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Version	Version Date (dd/mmm/yyyy)	Comments
2.0	09 SEP 2004	Roche Amplicor DNA Templates and VQA DNA control requirements.
3.0	21 SEP 2005	Added FSTRF email information for verifying VQA blinded pellet results.
4.0	28 AUG 2007	Formatted the document into a formal SOP.
5.0	13 MAR 2012	Added templates for Abbott DNA QL (AQ) and Roche TaqMan QL (TQ) assays and VQA DNA control requirements.
5.0	05 FEB 2013	Added notes to sections 4.3 and 6.3 indicating the minimum number of blinded pellets required per run. Formatting changed to include signature sections and revision history section. Clarified language. Updated Templates.
6.0	01 DEC 2016	Modified templates for Roche TaqMan and Abbott RealTime (qualitative and quantitative assays) for different matrices. Removed Roche Amplicor HIV DNA testing. Added RAP testing. Changed HIV DNA to HIV DNA/TNA (total nucleic acid) to reflect the change in HIV nucleic acid testing (NAT).
6.0	05 APR 2017	Added control requirements for GeneXpert and added VQA200 serum control language throughout the SOP. Added control storage information. Updated references.

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1. PRINCIPLE

1.1. The Virology Quality Assurance (VQA) Laboratory provides external quality control materials (QCMs) in several matrices (serum/EDT [SER], frozen whole blood [FWB], cell pellets [PEL], and dried blood spots [DBS]) for use in run control and assay/instrument validation for the qualitative or quantitative detection of HIV DNA/TNA (total nucleic acid) in HIV nucleic acid testing (NAT).

1.2. VQA HIV controls matrices are derived from a variety of sources. Commercially available true human serum (SER) is clarified by filtration and EDTA is added to achieve a final concentration of 6mM, which mirrors the EDTA concentration typically found in plasma derived from an EDTA Vacutainer. HIV seronegative peripheral blood mononuclear cells (PBMC) are generated from source leukocytes or a unit of anticoagulated whole blood to create PEL controls; FWB and DBS controls are generated from anticoagulated whole blood. Chronically infected cell lines containing a known pre-determined amount of HIV proviral DNA copies and cell-associated HIV RNA per cell are diluted in PBMC or whole blood to achieve a specified concentration of HIV nucleic acid per aliquot. The aliquot size for a SER control is 1.25mL; the aliquot size for a PEL control is 1 million PBMCs; the aliquot size for FWB is 0.1mL; the aliquot size for DBS is 0.075mL per spot.

1.3. This SOP describes the proper use, interpretation, and template setup for the use of VQA external run controls for the Abbott RealTime HIV-1 qualitative (AQ) or quantitative (AR) assays, the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 qualitative (TQv2) or quantitative (RTv2) assays, and for random access platforms (RAP) such as the Cepheid GenXpert HIV nucleic acid assay. These control templates may also be used for internally developed assays and the laboratory should review the templates to determine which is most applicable to their testing. VQA controls must be included in any run that contains VQA-related data (i.e. proficiency testing data) or clinical trial data. The use of kit controls is also included in templates as required by the kit manufacturers.

2. VQA CONTROLS

2.1. VQA HIV NAT controls must be ordered from the VQA Laboratory using the Reagent Order Form located on the HANC website at http://www.hanc.info/labs/labresources/vqaResources/Pages/default.aspx. Laboratories will need to register with HANC to gain access to this reagent order for. Contact the VQA (vqa@rush.edu) for information. The matrix must be included in the order using the comment section; a laboratory must use controls that are created in the same matrix used for testing of clinical samples. VQA SER, FWB and PEL quality control materials (QCMs) are shipped on dry ice and should be maintained at -70°C or colder until extracted; VQA DBS QCMs are shipped ambient and should be maintained at -70°C or colder for long-term storage.

Note: DBS QCMs must be thawed to room temperature prior to opening the bag; the condition of the humidity indicator should be noted and additional desiccants should be added to the control bag if indicated.

2.2. VQA HIV NAT copy controls (VQA HIV NAT CC) – These controls may be used as part of assay/instrument validations or for other purposes such as kit lot evaluation, but they are not required for run performance:

2.2.1. VQA200 serum [SER] controls contain HIV virus at a concentration of 200 copies/mL (1.25 mL per aliquot) and is intended for single use only.

