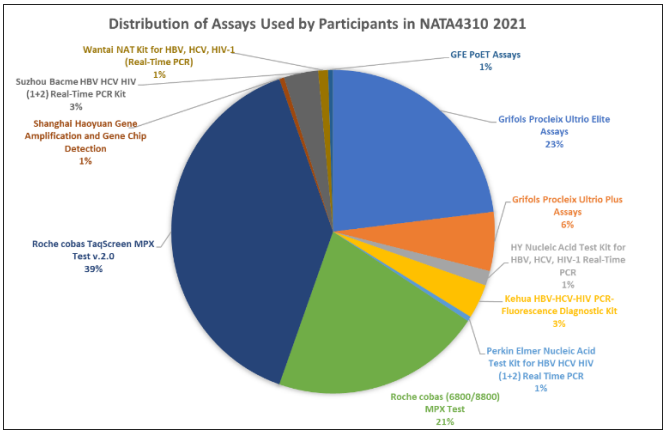

Figure 4. Distribution of eleven commercial assays used by participants in NATA4310, 2021.

Assay Statistics for Each Analyte

a. HIV Analyte

The HIV stock material contained HIV-1 Group M subtype B RNA calibrated against the WHO International Standard for HIV-1 (16/194). The HIV stock was also used for 2021 HIVL panels.

The three viral loads (100 IU/mL, 250 IU/mL and 500 IU/mLl) were diluted with NHP directly from the stock material. HIV500 was used for 2 panel samples, HIV250 was used for 2 panel samples, and HIV100 was used for 5 panel samples (Table 1).

Participants reported HIV (or HIV-1) detection (assay interpretation) and also the measurable values (S/Co or Ct) for NATA4310. Figures 5 and 6 present the mean HIV S/Co and Ct values of each peer group for each panel sample containing HIV across all three TEs.

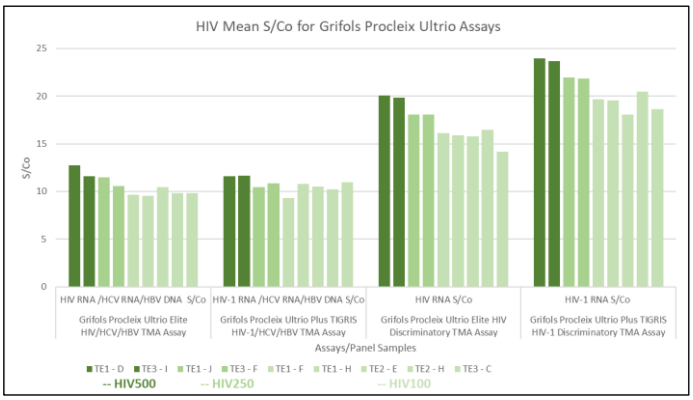

Figure 5. HIV mean S/Co for Grifols Procleix Ultrio Assays across three TEs in NATA4310 2021.

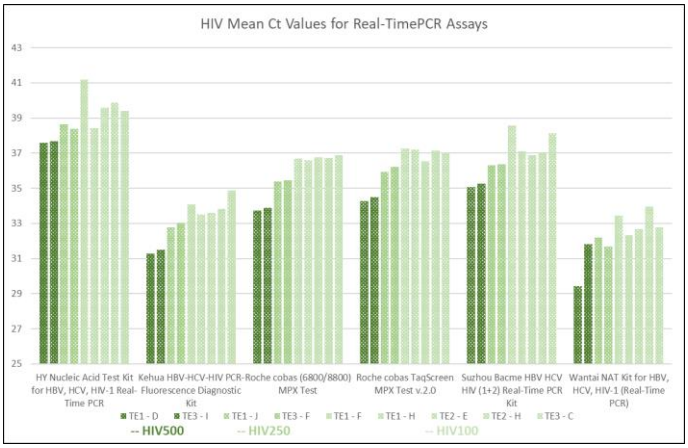

Figure 6. HIV mean Ct values for Real-time PCR Assays across three TEs in NATA4310, 2021.

b. HBV Analyte

The HBV stock material was calibrated against the WHO International Standard for HBV (10/266). The HBV stock was also used for 2021 HBVL panels.

The three viral loads (40 IU/mL, 100 IU/mL and 400 IU/mL) were diluted directly from the stock material with NHP. HBV400 was used for 2 panel samples, HBV100 was used for 2 panel samples, and HBV40 was used for 5 panel samples (Table 1).

Participants reported HBV detection (assay interpretation) and also the measurable values (S/Co or Ct) for NATA4310. Figures 7 and 8 present the mean HBV S/Co and Ct values of each peer group for each panel sample containing HBV across all three TEs.

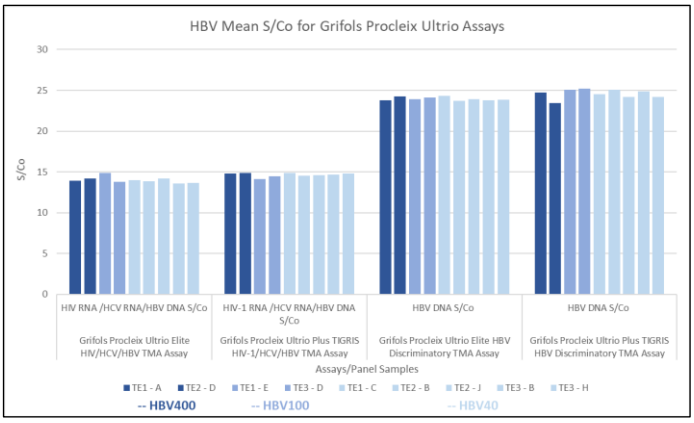

Figure 7. HBV mean S/Co for Grifols Procleix Ultrio Assays across three TEs in NATA4310 2021.

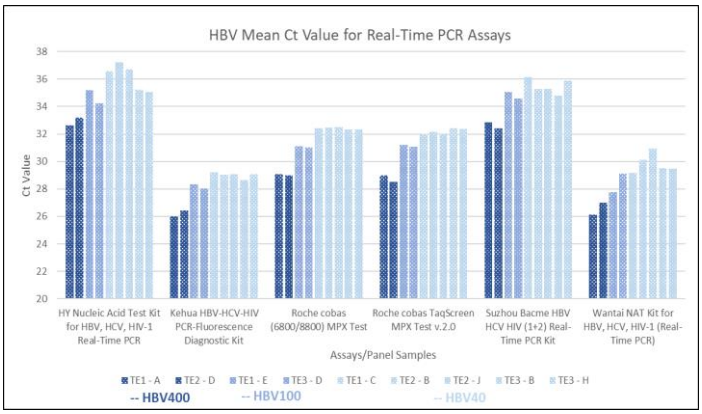

Figure 8. HBV mean Ct values for Real-time PCR Assays across three TEs in NATA4310 2021.

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c. HCV samples

The HCV stock material was calibrated against the WHO International Standard for HCV (18/184). The HCV stock was also used for 2021 HCVQ panels.

The three viral load (40 IU/mL, 100 IU/mL and 400 IU/mL) were diluted directly from the stock material with NHP. HCV400 was used for 2 panel samples, HCV100 was used for 2 panel samples, and HCV40 was used for 5 panel samples (Table 1).

Participants reported HCV detection (assay interpretation) and also the measurable values (S/Co or Ct) for NATA4310. Figures 9 and 10 present the mean HCV S/Co and Ct values of each peer group for each panel sample containing HCV across all three TEs.

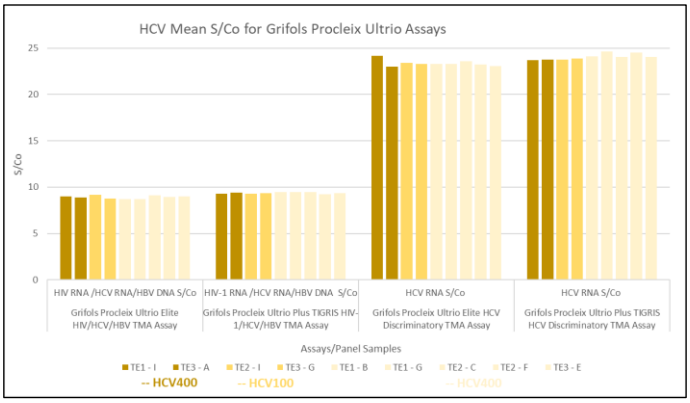

Figure 9. HCV mean S/Co for Grifols Procleix Ultrio Assays across three TEs in NATA4310 2021.

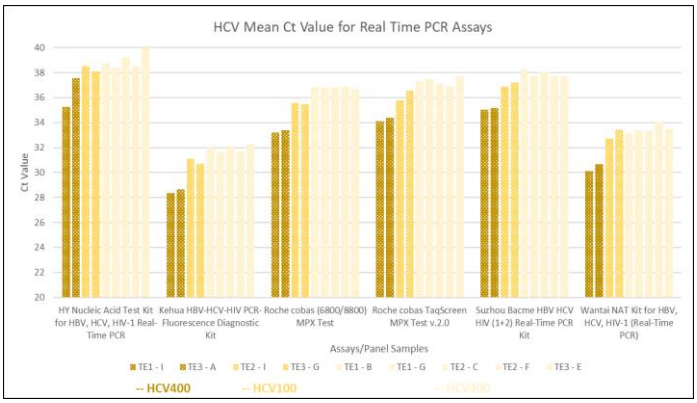

Figure 10. HCV mean Ct value for Real-Time PCR Assays across three TEs in NATA4310, 2021.

Single Sample vs. Minipool

To the best of our knowledge, approximately half of the participants that used Roche cobas TaqScreen MPX Test v.2.0 tested NATA4310 samples as single samples, while the other half of the participants used a Minipool of 6 samples strategy (6-Minipool), which mixed one NATA4310 sample with 5 equal volume NHP samples. Figures 11-13 demonstrate the difference in Ct values between single samples and 6-Minipools.

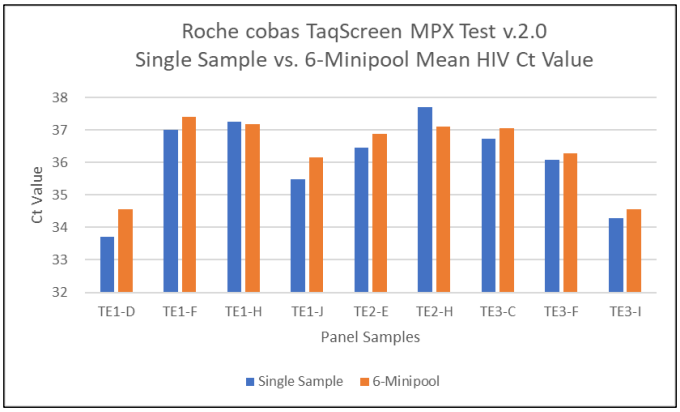

Figure 11. Roche cobas TaqScreen MPX Test v.2.0 Mean HIV Ct Values: Single Sample vs. 6-Minipool.

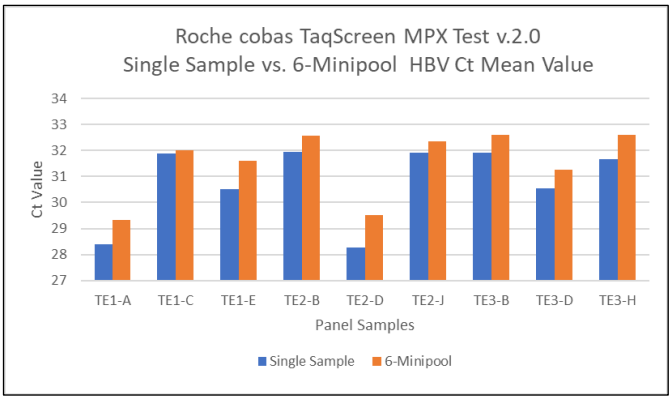

Figure 12. Roche cobas TaqScreen MPX Test v.2.0 Mean HBV Ct Values: Single Sample vs. 6-Minipool.