2.2.2. HIV NAT cell pellet [PEL] copy control samples contain 1 million PBMCs in a 0.1 mL suspension per aliquot which includes 0, 5, 10 or 20 copies of HIV proviral DNA (+cell-

associated RNA, depending on the cell line used). One set of copy controls consists of one vial of each level (4 tubes total); 20 sets contain 20 vials of each level (80 tubes total).

2.2.3. HIV NAT frozen whole blood [FWB] samples contain 0.1 mL of citrated whole blood (CPD) per aliquot which includes 0, 5, 10, or 20 copies of proviral HIV DNA (+cell-associated RNA, depending on the cell line used). One set of copy controls consists of one vial of each level (4 tubes total); 20 sets contain 20 vials of each level (80 tubes total).

2.2.4. HIV NAT dried blood spot [DBS] samples contain 0.075 mL of citrated whole blood (CPD) per spot which includes 0, 5, 10, or 20 copies of proviral HIV DNA (+cell-associated RNA, depending on the cell line used). One DBS card contains 4 spots of one level of control – sets of DBS copy controls are sent in multiples of 4; four sets consists of 4 cards – each card containing 4 spots of each level (4 DBS cards, 16 spots total). Twenty sets consist of 5 cards for each level of control (20 cards, 80 spots total).

2.3. VQA HIV NAT blinded extraction controls:

2.3.1. HIV NAT cell pellet [PEL] extraction control samples contain 1 million PBMCs in a 0.1 mL suspension per aliquot which includes 0 or 20 copies of HIV proviral DNA (+cell-associated RNA, depending on the cell line used). One set of blinded PEL extraction controls consists of fifty vials of cells that consist of different combinations of HIV Positive and HIV Negative samples (50 tubes total). A laboratory may only use each configuration one time – they must indicate their current lot of blinded extraction controls in their order to ensure they don't receive a duplicate panel.

2.3.2. HIV NAT frozen whole blood [FWB] samples contain 0.1 mL of citrated whole blood (CPD) per aliquot and may or may not contain any HIV. One set of blinded FWB extraction controls consists of 32 vials of blood that consist of different combinations of HIV Positive and HIV Negative samples (32 tubes total). A laboratory may only use each configuration one time – they must indicate their current lot of blinded extraction controls in their order to ensure they don't receive a duplicate panel.

2.3.3. HIV NAT dried blood spot [DBS] samples contain 0.075 mL of citrated whole blood (CPD) per spot and may or may not contain HIV. One set of blinded DBS extraction controls consists of 25 spots of blood that consist of different combinations of HIV Positive and HIV Negative samples (5 cards, 25 spots total). A laboratory may only use each configuration one time – they must indicate their current lot of blinded extraction controls in their order to ensure they don't receive a duplicate panel.

2.4. VQA HIV NAT Copy Control and Blinded Extract Control Storage and Handling:

2.4.1. VQA200 serum (SER) controls should be stored at -70°C or colder for long-term storage; short term storage (i.e. 1 month) at -30°C is acceptable. Care must be taken to maintain the cold chain while removing samples for testing; distributing controls into smaller storage boxes (i.e. 5x5 array freezer storage boxes) for routine use can help to preserve the stability of controls - store freezer boxes away from the door, especially if the freezer is opened frequently. Individual controls must be thawed prior to use and vortexed well to thoroughly mix prior to testing; do not remove more controls than are needed for a single assay. All VQA200 controls are intended for single use only and must not be reused or undergo multiple freeze/thaw cycles. Do not stockpile reagents. More frequent shipments may be required for high volume laboratories.