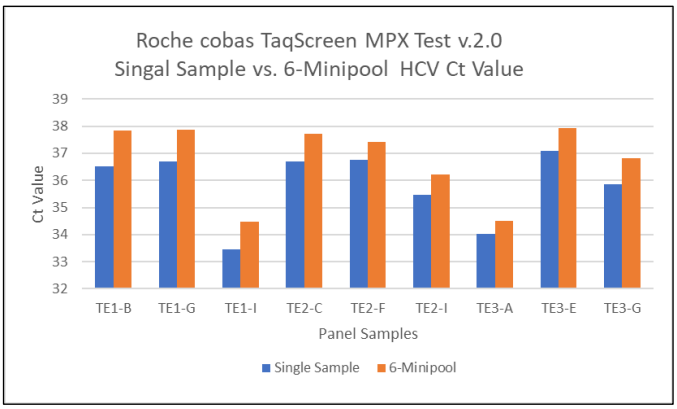

Figure 13. Roche cobas TaqScreen MPX Test v.2.0 Mean HCV Ct Values: Single Sample vs. 6-Minipool.

DISCUSSION

General Observation

The majority of results submitted were concordant with the reference results. Amongst the 1500 results that were received, less than 20 results were identified as unacceptable (discordant with the reference results).

Most unacceptable results appeared to be due to data entry error. NRL recommends double checking result entry to avoid such errors.

Assay Performance

a. Assays used

There were two major detection methods used for blood screening molecular testing for HIV, HBV and HCV:

- Transcription-Mediated Amplification (TMA) and Hybridization Protection Assay (HPA). Grifols Procleix Ultrio Elite and Ultrio Plus assays use this method.
- Real-Time PCR used by Roche cobas assays and most other manufacturers.

The assay sensitivity and reproducibility between these two methods were very similar.

b. Detection of Lowest concentrations

Participants were able to detect the lowest concentration of each analyte (HIV 100 IU/mL, HBV 40 IU/mL, HCV 40 IU/mL) on most occasions.

All results reported were concordant with reference results for HBV40 and HCV40. Six out of approximately 800 results reported false negative HIV interpretations for HIV100. The six discordant results were from various assays and various participants across different regions, and appear to be due to random errors.

The observation demonstrated high sensitivity of the assays on the market and high quality of the testing laboratories.

For all real-time PCR assays, although all the assays were qualitative, the differences in Ct values aligned with the viral load difference of each analyte for all assays.

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c. Assay Performance Testing Strategy: Single Sample vs. Minipool

To the best of our knowledge, one group of participants tested the EQAS samples using Minipools containing 6 samples or 8 samples depending on the assays (i.e. one EQAS sample was mixed with 5 or 7 unknown NHP samples according to their laboratories' Standard Operating Procedure (SOP)), while most other participants tested each EQAS sample as a single sample.

Therefore, the samples used in the Minipool strategy were diluted 6-8 times prior to testing. All results submitted by those participants who used the Minipool strategy were concordant with reference results, which demonstrated low limit of detection of these assays.

Approximately half of the participants that used Roche cobas TaqScreen MPX Test v.2.0 used a Single sample strategy, while the other half of the participants used a Minipool of 6 samples strategy (6-Minipool).

Figures 11-13 demonstrate the difference in Ct values between samples with the 0.5 to 1.0 log viral loads for each analyte when tested as single samples vs when tested in Minipools.

Concentration of Stock Material

One of the key aims of the NATA4310 program is to confirm the LOD of the assays used in participants' laboratories. The stock materials for HIV RNA, HBV DNA and HCV RNA were calibrated against WHO International Standards, in order to provide samples with accurate concentrations.

The same stock materials were also used in individual viral load EQA programs, and the calculated concentrations were compared by the quantitative viral load results obtained from various assays.

Calculated concentrations of HIV RNA and HBV DNA samples were very close to the viral load values reported by various assays in HIVL and HBVL programs, which indicated that the assigned viral load values of HIV RNA and HBV DNA stock material were accurate according to the WHO International Standard.

In the HCVQ EQAS annual report, it was mentioned that the calculated concentration of HCVQ samples was about 0.3 $\log_{10}$ IU/mL higher than the mean viral load values reported by participants, which indicated the possible over estimation of the concentration of HCV stock material. Therefore, the actual concentrations of HCV samples in NATA4310 panels may have been lower than stated in this report. The majority of participants however, still detected HCV at each concentration across all TEs. NRL is investigating the viral load of the HCV stock material, and further discussion and action will be conducted when the investigation has completed.

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2021 ANNUAL REPORT HIV Molecular (HIVL435)

INTRODUCTION

NRL EQAS HIV Molecular Program (HIVL) was designed as a comprehensive EQA program for laboratories that perform HIV RNA viral load testing. In 2021, three Test Events (TEs) were provided by NRL.

After each TE, the participants' results were evaluated based on peer groups containing datasets of five or more results. The TE performance report included statistical information to enable laboratories to assess their Limits of Detection (LOD), the reproducibility and repeatability of their assays, the linearity of ten-fold dilutions, and the coefficient of variation within their laboratory and peer group.

In addition to the OASYS generated performance reports, including NRL's comments, this annual report reviews the overall performance of various test kits from participant data across all three TEs of 2021.

Please note, the data analysis of this annual report focuses on the Detection Kit only, to capture all data derived from the same platform, and does not assess differences between different methods for extraction and amplification.

Aim

The aims of this report were to:

- Investigate the reproducibility and repeatability of participants' results of different HIV RNA viral loads tested across the three TEs;
- Investigate the overall assay performance and LOD;
- Discuss potential issues that were observed.

METHODS

Panel Samples

In 2021, panels for three TEs were provided by NRL. Each panel contained five samples of 1.2mL pooled plasma in each vial. All panels were produced concurrently and shipped on dry ice to participants in a single shipment prior to the first TE. Participants were requested to store panels below -20°C until tested.

Sample Concentrations

The 2021 HIVL435 panel samples were derived from a single plasma stock containing HIV genotype M(B), and comprised of four different concentrations (Very High, High, Medium and Low). The Very High concentration sample was diluted directly from the stock, which was calibrated against the 4th WHO International Standard for HIV-1 RNA for nucleic acid testing (NIBSC code: 16/194, NIBSC, Potters Bar, UK). A further three ten-fold dilutions were performed to produce the High, Medium and Low concentration samples, respectively. The diluent and the Negative sample were Normal Human Plasma (NHP), which was tested and confirmed negative to HIV RNA, HBV DNA and HCV RNA.

Panel Composition

All four concentrations, along with the Negative sample, were aliquoted at the same time and used for multiple samples across the three TEs (Table 1).

Table 1. Panel composite of each sample.

Sample	Panel composite	Reference Results (log_{10} copies/mL)	Target Concentration (log_{10} IU/mL)	Target Concentration (log_{10} copies/mL)*
Very High Concentration	TE1-A, TE2-E, TE3-C	5.53	5.70	5.48
High Concentration	TE1-E, TE2-A, TE2-B	4.59	4.70	4.48
Medium Concentration	TE1-C, TE2-C, TE3-A	3.60	3.70	3.48
Low Concentration	TE2-D, TE3-B, TE3-E	2.60	2.70	2.48
Negative	TE1-B, TE1-D, TE3-D	Not Detected	N/A	N/A

* The conversion from log_{10} IU/mL to log_{10} copies/mL was calculated as "0.60 cp/IU" as stated in the IFU of cobas HIV 1 Quantitative nucleic acid test for use on the cobas 6800/8800 Systems.

RESULTS

Results from 52 participants were received for at least one TE for HIVL435 2021. Thirty-nine participants submitted results for all three TEs.

Ten different assays (Detection kits) were used by participants in the program. Most assays reported results in Log_{10} copies/mL as the unit of measurement.

Assays used in the HIVL program

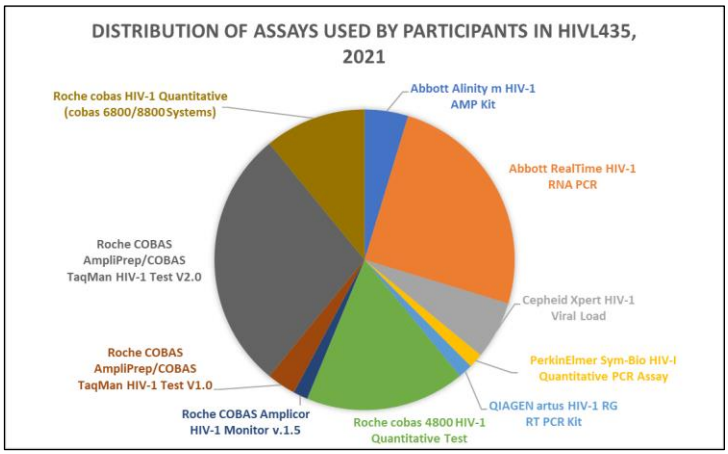

Figure 1. Distribution of the ten assays used by participants in HIVL435, 2021.

Assay Statistics for Each Concentration

a. Very High Concentration Sample

The highest concentration (Very High) sample was diluted directly from the stock material and had a target concentration of 5.48 log_{10} copies/mL (5.70 log_{10} IU/mL).

This sample was used for three panel samples (Table 1). The mean HIV viral load (log_{10} copies/mL) of each panel sample was calculated for each peer group that reported two or more results (Table 2 and Figure 2).

Table 2. Very High Concentration Sample: Mean HIV Viral Load (log_{10} copies/mL) across different TEs for each peer group.

Peer Group (Detection Kit)	TE1-A	TE2-E	TE3-C	Peer Group Mean	Coefficient of Variation (%)
Abbott RealTime HIV-1 RNA PCR	4.95	4.79	4.63	**4.79**	**6.96**
Cepheid Xpert HIV-1 Viral Load	5.34	5.29	4.95	**5.19**	**5.00**
Roche cobas 4800 HIV-1 Quantitative Test	5.50	5.31	5.26	**5.36**	**3.55**
Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test V2.0	5.24	5.06	5.04	**5.11**	**2.39**
Roche cobas HIV-1 Quantitative (cobas 6800/8800 Systems)	5.53	5.35	5.36	**5.41**	**2.44**

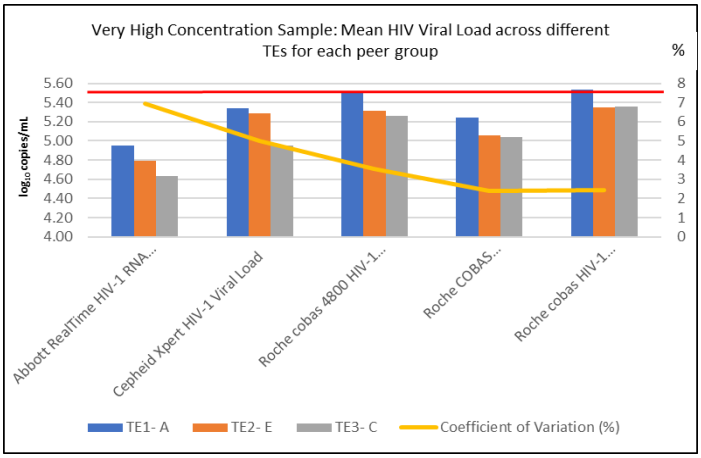

Figure 2. Very High Concentration Sample: Mean HIV Viral Load across different TEs for each peer group. (Red Line at 5.48 indicated the target concentration).

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b. High Concentration Sample

The High concentration sample was a ten-fold dilution of the Very High concentration sample, with the calculated target concentration of 4.48 $\log_{10}$ copies/mL (4.70 $\log_{10}$ IU/mL).

This sample was used for three panel samples (Table 1). The mean HIV viral load ($\log_{10}$ copies/mL) of each panel sample was calculated for each peer group that reported two or more results (Table 3 and Figure 3).

Table 3. High Concentration Sample: Mean HIV Viral Load ($\log_{10}$ copies/mL) across different TEs for each peer group.

Peer Group (Detection Kit)	TE1-E	TE2-A	TE2-B	Peer Group Mean	Coefficient of Variation (%)
Abbott RealTime HIV-1 RNA PCR	3.99	3.80	3.80	**3.86**	**7.80**
Cepheid Xpert HIV-1 Viral Load	4.37	4.27	4.29	**4.31**	**1.38**
Roche cobas 4800 HIV-1 Quantitative Test	4.48	4.31	4.28	**4.36**	**4.01**
Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test V2.0	4.41	4.28	4.30	**4.33**	**2.56**
Roche cobas HIV-1 Quantitative (cobas 6800/8800 Systems)	4.49	4.32	4.34	**4.38**	**2.85**

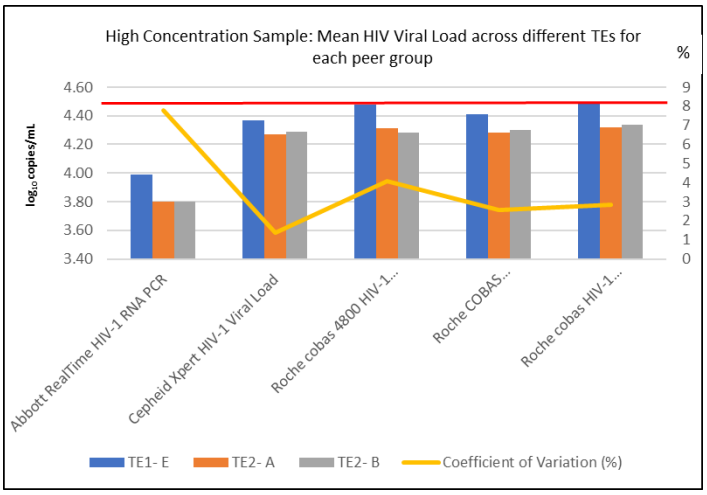

Figure 3. High Concentration Sample: Mean HIV viral load across different TEs for each peer group. (Red Line at 4.48 indicated the target concentration).

c. Medium Concentration Sample

The Medium concentration sample was a ten-fold dilution of the High concentration sample, with the calculated target concentration of 3.48 $\log_{10}$ copies/mL (3.70 $\log_{10}$ IU/mL).

This sample was used for three panel samples (Table 1). The mean HIV viral load ($\log_{10}$ copies/mL) of each panel sample was calculated for each peer group that contained two or more results reported (Table 4 and Figure 4).

Table 4. Medium Concentration Sample: Mean HIV Viral Load ($\log_{10}$ copies/mL) across different TEs for each peer group.

Peer Group (Detection Kit)	TE1-C	TE2-C	TE3-A	Peer Group Mean	Coefficient of Variation (%)
Abbott RealTime HIV-1 RNA PCR	2.98	2.89	2.81	**2.89**	**11.88**
Cepheid Xpert HIV-1 Viral Load	3.40	3.31	3.04	**3.25**	**7.29**
Roche cobas 4800 HIV-1 Quantitative Test	3.45	3.32	3.16	**3.31**	**4.91**
Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test V2.0	3.51	3.38	3.29	**3.39**	**4.82**
Roche cobas HIV-1 Quantitative (cobas 6800/8800 Systems)	3.57	3.29	3.28	**3.38**	**4.16**

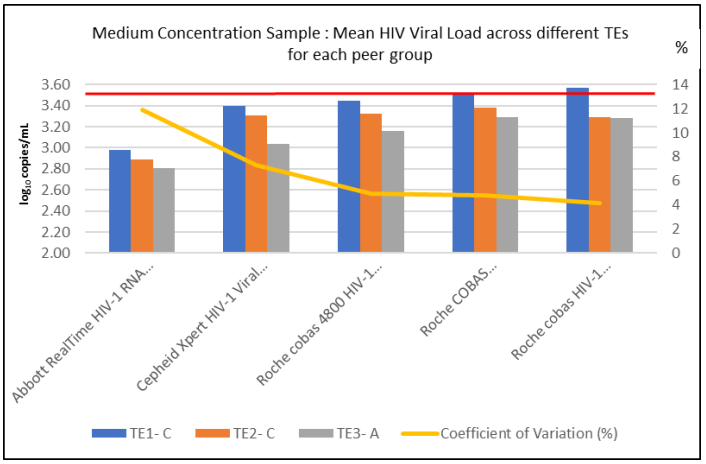

Figure 4. Medium Concentration Sample: Mean HIV viral load across different TEs for each peer group. (Red Line at 3.48 indicated the target concentration).

d. Low Concentration Sample

The Low concentration sample was a ten-fold dilution of the Medium concentration sample, with the calculated target concentration of 2.48 $\log_{10}$ copies/mL (2.70 $\log_{10}$ IU/mL).

This sample was used for three panel samples (Table 1). The mean HIV viral load ($\log_{10}$ copies/mL) of each panel sample was calculated for each peer group that contained two or more results reported (Table 5 and Figure 5).

Table 5. Low Concentration Sample: Mean HIV Viral Load ($\log_{10}$ copies/mL) across different TEs for each peer group.

Peer Group (Detection Kit)	TE2-D	TE3-B	TE3-E	Peer Group Mean	Coefficient of Variation (%)
Abbott RealTime HIV-1 RNA PCR	2.16	2.25	2.23	**2.21**	**8.31**
Cepheid Xpert HIV-1 Viral Load	2.33	2.27	2.31	**2.30**	**3.13**
Roche cobas 4800 HIV-1 Quantitative Test	2.33	2.35	2.42	**2.37**	**8.59**
Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test V2.0	2.42	2.36	2.33	**2.37**	**8.41**
Roche cobas HIV-1 Quantitative (cobas 6800/8800 Systems)	2.49	2.37	2.41	**2.42**	**6.52**

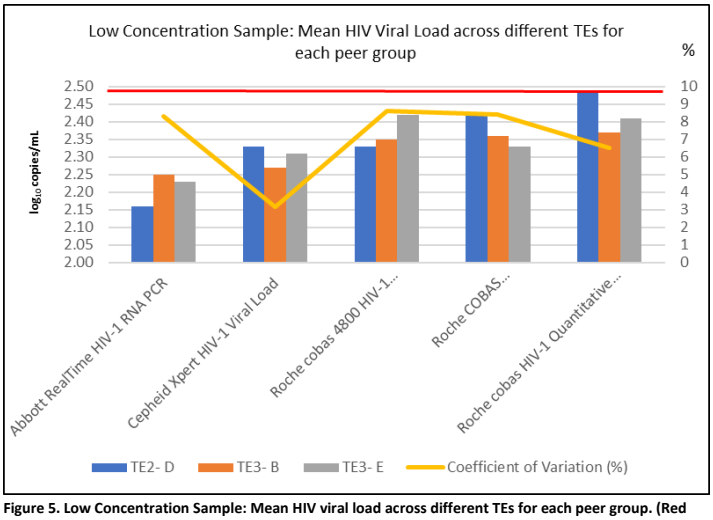

Figure 5. Low Concentration Sample: Mean HIV viral load across different TEs for each peer group. (Red Line at 2.48 indicated the target concentration).

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e. Assay Linearity Performance

The mean HIV viral load (log_{10} copies/mL) values from all three TEs for each concentration were used to investigate the linearity performance (ten-fold dilution series) of each assay (Figure 6).

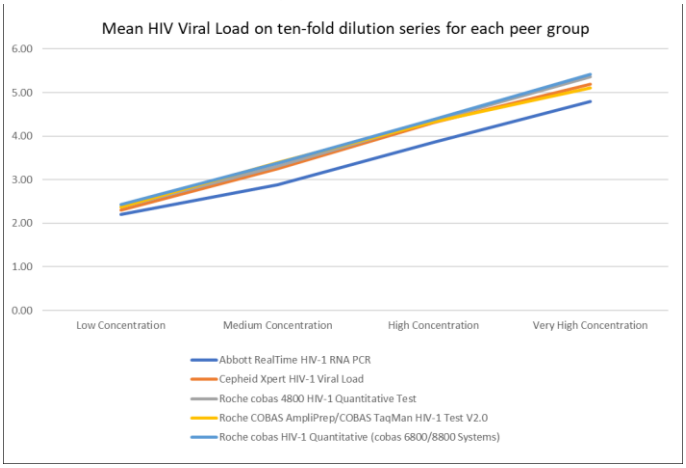

Figure 6. Mean HIV viral load (log_{10} copies/mL) on ten-fold dilution series for each peer group.

DISCUSSION

General Observation

Majority of results submitted were acceptable and met the evaluation criteria of HIV viral load within peer group mean ± 0.5 log_{10} copies/ml. Most results also displayed good reproducibility and linear intra- and inter-run quantification.

However, a few participants reported viral load results that were not log_{10} transformed. Results that were not log_{10} transformed were identified as unacceptable and removed from statistical analysis. Some participants reported "0" as the result for the negative samples. These results were also identified as unacceptable. NRL recommends double checking result entry to avoid such errors.

Assay Performance

Although statistical calculations on some assays may have low confidence due to the small numbers of results, all testing kits demonstrated good repeatability, reproducibility and linearity of quantification for samples in ten-fold dilution series. The coefficient of variation for all peer groups were low (<12%). This demonstrated consistent performance of each assay in different laboratories.

The variance between the mean results of each peer group were <0.62 log_{10} copies/ml. When excluding "Abbott RealTime HIV-1 RNA PCR", the variance between peer groups were <0.30 log_{10} copies/ml.

Some potential causes of variation are user performance, sample volume used for testing and the various user groups across the world.

Units of Measurement

Most assays allow the participants to report HIV viral load results in either copies/mL or IU/mL. Majority of clinical laboratories reported in log_{10} copies/mL. The NRL stock material used for this program was calibrated against the 4th WHO International Standard, therefore the viral load value of stock material and target concentrations of bulk samples were assigned in log_{10} IU/mL. To accurately compare participants' results against the calibrated stock material, the target concentrations were converted to log_{10} copies/mL according to the IFU of "Roche cobas HIV-1 Quantitative nucleic acid test" for use on the "Roche cobas 6800/8800 Systems" (Conversion as 0.60 cp/IU). The conversions between copies/mL and IU/mL vary between different assays, but all sit in the range of 1 IU = 0.55-0.75 copies/ml, which has low impact on log_{10} values.

Low Sample Concentration

Panel samples of Low concentration contained HIV RNA with the target concentration of 2.48 log_{10} copies/mL (2.70 log_{10} IU/mL).

Most participants were able to detect and quantify this sample. However, approximately 40% of participants who used "Abbott RealTime HIV-1 RNA PCR" Assay (especially with 0.2mL start volume), submitted "below the limit of quantification" or "not detected" for these samples across all three TEs, which indicated that the concentration was close to the limit of quantification for this assay.

Abbott RealTime HIV-1 RNA PCR

The participants that reported results using "Abbott RealTime HIV-1 RNA PCR" indicated higher variation, lower concentration and higher limit of detection/quantification compared to other test kits used in HIVL 2021. Several possible factors contributed to the observation:

- This test kit allows for different sample volumes to load onto the instrument and perform the test. The lowest sample volume 0.2 mL may have impacted the limit of detection/quantification and accuracy of samples with low concentrations;
- This test kit was used by participants from various regions. The different performance in those laboratories may have impacted the statistical data;
- The storage of samples at participants' sites may have also impacted on the results of later TEs.

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2021 ANNUAL REPORT HBV Molecular (HBVL435)

INTRODUCTION

NRL EQAS HBV Molecular Program (HBVL) was designed as a comprehensive EQA program for laboratories that perform HBV viral load testing. In 2021, three Test Events (TEs) were provided by NRL.

After each TE, the participants' results were evaluated based on peer groups containing datasets of five or more results. The TE performance reports included statistical information to enable laboratories to assess their Limits of Detection (LOD), the reproducibility and repeatability of their assays, the linearity of ten-fold dilutions, and the coefficient of variation within their laboratory and peer group.

In addition to the OASYS generated performance reports including NRL's comments, this annual report reviews the overall performance of various test kits from participants' data across all three TEs of 2021.

Please note, the data analysis of this annual report focuses on the Detection Kit only, to capture all data derived from the same platform, and does not assess differences between different methods for extraction and amplification.

Aim

The aims of this report were to:

- Investigate the reproducibility and repeatability of participants' results of different HBV DNA viral loads tested across the three TEs;
- Investigate the overall assay performance and LOD;
- Discuss potential issues that were observed.

METHODS

Panel Samples

In 2021, panels for three TEs were provided by NRL. Each panel contained five samples of 1.2mL pooled plasma in each vial. All panels were produced concurrently and shipped on dry ice to participants in a single shipment prior to the first TE. Participants were requested to store panels below -20°C until tested.

Sample Concentrations

The 2021 HBVL435 panel samples were derived from a single plasma stock containing HBV genotype A, and comprised of three different concentrations (High, Medium and Low). The High concentration sample was diluted directly from the stock, which was calibrated against 4th WHO International Standard for HBV DNA for nucleic acid testing (NIBSC code: 10/266; NIBSC, Potters Bar, UK). A further two ten-fold dilutions were performed to produce the Medium and Low concentration samples, respectively. The diluent and the Negative sample were Normal Human Plasma (NHP), which was tested and confirmed negative to HIV RNA, HBV DNA and HCV RNA.