2.4.2. VQA HIV NAT PEL controls should be stored at -70°C or colder for long-term storage; short term storage (i.e. 1 month) at -30°C is acceptable. Care must be taken to maintain the cold chain while removing samples for testing; distributing controls into smaller storage boxes (i.e. 5x5 array freezer storage boxes) for routine use can help to preserve the stability of controls - store freezer boxes away from the door, especially if the freezer is opened frequently. Individual controls must be thawed prior to use and vortexed well to thoroughly mix prior to testing; do not remove more controls than are needed for a single assay. Individual controls must be thawed prior to use and centrifuged for 1 minute to ensure the suspension volume is present and visible. Discard the control if a cell suspension is not visible prior to extraction. All VQA HIV NAT PEL controls are intended for single use only and must not be reused or undergo multiple freeze/thaw cycles. Do not stockpile reagents. More frequent shipments may be required for high volume laboratories.

2.4.3. VQA HIV NAT FWB controls should be stored at -70°C or colder for long-term storage; short term storage (i.e. 1 month) at -30°C is acceptable. Care must be taken to maintain the cold chain while removing samples for testing; distributing controls into smaller storage boxes (i.e. 5x5 array freezer storage boxes) for routine use can help to preserve the stability of controls - store freezer boxes away from the door, especially if the freezer is opened frequently. Individual controls must be thawed prior to use and vortexed well to thoroughly mix prior to testing; do not remove more controls than are needed for a single assay. Individual controls must be thawed prior to use and centrifuged for 1 minute to ensure the blood volume is present and visible. Discard the control if blood is not visible prior to extraction. All VQA HIV NAT FWB controls are intended for single use only and must not be reused or undergo multiple freeze/thaw cycles. Do not stockpile reagents. More frequent shipments may be required for high volume laboratories.

2.4.4. VQA HIV NAT DBS cards should be stored at -70°C or colder for long-term storage; short term storage (i.e. 1 month) at room temperature, 4°C or at -20°C (3 months) is acceptable; humidity indicators should be monitored frequently (e.g. weekly) if DBS cards are not frozen and monthly if they are frozen at -20°C. DBS cards must be thawed to room temperature prior to opening the zip lock bag. The user must inspect the humidity indicator and note if there was any exposure to humidity. Once a spot has been removed, the user must repackage the unused HIV NAT DBS and added new/additional desiccants/humidity indicator card, as needed and return the controls to the appropriate storage condition; humidity can reduce the ability to elute material off the DBS card or result in fungal or bacterial growth on the card; discard the control if there is any visible contamination or if the sample has been compromised in any way. Each VQA HIV NAT DBS SPOT is intended for single use only and must not be reused. Do not stockpile reagents. More frequent shipments may be required for high volume laboratories.

3. PROCEDURE: Abbott RealTime HIV-1 Qual Assay (AQ)

Refer to Templates 1-4 in Appendix II.

3.1. Kit controls (1 positive and 1 negative) must be included in every run per the manufacturer's requirement.

3.2. A minimum of two (2) HIV NAT blinded extraction controls must be included in batch of 24 samples. If multiple sample matrices are included in a single assay then a minimum of 1 blinded extraction for each sample matrix must be included in each batch of 24 samples; the extraction controls should be evenly spaced throughout the run. One additional blinded extraction control will be added after every 10 samples; the matrix of the extraction control may alternate as new controls are added. At least 1 blinded extraction control for each sample matrix must be included

in each run regardless of the batch size.

Note: If one of the control matrices is a plasma matrix, then the VQA200 control should be included as the VQA sensitivity control for that matrix. No blinded extraction controls for plasma are available; simply substitute the HIV NAT blinded extraction controls for the other sample matrices included in the run. If the entire run consists only of plasma samples then only a single VQA200 control needs to be included in the run beyond the required kit controls; the VQA200 control result must be detected for the run to be valid.

4. PROCEDURE: Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Qual Test, v2 (TQv2)

Refer to Templates 5-6 in Appendix II.

4.1. Kit controls (1 positive and 1 negative) must be included in every run per the manufacturer's requirement.

4.2. A minimum of two (2) blinded extraction controls must be included in batch of 24 samples. If multiple sample matrices are included in a single assay then a minimum of 1 blinded extraction for each sample matrix must be included in each batch of 24 samples; the extraction controls should be evenly spaced throughout the run.

Note: If one of the control matrices is a plasma matrix, then the VQA200 control should be included as the VQA sensitivity control for that matrix. No blinded extraction controls for plasma are available; simply substitute the HIV NAT blinded extraction controls for the other sample matrices included in the run. If the entire run consists only of plasma samples then only a single VQA200 control needs to be included in the run beyond the required kit controls; the VQA200 control must be detected for the run to be valid.

5. PROCEDURE: Abbott RealTime HIV-1 Quantitative Assay (AR)

Refer to Templates 7-10 in Appendix II.

5.1. Kit controls (1 low positive, 1 high positive, and 1 negative) must be included in every run per the manufacturer's requirement.

5.2. A minimum of two (2) HIV NAT blinded extraction controls must be included in batch of 24 samples. If multiple sample matrices are included in a single assay then a minimum of 1 blinded extraction for each sample matrix must be included in each batch of 24 samples; the extraction controls should be evenly spaced throughout the run. One additional blinded extraction control will be added after every 10 samples; the matrix of the extraction control may alternate as new controls are added. At least 1 blinded extraction control for each sample matrix must be included in each run regardless of the batch size.

Note: If one of the control matrices is a plasma matrix, then the VQA200 control should be included as the VQA sensitivity control for that matrix. No blinded extraction controls for plasma are available; simply substitute the HIV NAT blinded extraction controls for the other sample matrices included in the run. If the entire run consists only of plasma samples then only a single VQA200 control needs to be included in the run beyond the required kit controls; the VQA200 control result must fall within the specified range for the run to be valid.

6. PROCEDURE: Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Quantitative Test, v2 (RTv2)

Refer to Templates 11-12 in Appendix II.

6.1. Kit controls (1 low positive, 1 high positive, and 1 negative) must be included in every run per the manufacturer's requirement.

6.2. A minimum of two (2) blinded extraction controls must be included in batch of 24 samples. If multiple sample matrices are included in a single assay then a minimum of 1 blinded extraction for each sample matrix must be included in each batch of 24 samples; the extraction controls should be evenly spaced throughout the run.

Note: If one of the control matrices is a plasma matrix, then the VQA200 control should be included as the VQA sensitivity control for that matrix. No blinded extraction controls for plasma are available; simply substitute the HIV NAT blinded extraction controls for the other sample matrices included in the run. If the entire run consists only of plasma samples then only a single VQA200 control needs to be included in the run beyond the required kit controls; the VQA200 control result must fall within the specified range for the run to be valid.

7. PROCEDURE: Internally Developed Assays (IH)

7.1. Assay controls must be included in every run per the laboratory's SOP.

7.2. A minimum of two (2) blinded extraction controls must be included in batch of 24 samples. The laboratory should review the templates provided for the Abbott and Roche templates and determine which templates are applicable to their test. A minimum of one blinded extraction control for each sample matrix must be included in any run size.

8. Random Access Platforms (e.g. GenXpert)

8.1. Internal sample controls are included with each sample; no external control is required.

8.2. A minimum of one blinded extraction control (or VQA200 control for the viral load assay) should be run weekly with the result confirmed as accurate, whenever patient samples are tested in that week; or after every 20 patient samples, if more than 20 samples are assayed in a week.

8.3. A minimum of two (2) blinded extraction controls (or VQA200 controls for the viral load assay) must be assayed and the result confirmed before implementing a new cartridge lot or a new shipment of that same cartridge lot. The extraction control matrix must match the matrix used for clinical sample testing.

9. INTERPRETATIONS

9.1. Run Validity:

9.1.1. Whenever kit controls are included for testing, they must be deemed valid per the package insert for that kit and yield a correct result.

9.1.1.1. An invalid kit control invalidates the entire run and all the samples included in the run.

9.1.2. VQA blinded extraction controls must yield the correct result as defined by the key for that production lot number. For all kits, a positive control sample must be valid and yield a POSITIVE result; a negative control sample must be valid and yield a NEGATIVE result per the package insert or laboratory SOP.

9.1.2.1. An invalid extraction control (or VQA control) result invalidates the entire run and all the samples included in the run if only one sample matrix is included in the run.

9.1.2.2. If the run includes more than one sample matrix, and blinded extraction control of only one sample matrix fails, then only samples that consist of the same matrix are deemed invalid.

Note: all other controls included in the run must also be valid.