Panel Composition

All three concentrations, along with the Negative sample, were aliquoted at the same time and used for multiple samples across the three TEs (Table 1).

Table 1. Panel composite of each sample.

Sample	Panel composite	Reference Results (log_{10} IU/mL)	Target Concentration (log_{10} IU/mL)
High Concentration	TE1-C, TE2-B, TE2-E, TE3-B	5.64	5.70
Medium Concentration	TE1-A, TE1-D, TE2-A, TE3-D	4.61	4.70
Low Concentration	TE1-B, TE2-C, TE2-D, TE3-E	3.60	3.70
Negative	TE1-E, TE3-A, TE3-C	Not Detected	N/A

RESULTS

Results from 42 participants were received for at least one TE for HBVL435 2021. Thirty-five participants submitted results for all three TEs.

Ten different assays (Detection kits) were used by participants in the program. All assays reported results in Log_{10} IU/mL as the unit of measurement.

Assays used in the program

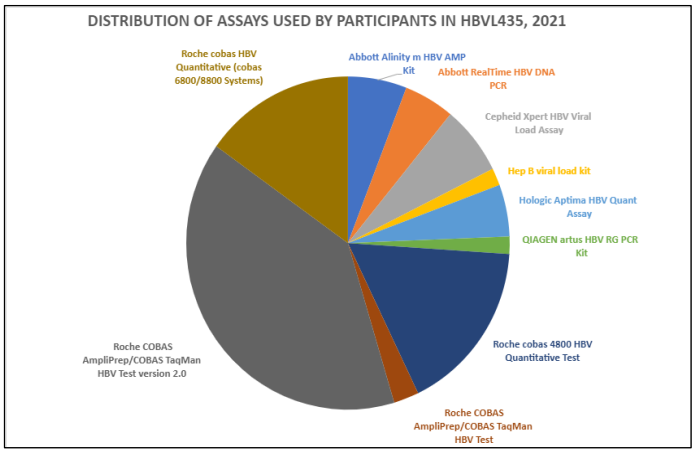

Figure 1. Distribution of Ten assays used by participants in HBVL435, 2021.

Assay Statistics for Each Concentration

a. High Concentration Sample

The High concentration sample was diluted directly from the stock material and had a target concentration of 5.70 log_{10} IU/mL.

This sample was used for four panel samples (Table 1). The mean HBV viral load (log_{10} IU/mL) of each panel sample was calculated for each peer group that reported two or more results (Table 2 and Figure 2).

Table 2. High Concentration Sample: Mean HBV Viral Load (log_{10} IU/mL) across different TEs for each peer group.

Peer Group (Detection Kit)	TE1-C	TE2-B	TE2-E	TE3-B	Peer Group Mean	Coefficient of Variation (%)
Abbott Alinity m HBV AMP Kit	5.69	5.75	5.73	5.65	**5.71**	**1.53**
Abbott RealTime HBV DNA PCR	5.49	5.49	5.47	5.58	**5.51**	**0.97**
Cepheid Xpert HBV Viral Load Assay	5.77	5.69	5.73	5.74	**5.73**	**1.26**
Hologic Aptima HBV Quant Assay	5.60	5.28	5.36	5.17	**5.35**	**2.01**
Roche cobas 4800 HBV Quantitative Test	5.64	5.75	5.70	5.77	**5.72**	**1.21**
Roche COBAS AmpliPrep/COBAS TaqMan HBV Test version 2.0	5.54	5.51	5.50	5.49	**5.51**	**1.37**
Roche cobas HBV Quantitative (cobas 6800/8800 Systems)	5.70	5.66	5.64	5.67	**5.67**	**1.21**

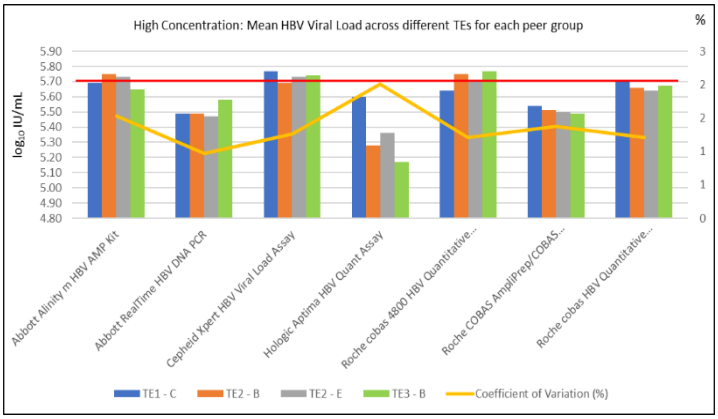

Figure 2. High Concentration Sample: Mean HBV Viral Load across different TEs for each peer group. (Red Line at 5.70 indicated the target concentration).

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b. Medium Concentration Sample

The Medium concentration sample was a ten-fold dilution of the High concentration sample, with the calculated target concentration of 4.70 $\log_{10}$ IU/mL.

This sample was used for four panel samples (Table 1). The mean HBV viral load ($\log_{10}$ IU/mL) of each panel sample was calculated for each peer group that reported two or more results (Table 3 and Figure 3).

Table 3. Medium Concentration Sample: Mean HBV Viral Load ($\log_{10}$ IU/mL) across different TEs for each peer group.

Peer Groups (Detection Kit)	TE1-A	TE1-D	TE2-A	TE3-D	Peer Group Mean	Coefficient of Variation (%)
Abbott Alinity m HBV AMP Kit	4.75	4.74	4.77	4.69	**4.74**	**2.22**
Abbott RealTime HBV DNA PCR	4.55	4.52	4.47	4.57	**4.53**	**2.18**
Cepheid Xpert HBV Viral Load Assay	4.51	4.73	4.67	4.66	**4.64**	**1.03**
Hologic Aptima HBV Quant Assay	4.48	4.54	4.41	4.21	**4.41**	**1.51**
Roche cobas 4800 HBV Quantitative Test	4.61	4.63	4.71	4.77	**4.68**	**1.80**
Roche COBAS AmpliPrep/COBAS TaqMan HBV Test version 2.4	4.75	4.75	4.70	4.68	**4.72**	**1.38**
Roche cobas HBV Quantitative (cobas 6800/8800 Systems)	4.67	4.66	4.63	4.61	**4.64**	**1.32**

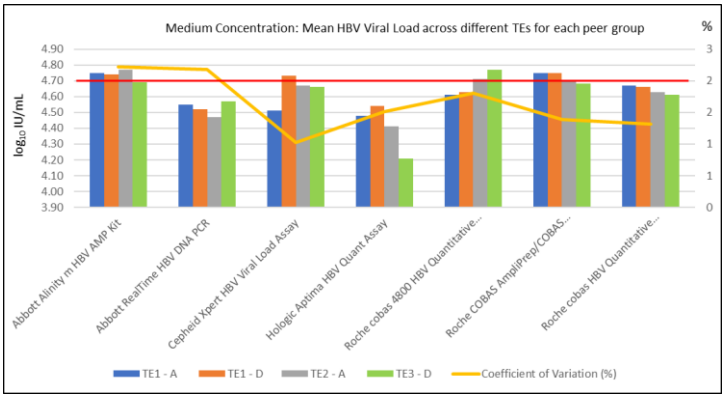

Figure 3. Medium Concentration Sample: Mean HBV viral load across different TEs for each peer group. (Red Line at 4.70 indicated the target concentration).

c. Low Concentration Sample

The Low concentration sample was a ten-fold dilution of the Medium concentration sample, with the calculated target concentration of 3.70 $\log_{10}$ IU/mL.

This sample was used for four panel samples (Table 1). The mean HBV viral load ($\log_{10}$ IU/mL) of each panel sample was calculated for each peer group that contained two or more results reported (Table 4 and Figure 4).

Table 4. Low Concentration Sample: Mean HBV Viral Load ($\log_{10}$ IU/mL) across different TEs for each peer group

Peer Group (Detection Kit)	TE1-B	TE2 -C	TE2-D	TE3 -E	Peer Group Mean	Coefficient of Variation (%)
Abbott Alinity m HBV AMP Kit	3.77	3.66	3.73	3.70	**3.72**	**1.29**
Abbott RealTime HBV DNA PCR	3.60	3.46	3.47	3.56	**3.52**	**5.37**
Cepheid Xpert HBV Viral Load Assay	3.68	3.61	3.69	3.72	**3.68**	**1.83**
Hologic Aptima HBV Quant Assay	3.66	3.44	3.37	3.50	**3.49**	**1.80**
Roche cobas 4800 HBV Quantitative Test	3.63	3.68	3.69	3.74	**3.69**	**1.92**
Roche COBAS AmpliPrep/COBAS TaqMan HBV Test version 2.0	3.69	3.71	3.69	3.71	**3.70**	**1.96**
Roche cobas HBV Quantitative (cobas 6800/8800 Systems)	3.62	3.64	3.63	3.61	**3.63**	**1.38**

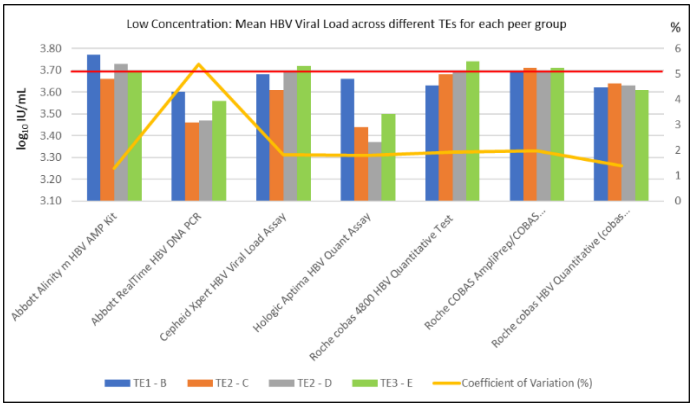

Figure 4. Low Concentration Sample: Mean HBV viral load across different TEs for each peer group. (Red Line at 3.70 indicated the target concentration).

d. Assay Linearity Performance

The mean HBV viral load ($\log_{10}$ IU/mL) values from all three TEs for each concentration were used to investigate the linearity performance (ten-fold dilution series) of each assay (Figure 5).

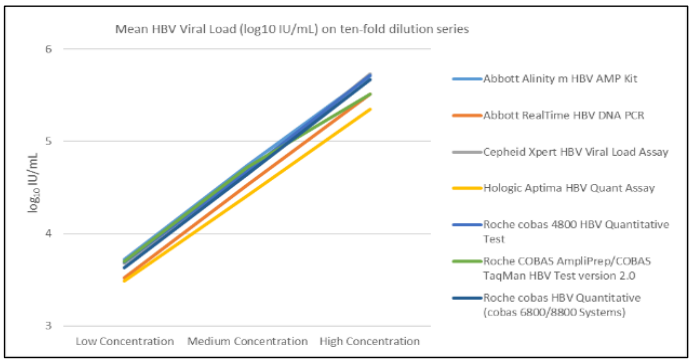

Figure 5. Mean HBV viral load ($\log_{10}$ IU/mL) on ten-fold dilution series for each peer group.

DISCUSSION

General Observation

Majority of results submitted were acceptable and met the evaluation criteria of HBV viral load within peer group mean ± 0.5 $\log_{10}$ IU/ml. Most results also displayed good reproducibility and linear intra- and inter-run quantification.

However, few participants reported viral load results that were not $\log_{10}$ transformed. Results that were not $\log_{10}$ transformed were identified as unacceptable and removed from statistical analysis. Some participants reported "0" as the result for the negative samples. These results were also identified as unacceptable. NRL recommends double checking result entry to avoid such errors.

Assay Performance

Although statistical calculations on some assays may have low confidence due to the small numbers of results in some peer groups, all testing kits demonstrated good repeatability, reproducibility and linearity of quantification for samples in ten-fold dilution series. The coefficient of variation for each peer group was very low (<4%) for all peer groups. It was observed that the "Abbott RealTime HBV DNA PCR" displayed a higher coefficient of variation when compared to other assays.