10. REAL-TIME BLINDED CONTROL RESULT VERIFICATION

10.1. VQA blinded controls may be validated by use of the Laboratory Data Management System (see LDMS user manual, https://www.fstrf.org/apps/cfmx/apps/common/Portal/index.cfm).

10.1.1. The blinded pellet code must be logged into the LDMS exactly as it appears on the sample label.

10.1.2. Failed runs will be tracked by the LDMS. Two consecutive failures or three failures out of 10 runs will result in the user being locked out of the HIV DNA assay module. Users will need to contact the VQA lab (vqa@rush.edu) and LDMS User Support (ldmshelp@fstrf.org) if they are locked out of their assay module.

10.2. Users who do not have access to an LDMS must verify their blinded pellet control results by emailing the control results to vqa.blind@fstrf.org (*Note*: This is a **new** email logon). The user must send their data using the following template included in the text of the email (see the template in Appendix I):

11. REFERENCES

11.1. Jackson, J.B., J. Drew, H.J. Lin, P. Otto, J.W. Bremer, F.B. Hollinger, S.M. Wolinsky, The ACTG PCR Working Group, and The ACTG PCR Virology laboratories. 1993. Establishment of a Quality Assurance Program for Human Immunodeficiency Virus Type 1 DNA Polymerase Chain Reaction Assays by the AIDS Clinical Trials Group. J. Clin. Microbiol. 31:3123-3128.

11.2. Abbott RealTime HIV-1 package insert [IVD]. June 2013. Abbott Molecular, Inc. Des Plaines, IL 60018, USA.

11.3. Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0 package insert [IVD]. May 2013. Roche Molecular Systems, Inc. Branchburg, NJ 08876, USA.

11.4. Abbott RealTime HIV-1 Qualitative package insert[CE]. September 2010. Abbott Molecular, Inc. Des Plaines, IL 60018, USA.

11.5. Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 package insert [RUO]. March 2014. Roche Molecular Systems, Inc., Branchburg, NJ, 08876, USA.

11.6. Xpert® HIV-1 Viral Load package insert [CE]. January 2015. Cepheid AB, Solna, Sweden.

11.7. Xpert® HIV-1 Qual package insert [CE]. March 2015. Cepheid AB, Solna, Sweden.

12. Appendix I – Template for Validating Blinded Extraction Controls by Email:

Note: This is only a template – report the result per your laboratory SOP.

TQ or AQ Email Notification Template:

Note: AQ does not report CT in the Run Report so CTs need not be reported for AQ

Dear FSTRF Staff:
Please verify the following results for VQA HIV DNA real-time blinded control pellets. The results are as follows:
Lab Number: 078
Requested By: Cheryl Jennings
Method/Assay: **Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Qual Test**
Date of Request: 10 March 2012

Blinded Pellet ID	HIV CT	IC CT	Result
022d.019	28.2	26.5	Reactive
022d.020	--	27.1	Non-Reactive

13. Appendix II – Assay Templates:

Template 1: Abbott HIV Qual – 24 samples

Template 1: VQA HIV Nucleic Acid Control Requirements - Abbott HIV QUAL assay - 24 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	1	1	VQA extraction Control #1 (blinded) in matrix #1
4	2	2	1
5	3	3	2
6	4	4	3
7	5	5	4
8	6	6	5
9	7	7	6
10	8	8	7
11	9	9	8
12	10	10	9
13	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
14	11	11	10
15	12	12	11
16	13	13	12
17	14	14	13
18	15	15	14
19	16	16	15
20	17	17	16
21	18	18	17
22	19	19	18
23	20	20	19
24	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 2: Abbott HIV Qual – 48 samples

Template 2: VQA HIV Nucleic Acid Control Requirements - Abbott HIV QUAL assay - 48 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	1	1	VQA extraction Control #1 (blinded) in matrix #1
4	2	2	1
5	3	3	2
6	4	4	3
7	5	5	4
8	6	6	5
9	7	7	6
10	8	8	7
11	9	9	8
12	10	10	9
13	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
14	11	11	10
15	12	12	11
16	13	13	12
17	14	14	13
18	15	15	14
19	16	16	15
20	17	17	16
21	18	18	17
22	19	19	18
23	20	20	19
24	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3
25	21	21	20
26	22	22	21
27	23	23	22
28	24	24	23
29	25	25	24
30	26	26	25
31	27	27	26
32	28	28	27
33	29	29	28
34	30	30	29
35	VQA extraction Control #3 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #3 (blinded) in matrix #1	VQA extraction Control #4 (blinded) in matrix #1, 2 or 3
36	31	31	30
37	32	32	31
38	33	33	32
39	34	34	33
40	35	35	34
41	36	36	35
42	37	37	36
43	38	38	37
44	39	39	38
45	40	40	39
46	VQA extraction Control #4 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #4 (blinded) in matrix #2	VQA extraction Control #5 (blinded) in matrix #1, 2, or 3
47	41	41	40
48	42	42	41

Labs running multiple matrices can mix and match blinded extraction controls to ensure even use of control sets. A minimum of one control for each matrix must be included in each run.

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 3: Abbott HIV Qual – 72 samples

Template 3: VQA HIV Nucleic Acid Control Requirements -Abbott HIV QUAL assay - 72 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	1	1	VQA extraction Control #1 (blinded) in matrix #1
4	2	2	1
5	3	3	2
6	4	4	3
7	5	5	4
8	6	6	5
9	7	7	6
10	8	8	7
11	9	9	8
12	10	10	9
13	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
14	11	11	10
15	12	12	11
16	13	13	12
17	14	14	13
18	15	15	14
19	16	16	15
20	17	17	16
21	18	18	17
22	19	19	18
23	20	20	19
24	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3
25	21	21	20
26	22	22	21
27	23	23	22
28	24	24	23
29	25	25	24
30	26	26	25
31	27	27	26
32	28	28	27
33	29	29	28
34	30	30	29
35	VQA extraction Control #3 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #3 (blinded) in matrix #1	VQA extraction Control #4 (blinded) in matrix #1
36	31	31	30
37	32	32	31
38	33	33	32
39	34	34	33
40	35	35	34
41	36	36	35
42	37	37	36
43	38	38	37
44	39	39	38
45	40	40	39
46	VQA extraction Control #4 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #4 (blinded) in matrix #2	VQA extraction Control #5 (blinded) in matrix #2
47	41	41	40
48	42	42	41
49	43	43	42
50	44	44	43
51	45	45	44
52	46	46	45
53	47	47	46
54	48	48	47
55	49	49	48
56	50	50	49
57	VQA extraction Control #5 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #5 (blinded) in matrix #1	VQA extraction Control #6 (blinded) in matrix #3
58	51	51	50
59	52	52	51
60	53	53	52
61	54	54	53
62	55	55	54
63	56	56	55
64	57	57	56
65	58	58	57
66	59	59	58
67	60	60	59
68	VQA extraction Control #6 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #6 (blinded) in matrix #2	VQA extraction Control #7 (blinded) in matrix #1, 2, or 3
69	61	61	60
70	62	62	61
71	63	63	62
72	64	64	63

Labs running multiple matrices can mix and match blinded extraction controls to ensure even use of control sets. A minimum of one control for each matrix must be included in each run.

The VQA sensitivity control will be the 5 copy control until a new sensitivity control is established.

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 4: Abbott HIV Qual – 96 samples