All assays gave similar mean viral load values, which were close to the calculated target concentration, which was calibrated by NRL against the WHO International Standard. The variance between the mean results of each peer group were less than <0.40 $\log_{10}$ IU/ml, which demonstrated consistent performance of the assay in different laboratories. Relatively, the "Hologic Aptima HBV Quant DX" Assay and the "Abbott RealTime HBV DNA PCR" Assay had the lower peer group mean viral load values for all concentrations when compared to the other assays.

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2021 ANNUAL REPORT HCV Molecular (HCVQ435)

INTRODUCTION

NRL EQAS HCV Molecular Program (HCVQ) was designed as a comprehensive EQA program for laboratories that perform HCV RNA viral load testing and HCV RNA detection. In 2021, three Test Events (TEs) were provided by NRL.

After each TE, the participants' quantitative results were evaluated based on peer groups containing datasets of five or more results. For qualitative results, the participants' results were evaluated against the reference results. The TE performance report included statistical information to enable laboratories to assess their Limits of Detection (LOD), the reproducibility and repeatability of their assays, the linearity of ten-fold dilutions, the coefficient of variation within their laboratory and peer group.

In addition to the OASYS generated performance reports including NRL's comments, this annual report reviews the overall performance of various test kits from participants' data across all three TEs of 2021.

Please note, the data analysis of this annual report focuses on the Detection Kit only, to capture all data derived from the same platform, and does not assess differences between different methods for extraction and amplification.

Aim

The aims of this report were to:

- Investigate the reproducibility and repeatability of participants' results of different HCV RNA viral loads tested across the three TEs;
- Investigate the overall assay performance and LOD;
- Discuss potential issues that were observed.

METHODS

Panel Samples

In 2021, panels for three TEs were provided by NRL. Each panel contained five samples of 1.2mL pooled plasma in each vial. All panels were produced concurrently and shipped on dry ice to participants in a single shipment prior to the first TE. Participants were requested to store panels below -20°C until tested.

Sample Concentrations

The 2021 HCVQ435 panel samples comprised of five different concentrations (Very High, High, Medium, Low and Very Low), and were derived from a single plasma stock containing HCV genotype 1a. The Very High concentration sample was diluted directly from the stock, which was calibrated against 6th WHO International Standard for HCV RNA for nucleic acid testing (NIBSC code: 18/184; NIBSC, Potters Bar, UK). A further four ten-fold dilutions were performed to produce the High, Medium, Low and Very Low concentration samples, respectively. The diluent and the Negative sample were Normal Human Plasma (NHP), which was tested and confirmed negative to HIV RNA, HBV DNA and HCV RNA.

Panel Composition

All five concentrations, along with the Negative sample, were aliquoted at the same time and used for multiple samples across the three TEs (Table 1).

Table 1. Panel composite of each sample.

Sample	Panel composite	Reference Results (log_{10} IU/mL)	Target Concentration (log_{10} IU/mL)
Very High Concentration	TE2-D	4.61	4.81
High Concentration	TE1-A, TE1-D, TE2-C	3.52	3.81
Medium Concentration	TE2-E, TE3-A, TE3-D	2.50	2.81
Low Concentration	TE1-B, TE2-B, TE3-B	1.65	1.81
Very Low Concentration	TE2-A, TE3-C	1.35	0.81
Negative	TE1-C, TE1-E, TE3-E	Not Detected	N/A

RESULTS

Results from 40 participants were received for at least one TE for HCVQ435 2021. Thirty-four participants submitted results for all three TEs.

Eleven different assays (Detection kits) were used by participants in the program (Figure 1). All assays reported results in Log_{10} IU/mL as the unit of measurement.

Assays used in the program

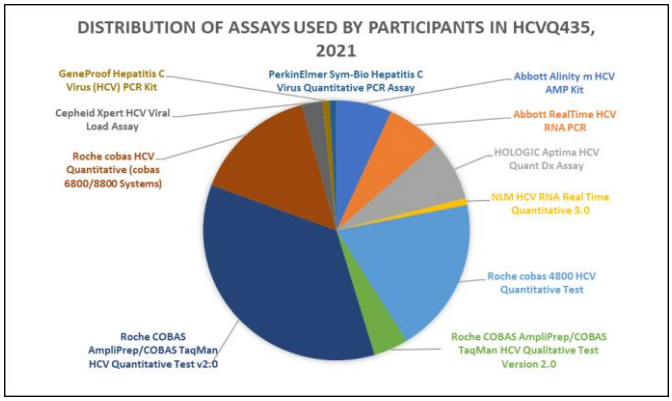

Figure 1. Distribution of 11 assays used by participants in HCVQ435, 2021.

Assay Statistics for Each Concentration

a. Very High Concentration Sample

The highest concentration (Very High) sample was diluted directly from the stock material and had a target concentration of 4.81 log_{10} IU/mL.

This sample was used for one panel sample (Table 1). The mean HCV viral load (log_{10} IU/mL) was calculated for each peer group that reported two or more results (Table 2 and Figure 2).

Table 2. Very High Concentration Sample: Mean HCV Viral Load (log_{10} IU/mL) for each peer group.

Peer Group (Detection Kit)	TE2-D (Peer Group Mean)	Coefficient of Variation (%)
Abbott Alinity m HCV AMP Kit	4.46	1.25
Abbott RealTime HCV RNA PCR	4.26	3.71
Cepheid Xpert HCV Viral Load Assay	4.23	N/A
GeneProof Hepatitis C Virus (HCV) PCR Kit	3.94	N/A
HOLOGIC Aptima HCV Quant Dx Assay	4.12	2.02
Roche cobas 4800 HCV Quantitative Test	4.36	4.25
Roche COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v2.0	4.25	2.70
Roche cobas HCV Quantitative (cobas 6800/8800 Systems)	4.28	3.56

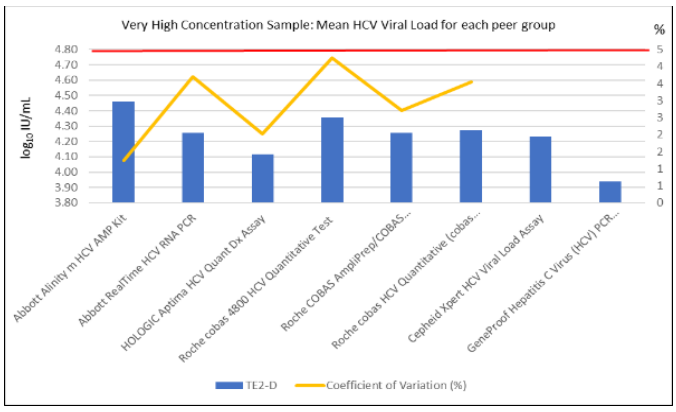

Figure 2. Very High Concentration Sample: Mean HCV Viral Load for each peer group. (Red Line at 4.81 indicated the target concentration).

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b. High Concentration Sample

The High concentration sample was a ten-fold dilution of the Very High concentration sample, with the calculated target concentration of 3.81 $\log_{10}$ IU/mL.

This sample was used for three panel samples (Table 1). The mean HCV viral load ($\log_{10}$ IU/mL) of each panel sample was calculated for each peer group that reported two or more results (Table 3 and Figure 3).

Table 3. High Concentration Sample: Mean HCV viral load ($\log_{10}$ IU/mL) across different TEs for each peer group.

Peer Group (Detection Kit)	TE1-A	TE1-D	TE-C	Peer Group Mean	Coefficient of Variation (%)
Abbott Alinity m HCV AMP Kit	3.20	3.30	3.41	**3.30**	**5.74**
Abbott RealTime HCV RNA PCR	3.35	3.41	3.27	**3.34**	**2.16**
HOLOGIC Aptima HCV Quant Dx Assay	3.15	3.13	3.13	**3.14**	**3.85**
Roche cobas 4800 HCV Quantitative Test	3.20	3.18	3.31	**3.23**	**7.01**
Roche COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v2.0	3.32	3.27	3.32	**3.30**	**6.19**
Roche cobas HCV Quantitative (cobas 6800/8800 Systems)	3.47	3.35	3.24	**3.35**	**2.62**

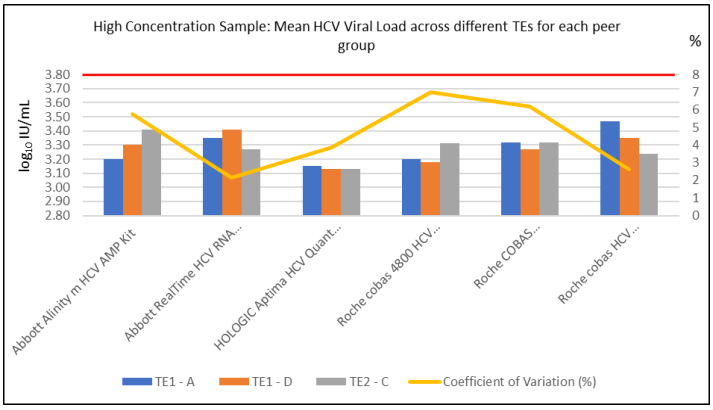

Figure 3. High Concentration Sample: Mean HCV viral load across different TEs for each peer group. (Red Line at 3.81 indicated the target concentration).

c. Medium Concentration Sample

The Medium concentration sample was a ten-fold dilution of the High concentration sample, with the calculated target concentration of 2.81 $\log_{10}$ IU/mL.

This sample was used for three panel samples (Table 1). The mean HCV viral load ($\log_{10}$ IU/mL) of each panel sample was calculated for each peer group that contained two or more results reported (Table 4 and Figure 4).

Table 4. Medium Concentration Sample: Mean HCV Viral Load ($\log_{10}$ IU/mL) across different TEs for each peer group.

Peer Group (Detection Kit)	TE2-E	TE3-A	TE3-D	Peer Group Mean	Coefficient of Variation (%)
Abbott Alinity m HCV AMP Kit	2.44	2.26	2.6	**2.43**	**8.28**
Abbott RealTime HCV RNA PCR	2.3	2.44	2.47	**2.40**	**2.42**
Cepheid Xpert HCV Viral Load Assay	2.27	2.39	2.28	**2.31**	**1.37**
HOLOGIC Aptima HCV Quant Dx Assay	2.19	2.03	2.04	**2.09**	**8.18**
Roche cobas 4800 HCV Quantitative Test	2.34	2.2	2.26	**2.27**	**8.85**
Roche COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v2.0	2.37	2.42	2.31	**2.37**	**9.13**
Roche cobas HCV Quantitative (cobas 6800/8800 Systems)	2.27	2.28	2.23	**2.26**	**5.64**

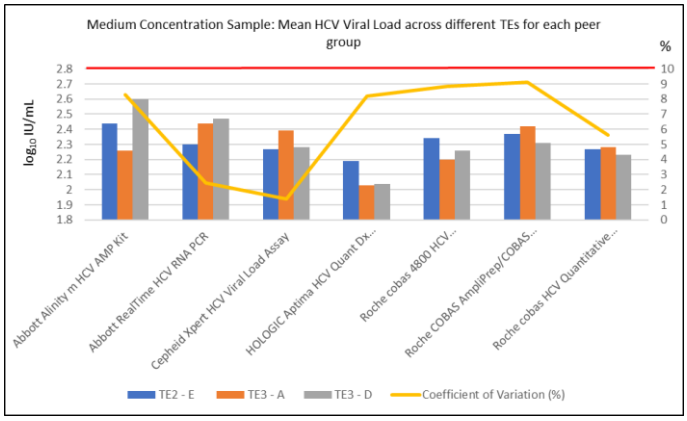

Figure 4. Medium Concentration Sample: Mean HCV Viral Load across different TEs for each peer group. (Red Line at 2.81 indicated the target concentration).

d. Low Concentration Sample

The Low concentration sample was a ten-fold dilution of the Medium concentration sample, with the calculated target concentration of 1.81 $\log_{10}$ IU/mL.

This sample was used for three panel samples (Table 1). About 20% of participants submitted "below the limit of quantification" for these samples in various assays across all three TEs.

The mean of valid HCV viral load results ($\log_{10}$ IU/mL) of each panel sample was calculated for each peer group that contained two or more results reported (Table 5 and Figure 5).

Table 5. Low Concentration Sample: Mean HCV Viral Load ($\log_{10}$ IU/mL) across different TEs for each peer group.

Peer Group (Detection Kit)	TE1-B	TE2-B	TE3 -B	Peer Group Mean	Coefficient of Variation (%)
Abbott Alinity m HCV AMP Kit	1.39	1.37	1.3	**1.35**	**15.11**
Abbott RealTime HCV RNA PCR	1.55	1.55	1.66	**1.59**	**13.52**
HOLOGIC Aptima HCV Quant Dx Assay	1.4	1.3	1.24	**1.31**	**7.03**
Roche cobas 4800 HCV Quantitative Test	1.51	1.5	1.45	**1.49**	**12.94**
Roche COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v2.0	1.58	1.46	1.32	**1.45**	**16.79**
Roche cobas HCV Quantitative (cobas 6800/8800 Systems)	1.6	1.42	1.47	**1.57**	**9.54**

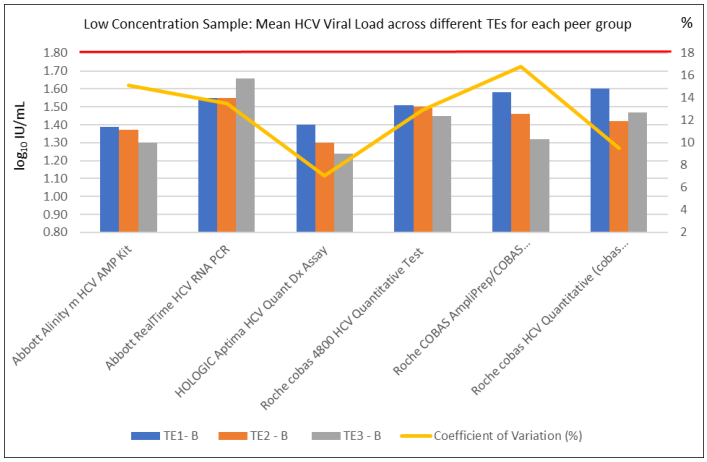

Figure 5. Low Concentration Sample: Mean HCV Viral Load across different TEs for each peer group. (Red Line at 1.81 indicated the target concentration).

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e. Very Low Concentration Sample

The Very Low concentration sample was a ten-fold dilution of the Low concentration sample, with the calculated target concentration of 0.81 log_{10} IU/mL. The Very Low concentration sample was used for two panel samples (Table 1). Few participants detected and/or quantified the sample; most participants submitted "not detected" and/or "below the limit of quantification".

f. Assay Linearity Performance

The mean HCV viral load (log_{10} IU/mL) values from all three TEs for Very High, High, Medium and Low concentrations were used to investigate the linearity performance (ten-fold dilution series) of each assay (Figure 6).

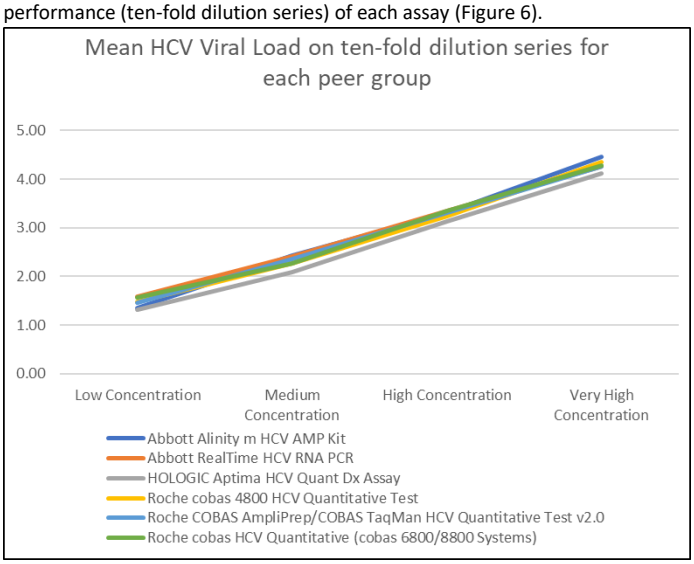

Figure 6. Mean HCV viral load (log_{10} IU/mL) on ten-fold dilution series for each peer group.

DISCUSSION

General Observation

Majority of results submitted were acceptable and met the evaluation criteria of HCV viral load within peer group mean ± 0.5 log_{10} IU/ml. Most results demonstrated good reproducibility and linear intra- and inter-run quantification.

However, few participants reported viral load results that were not log_{10} transformed. Results that were not log_{10} transformed were identified as unacceptable and removed from statistical analysis. Some participants reported "0" as the result for the negative samples. These results were also identified as unacceptable. NRL recommends double checking result entry to avoid such errors.

Assay Performance

Although statistical calculations on some assays may have low confidence due to the small number of results, all testing kits demonstrated good repeatability, reproducibility and linearity of quantification for samples in ten-fold dilution series. The coefficient of variation (CV%) for each peer group ranged between 1-17%. The highest CV% were observed in the Low concentration samples which were expected, as the nature of low concentration samples can be a source of variation when the sample concentration approaches the limit of detection.

All assays gave similar mean viral load values, with a variance between the mean results of each peer group being <0.35 log_{10} IU/mL, which demonstrated consistent performance of the assay in different laboratories and on different platforms. Relatively, the "Hologic Aptima HCV Quant DX" Assay had lower peer group mean viral load values for all concentrations when compared to other assays.

Low and Very Low Sample Concentrations

Panel samples Low and Very Low contained concentrations of HCV RNA with the target concentration of 1.81 log_{10} IU/mL and 0.81 log_{10} IU/mL, respectively.

For the Low concentration sample, approximately 20% of participants submitted "below the limit of quantification" for these samples in various assays across all three TEs, which indicated that the concentration was close to the limit of quantification for most assays. For qualitative results, all assays reported "detected".

For the Very Low concentration sample, most participants reported results as "not detected" and/or "below the limit of quantification". The concentration of the Very Low sample was determined to be below the limit of quantification/detection for most assays.

Target Concentrations

The Target Concentration for each sample was between 0.35-0.57 log_{10} IU/mL above the mean viral load value reported by participants. NRL is investigating the cause of this observation. Variation could be introduced by stock storage and freeze-thaw cycles, stock calibration and calculation, bulk material preparation and dilution. In addition, this specific EQAS stock was calibrated against a different WHO International Standard compared to most assays. The majority of assays used by participants were calibrated against the 4th WHO International Standard, whereas NRL calibrated the stock material against the 6th WHO International Standard. Further discussion and action will be conducted when the investigation has completed.

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2021 ANNUAL REPORT Viral Respiratory Molecular (RESP435)

INTRODUCTION

In 2021, NRL EQAS introduced a new program: Viral Respiratory Molecular Program (RESP) in response to the increasing demand for respiratory testing. There are three analytes of interest in this program: SARS-CoV-2 RNA, Influenza RNA and RSV RNA. The RESP program was designed as a comprehensive EQA program for both sophisticated laboratories, as well as Point-of-Care (POC) facilities in community and remote areas. Therefore, the RESP panels are validated for ambient shipment and 2-8°C storage. In 2021, three panels for three Test Events (TEs) were provided by NRL.

After each TE, the assay interpretations reported by participants were evaluated against the reference results. Additional statistical analyses of participants' assay interpretations compared with their peer groups were presented in tabular and graphical displays.

In addition to the OASYS generated performance reports, including NRL's comments, this annual report reviews the overall performance of various test kits from participant data across all three TEs of 2021. The measurable analytes, such as Ct values or S/Co, were submitted by participants, but not displayed in the performance reports. This annual report also includes some analysis of Ct values.

Please note, the data analysis of this annual report focuses on the Detection Kit only, to capture all data derived from the same platform, and does not assess differences in results between methods for extraction and amplification.

Aim

The aims of this report were to:

- Examine the overall detection rates and reproducibility of Flu A, Flu B, RSV and SARS-CoV-2 testing across the three TEs;
- Investigate the overall assay performance, LOD and measurable analytes;
- Discuss potential issues that were observed.

METHODS

Panel Samples

In 2021, three panels for three TEs were provided by NRL. Each panel contained five samples with 1.2mL in each vial. All panels were produced concurrently and shipped to participants at ambient temperature prior to the opening of each TE. All panels were required to be stored at 2-8°C until the opening of the relevant TE.

Analytes

All analytes used for RESP435 2021 EQAS panel samples were derived from cell culture supernatants and were gamma-irradiated at 50kGy to inactivate the virus. The subtypes or variants of each analyte (strain) were:

- Influenza A (H1N1)
- Influenza B (Victoria)
- RSV (A)
- SARS-CoV-2 (original strain)

Panel Composition

In the 2021 panels, each positive panel sample contained a single analyte. The bulk material of each analyte was produced by diluting the cell culture supernatant into diluent to a pre-determined concentration. Only one bulk (*i.e.* one concentration) of each analyte was used across all three TEs. The Negative sample was the diluent containing Minimum Essential Media (MEM) to mimic viral transport medium.

Each panel sample was included two to four times across the 3 TEs (Table 1).

Table 1. Panel composite of each sample.

Sample	Panel composite
SARS-CoV-2	TE1-A, TE1-C, TE2-D
Influenza A	TE1-E, TE2-B, TE3-E
Influenza B	TE1-D, TE2-C, TE3-A, TE3-D
RSV	TE2-A, TE3-B
Negative	TE1-B, TE2-E, TE3-C

RESULTS

Results from 32 participants were received for at least one TE for RESP435 2021. Twenty-two participants submitted results for all three TEs.

Thirty different commercial assays (Detection kits) were used by participants in the program. Figure 1 shows the distribution of these assays used by participants.

Assays used in the program

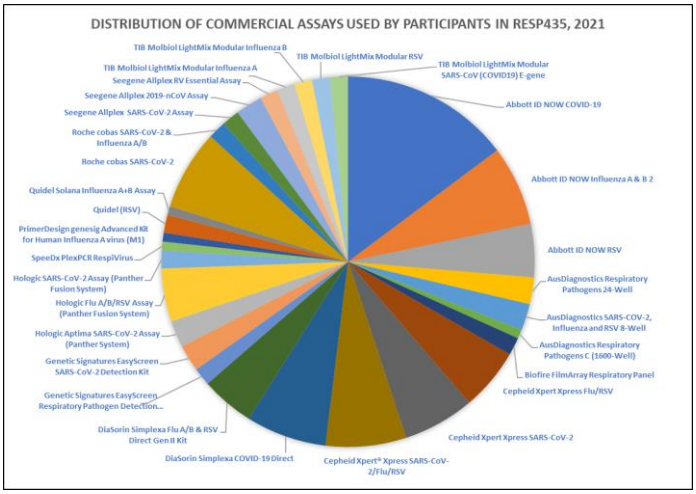

Figure 1. Distribution of 30 commercial assays used by participants in RESP435 2021.

A vast number of different commercial assays were used in RESP435 in 2021. Majority of assays displayed high levels of detection rates for all analytes across all panel samples.

Among the 30 commercial assays used in RESP435 2021, about half were single analyte assays and the other half were multiplex assays (Figure 2), which demonstrated the trend in the diagnostic field.

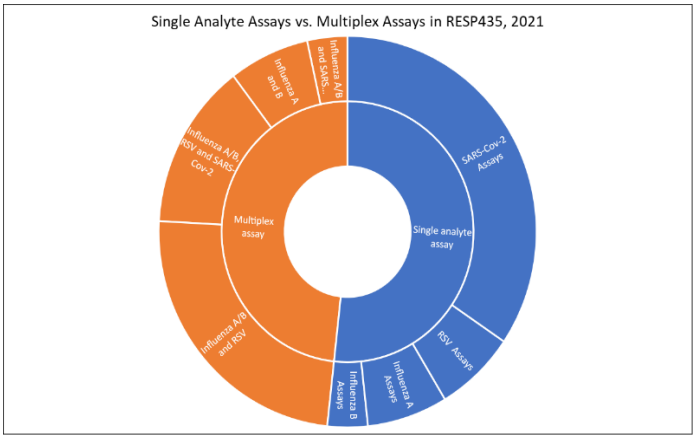

Figure 2. Single Analyte Assays vs. Multiplex Assays in RESP435, 2021.

Detection of SARS-CoV-2

From 2020, many molecular assays for SARS-CoV-2 have been developed, either as a single analyte assay or as part of a multiplex assay. Different from other analytes, most SARS-CoV-2 assays target two gene fragments. However, different assays use different sequence ranges.

Table 2 summarized the target genes used for detecting SARS-CoV-2 across the assays used in RESP435 2021.

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Table 2. Target genes for each assay detecting SARS-CoV-2.

Detection Kit	ORF1 gene	ORF1ab gene	ORF8 gene	E gene	N2 gene	N gene	S gene	M gene	RdRp gene
AusDiagnostics Respiratory Pathogens 24-Well	√		√						
AusDiagnostics SARS-COV-2, Influenza and RSV 8-Well	√		√						
Cepheid Xpert Xpress SARS-CoV-2				√	√				
Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV				√					
DiaSorin Simplexa COVID-19 Direct		√					√		
Genetic Signatures EasyScreen SARS-CoV-2 Detection Kit						√		√	
Roche cobas SARS-CoV-2		√		√					
Seegene Allplex SARS-CoV-2 Assay				√		√	√		√
Seegene Allplex 2019-nCoV Assay				√		√			√
TIB Molbiol LightMix Modular				√					

Assay Interpretations for each target analyte

Participants submitted assay interpretations for Influenza A RNA, Influenza B RNA, RSV RNA and/or SARS-CoV-2 RNA. Most assay interpretations reported were concordant to the reference results. Table 3 and Figure 3 displayed the concordant, false positive, false negative and equivocal results for each analyte in each TE.

Table 3. Concordant rate of assay interpretations for each analyte and TE.

Analyte	TE	Concordant	False Negative	False Positive	Equivocal
Influenza A RNA Interpretation	TE1	85	0	0	0
	TE2	100	0	0	0
	TE3	98	1	1	0
Influenza B RNA Interpretation	TE1	78	2	0	0
	TE2	99	1	0	0
	TE3	97	3	0	0
RSV RNA Interpretation	TE1	80	0	0	0
	TE2	94	1	0	0
	TE3	93	1	1	0
SARS-CoV-2 RNA Interpretation	TE1	129	0	0	1
	TE2	140	1	4	0
	TE3	120	0	0	0

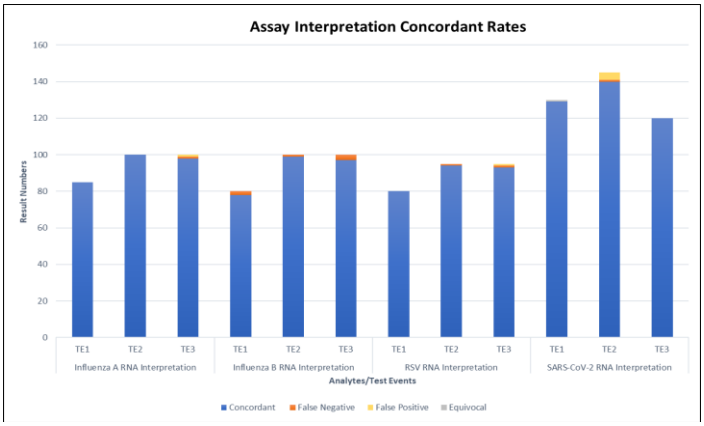

Figure 3. Concordant rate of assay interpretations for each analyte and TE.

Measurable Analytes for each Target Analyte

Some participants also reported the measurable analyte values, most frequently Ct values for real time PCR. The mean Ct values of each analyte for each peer group in each TE are summarised in Figure 4 to Figure 7. Please note, due to the vast array of assays and small datasets for each assay, statistical calculations may have low confidence. The mean Ct values are for indication only.

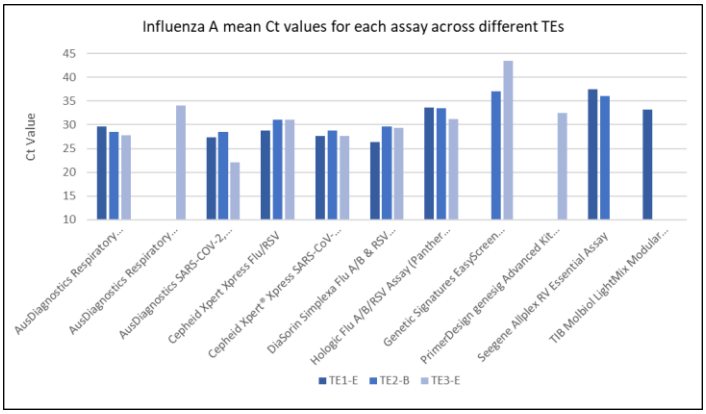

Figure 4. Influenza A mean Ct Values for each assay in each TE.

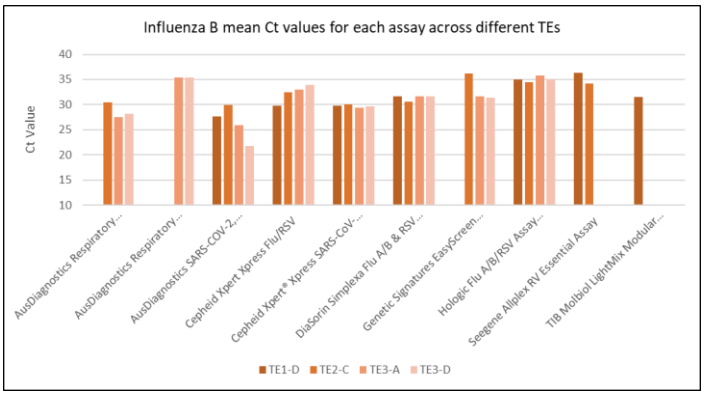

Figure 5. Influenza B mean Ct Values for each assay in each TE.

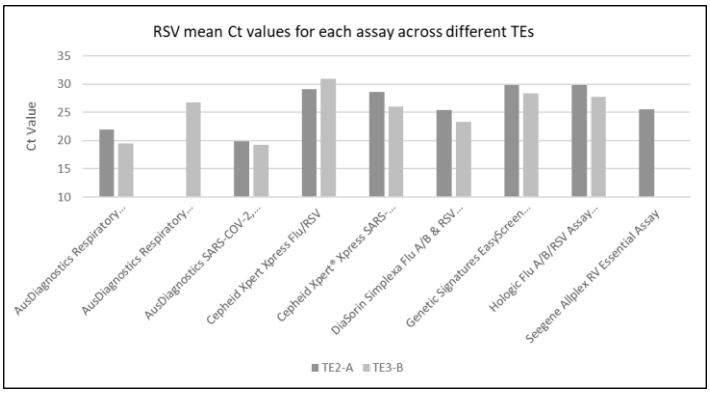

Figure 6. RSV mean Ct Values for each assay in each TE.

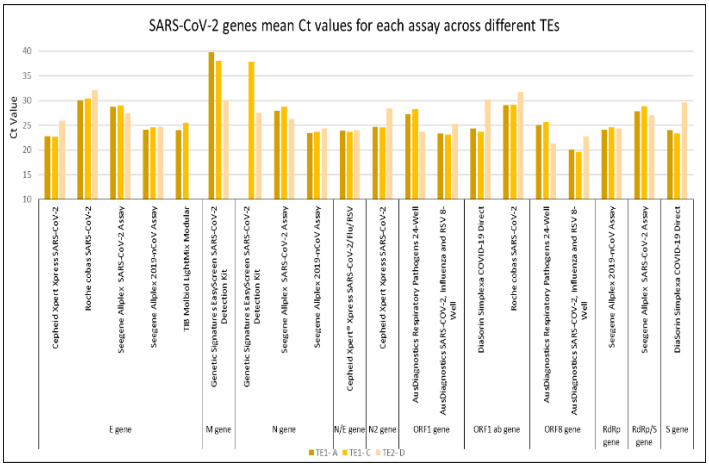

Figure 7. SARS-CoV-2 Genes mean Ct Values for each assay in each TE.

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Figures 8 and 9 compare the Ct values reported by participants using multiplex assays.

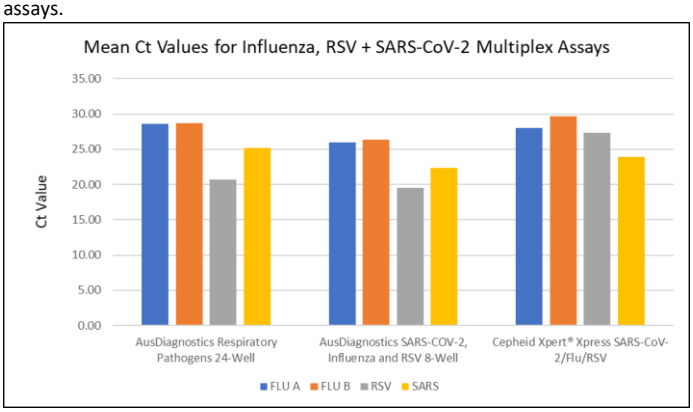

Figure 8. SARS-CoV-2, Influenza and RSV Multiplex assay mean Ct Values across all TEs.

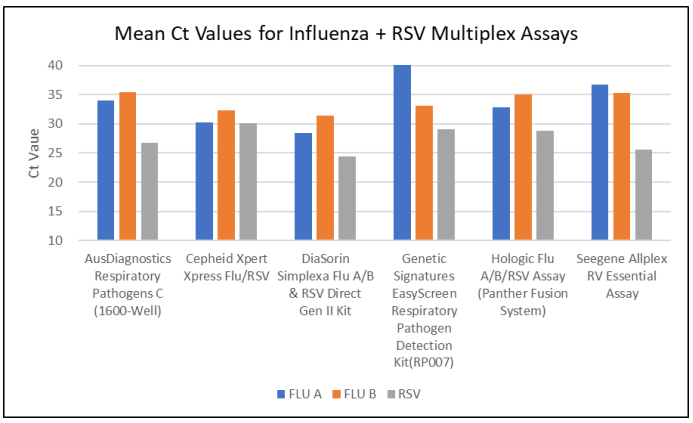

Figure 9. Mean Ct values for Influenza and RSV Multiplex assays across all TEs.

Concentration of Analytes

The overall Ct values reported for each analyte for each assay for all TEs, and the overall Ct values for each analyte for all assays combined across the different TEs are displayed in Figure 10 and Figure 11, respectively.

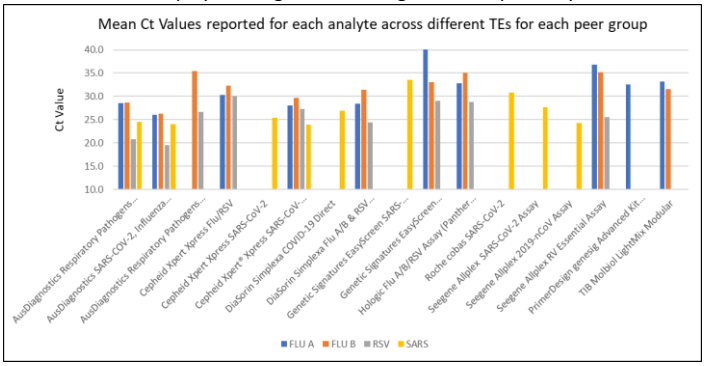

Figure 10. Mean Ct values reported for each analyte for all TEs for each peer group.

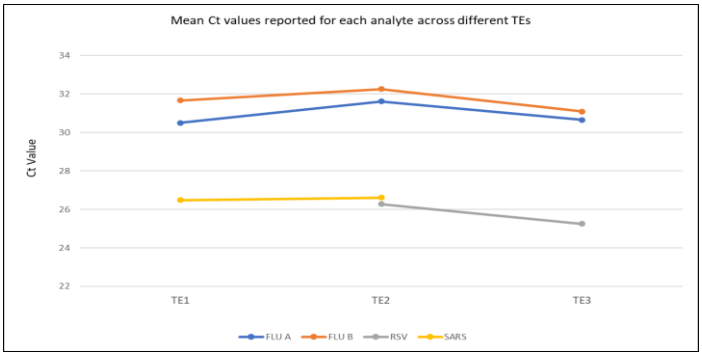

Figure 11. Overall mean Ct values reported for each analyte across different TEs.

DISCUSSION

General Observation

The majority of results submitted were concordant with the reference results (Table 3 and Figure 3). Most discordant results appeared to be due to data entry error. NRL recommends double checking result entry to avoid such errors.

Detection of SARS-CoV-2

Due to the Covid-19 pandemic, there has been an increase in demand for the manufacture of molecular assays for detecting SARS-CoV-2 RNA. From 2020, many molecular assays for SARS-CoV-2 have been developed, either as a single analyte assay or as part of a multiplex assay. Different from assays for other analytes, majority of SARS-CoV-2 assays target at least two various gene regions (Table 2). This observation reflects the gradual understanding of this new emerging virus and its high mutation frequency.

Measurable Analytes for each Target Analyte

Due to the vast array of assays used in the RESP435 program, statistical calculations on measurable values may have low confidence as a result of the small peer group datasets.

Nevertheless, the Ct values reported by participants for each individual analyte showed minor variation within the assays across the TEs (Figure 4-7). This demonstrated good assay reproducibility in different labs, tested on different dates. The assay with the highest observed variation appears to be 'Genetic Signatures EasyScreen SARS-CoV-2 Detection Kit' and 'Genetic Signatures EasyScreen Respiratory Pathogen Detection Kit(RP007)' with higher overall Ct values when compared with other assays. This may be due to lower limit of detection of the assay or potential user error. It was also the only assay used that targets the M gene for the detection of SARS-CoV-2.

Figure 10 shows the overall mean values for each of the assays where participants reported Ct values, inclusive of all TEs. The mean Ct values encompassing all assays across the 3 TEs (Figure 11) show that that the Flu A and Flu B analytes were lower concentration samples than RSV and SARS-CoV-2 analytes. Overall stability of the samples was demonstrated, as there was no drop in sample concentration over the 3 TEs for each analyte.

Multiplex Assays

There were 30 different commercial detection kits used by participants. Of these, approximately half were single analyte assays and the other half were multiplex assays (Figure 2). Among the multiplex assays, most participants used assays for the detection of Influenza A/B and RSV, or assays for the detection of Influenza A/B, RSV and SARS-CoV-2.

The multiplex assays demonstrated good assay performance for the detection of multiple analytes simultaneously (Figure 8 and Figure 9), and displayed similar concordant rates and Ct values with assays for signal analyte detection.

With the technology improvement and market desire, more manufacturers launched more test kits with wider range of respiratory analytes beyond Influenza, RSV and SARS-CoV-2. Some assays in the market can detect more than ten respiratory analytes simultaneously, which increase the testing efficiency of a range of analytes whilst providing a more clinically significant outcome.

RESP Program in 2023

Due to the high demand of syndromic respiratory testing and the well-received uptake of the RESP435 program in 2021, NRL EQAS is planning to expand the respiratory program offering, and provide a wide range of both viral and bacterial analytes that cause upper and lower respiratory infections. This will most likely remain as a comprehensive EQA program for both sophisticated laboratories, as well as Point-of-Care (POC) facilities in community and remote areas. This will also be suitable for single-analyte and/or multiplex assays. Feedback from participants, manufacturers or other customers are always welcome to assist NRL to continually improve and tailor our programs to suit the current needs of the community.

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2021 ANNUAL REPORT Blood Borne Serology POC (POCS435)

INTRODUCTION

The NRL EQAS Bloodborne Serology POC Program (POCS) was designed

as a Point of Care (POC) EQA program for POC sites and resource-limited laboratories that routinely use rapid test devices (RTDs) for infectious disease serology. All main blood-borne infections were represented in the POCS345 program including antibodies to HIV, HCV and Treponema as well as HBsAg and HIV-1 p24 Ag. In 2021, one panel for each of three Test Events (TEs) were provided by NRL.

After each TE, the assay interpretations reported by participants were compared to the reference results. Additional statistical analysis of participants' assay interpretations compared to their peer group was presented in tabular and graphical displays.

In addition to the OASYS generated performance reports, including NRL comments, this annual report reviews the overall performance of various test kits from participant data across all three TEs of 2021.

Aim

The aims of this report were to discuss the:

- program and panel design;
- overall performance of various test kits;
- general observation for result submission.

METHODS

Panel Samples

In 2021, NRL provided panels for three TEs. Each panel contained five samples, which were fully characterised for all the analytes using NRL's validated testing algorithms. Polybead® Microspheres (Polysciences, Inc.) were added into each sample to mimic whole blood. The samples were provided in dropper bottles together with finger-prick analogues to mimic sample collection and testing with RTDs. All panels for a given TE were produced and shipped at ambient temperature prior to the opening of each TE. All panels were required to be stored at 2-8°C until the opening of the relevant TE.

Analyte Frequency

Multiple analytes can be tested and reported in POCS, including anti-HIV, anti-HCV, anti-*T. pallidum* (syphilis), HBsAg and HIV-1 p24 Ag. Figure 1 presents the frequently reactive/positive samples for a particular analyte were included in the 15 samples for 2021. Each of the analytes were included approximately in the same frequency.

Of particular note:

- One sample that was negative for all analytes [Normal Human plasma (NHP)] was included in the TE 1 panel;
- A sample that was reactive/positive for both anti-HIV-1/2 and syphilis was included in the TE 2 panel;
- Included in duplicate in TE 2 panel was a manufactured sample that was negative for anti-HIV and positive for HIV-1 p24 antigen. The sample was manufactured by spiking NHP with 8E5 Cell culture supernatant to mimic HIV early infection;
- All other samples except the p24 manufactured samples were different samples and only used once in the 2021 POCS panels.

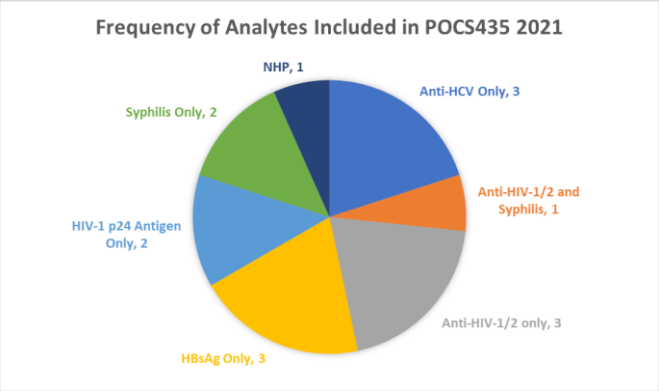

Figure 1. Frequency of analytes included in POCS435 panels in 2021

RESULTS

Participants

Overall, 14 participants from six countries/regions reported results in POCS435 program in 2021 (Figure 2). However, not all participants returned results and, of those who did, not all reported results for each TE.

Among the Australian participants, the majority were community-based testing sites.

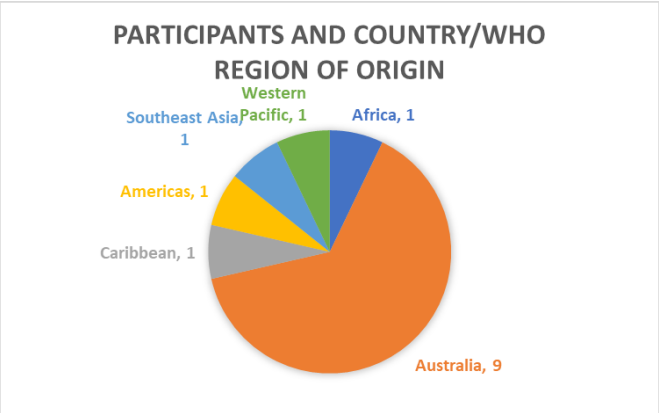

Figure 2. Country/World Health Organization (WHO) region of origin of participants that returned results before TE closing for POCS435 in 2021

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Testing Profile

The participants did not all test for all of analytes included in the POCS435 program. There were five groupings of analytes tested by participants (Figure 3). The majority of participants tested the panel samples for HIV only.

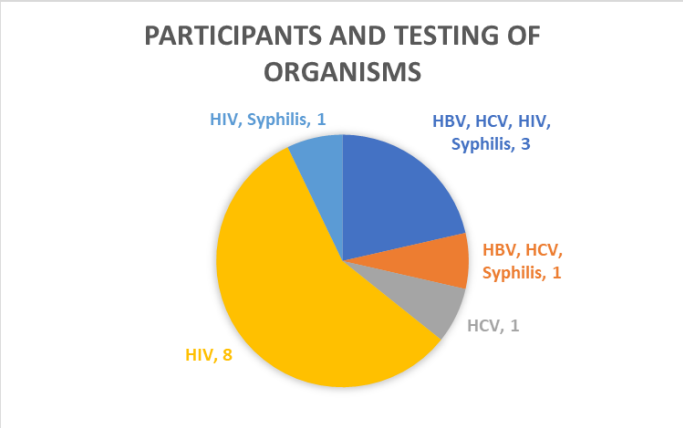

Figure 3. Groupings of analytes tested for by participants in POCS435 in 2021

Overall, participants reported results from 18 assays to detect the analytes in the POCS435 panel samples in 2021: four to identify the presence of HBsAg, four to detect the presence of anti-HCV, two to detect the presence of anti-HIV only, four to detect the presence of both anti-HIV and HIV-1 p24 Ag and four to detect the presence of anti-syphilis (Table 1).

Table 1. Assays used by participants in POCS435 2021

Assay	Analyte	Participant No.
Abbott Determine HBsAg 2 Rapid Test (S/P/WB)	HBsAg	1
Abbott Determine HBsAg Rapid Test (S/P/WB)	HBsAg	2
Abbott Determine HIV Ultra Rapid Test (S/P/WB)	Anti-HIV-1/2 p24 Ag	3
Abbott Determine HIV-1/2 Rapid Test (S/P/WB)	Anti-HIV-1/2	2
ABON Syphilis Ultra Rapid Test Device (WB/S/P)	Anti-T. pallidum	1
Alere Determine HIV-1/2 Ag/Ab Combo Rapid Test	Anti-HIV-1/2 p24 Ag	3
Alere Determine Syphilis TP Rapid Test	Anti-T. pallidum	2
Alere HIV Combo Rapid Test	Anti-HIV-1/2 p24 Ag	3
CTKB OnSite HCV Ab Plus Rapid Test-Cassette (Serum/Plasma)	Anti-HCV	1
InTec ADVANCED QUALITY ONE STEP Anti-TP (Treponema Pallidum/Syphilis) Rapid Test	Anti-T. pallidum	1
InTec ADVANCED QUALITY ONE STEP HBsAg Rapid Test (WB/S/P)	HBsAg	1
InTec ADVANCED QUALITY Rapid Anti-HCV Test (S/P/WB)	Anti-HCV	1
Meril MERISCREEN HIV 1-2 WB Rapid Test (S/P/WB)	Anti-HIV-1 Anti-HIV-2	1
OraSure OraQuick ADVANCE Rapid HIV-1/2 Antibody Test	Anti-HIV-1/2	1
OraSure OraQuick HCV Rapid Antibody Test	Anti-HCV	1
Standard Diagnostics SD BIOLINE HBsAg	HBsAg	2
Standard Diagnostics SD BIOLINE HCV	Anti-HCV	3
Standard Diagnostics SD BIOLINE Syphilis 3.0 Rapid Test	Anti-T. pallidum	2

DISCUSSION

General Observation

Majority of results submitted were acceptable and concordant with the reference results.

Most aberrant results appeared to be due to data entry error. It is recommended that a second individual review any manually submitted results before the TE closing date.

A few participants reported results under a wrong assay. NRL did not make the results unacceptable in most of circumstances if the results were concordant with the reference results for the target analyte, as we understood some assay names were very similar. However, selecting a wrong assay not only affected the analysis of the participant's own data, but also affected the statistical analysis of the whole peer group. Selection of the correct test kits is very critical for EQAS data submission.

Sample Integrity

Some participants reported that a few samples were clogged and were difficult to remove the samples from the bottle. These comments were also received in the previous years.

The plasma used for POCS435 was clarified by centrifugation prior to production to remove particulate matter and clots. The plasma was not defibrinated thus fibrin clots could have potentially formed after spin clarification. Moreover, the addition of Polybead® Microspheres sometimes increased the viscosity of the sample and may have caused the clots in the samples.

Discontinuation of POCS

NRL decided to discontinue POCS in the end of 2021. We want to express our gratitude to all participants who supported the program in the past years. We initially developed POCS for the purpose of helping POC sites to monitor the testing performance of their RTDs. Due to production issues and enrolment numbers, we had to make the decision to discontinue POCS at the end of 2021. As more and more participants report RTD results in our mainstream serology programs (MMBS, HEPM and RVSS), we recommend that all our POCS participants consider switching to other NRL serology programs. If participants need any assistance to select the most suitable program, please do not hesitate to contact NRL EQAS via qa@nrlquality.org.au.

Meanwhile, we are continuing to improve the production process and sample matrix for POCS, and hope POCS will come back to the market with a new format. If you have any suggestions and/or questions, please let us know via qa@nrlquality.org.au.

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