Template 4: VQA HIV Nucleic Acid Control Requirements -Abbott HIV QUAL assay - 96 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	1	1	VQA extraction Control #1 (blinded) in matrix #1
4	2	2	1
5	3	3	2
6	4	4	3
7	5	5	4
8	6	6	5
9	7	7	6
10	8	8	7
11	9	9	8
12	10	10	9
13	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
14	11	11	10
15	12	12	11
16	13	13	12
17	14	14	13
18	15	15	14
19	16	16	15
20	17	17	16
21	18	18	17
22	19	19	18
23	20	20	19
24	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3
25	21	21	20
26	22	22	21
27	23	23	22
28	24	24	23
29	25	25	24
30	26	26	25
31	27	27	26
32	28	28	27
33	29	29	28
34	30	30	29
35	VQA extraction Control #3 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #3 (blinded) in matrix #1	VQA extraction Control #4 (blinded) in matrix #1
36	31	31	30
37	32	32	31
38	33	33	32
39	34	34	33
40	35	35	34
41	36	36	35
42	37	37	36
43	38	38	37
44	39	39	38
45	40	40	39
46	VQA extraction Control #4 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #4 (blinded) in matrix #2	VQA extraction Control #5 (blinded) in matrix #2
47	41	41	40
48	42	42	41
49	43	43	42
50	44	44	43
51	45	45	44
52	46	46	45
53	47	47	46
54	48	48	47
55	49	49	48
56	50	50	49
57	VQA extraction Control #5 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #5 (blinded) in matrix #1	VQA extraction Control #6 (blinded) in matrix #3
58	51	51	50
59	52	52	51
60	53	53	52
61	54	54	53
62	55	55	54
63	56	56	55
64	57	57	56
65	58	58	57
66	59	59	58
67	60	60	59
68	VQA extraction Control #6 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #6 (blinded) in matrix #2	VQA extraction Control #7 (blinded) in matrix #1, 2, or 3
69	61	61	60
70	62	62	61
71	63	63	62
72	64	64	63
73	65	65	64
74	66	66	65
75	67	67	66
76	68	68	67
77	69	69	68
78	70	70	69
79	VQA extraction Control #7 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #7 (blinded) in matrix #1	VQA extraction Control #8 (blinded) in matrix #2
80	71	71	70
81	72	72	71
82	73	73	72
83	74	74	73
84	75	75	74
85	76	76	75
86	77	77	76
87	78	78	77
88	79	79	78
89	80	80	79
90	VQA extraction Control #8 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #8 (blinded) in matrix #2	VQA extraction Control #9 (blinded) in matrix #3
91	81	81	80
92	82	82	81
93	83	83	82
94	84	84	83
95	85	85	84
96	86	86	85

Labs running multiple matrices can mix and match blinded extraction controls to ensure even use of control sets. A minimum of one control for each matrix must be included in each run.

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 5: Roche TaqMan Qual – 24 samples

Template 5: VQA HIV Nucleic Acid Control Requirements - Roche TaqMan HIV QUAL assay - 24 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	1	1	VQA extraction Control #1 (blinded) in matrix #1
4	2	2	1
5	3	3	2
6	4	4	3
7	5	5	4
8	6	6	5
9	7	7	6
10	8	8	7
11	9	9	8
12	10	10	9
13	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
14	11	11	10
15	12	12	11
16	13	13	12
17	14	14	13
18	15	15	14
19	16	16	15
20	17	17	16
21	18	18	17
22	19	19	18
23	20	20	19
24	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 6: Roche TaqMan Qual – 48 samples

Template 6: VQA HIV Nucleic Acid Control Requirements - Roche TaqMan HIV QUAL assay - 48 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	1	1	VQA extraction Control #1 (blinded) in matrix #1
4	2	2	1
5	3	3	2
6	4	4	3
7	5	5	4
8	6	6	5
9	7	7	6
10	8	8	7
11	9	9	8
12	10	10	9
13	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
14	11	11	10
15	12	12	11
16	13	13	12
17	14	14	13
18	15	15	14
19	16	16	15
20	17	17	16
21	18	18	17
22	19	19	18
23	20	20	19
24	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3
25	kit control - negative	kit control - negative	kit control - negative
26	kit control - low positive	kit control - low positive	kit control - low positive
27	21	21	20
28	22	22	21
29	23	23	22
30	24	24	23
31	25	25	24
32	26	26	25
33	27	27	26
34	28	28	27
35	29	29	28
36	30	30	29
37	VQA extraction Control #3 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #3 (blinded) in matrix #1	VQA extraction Control #4 (blinded) in matrix #1, 2 or 3
38	31	31	30
39	32	32	31
40	33	33	32
41	34	34	33
42	35	35	34
43	36	36	35
44	37	37	36
45	38	38	37
46	39	39	38
47	40	40	39
48	VQA extraction Control #4 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #4 (blinded) in matrix #2	VQA extraction Control #5 (blinded) in matrix #1, 2, or 3

Labs running multiple matrices can mix and match blinded extraction controls to ensure even use of control sets. A minimum of one control for each matrix must be included in each run.

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 7: Abbott HIV Quant – 24 samples

Template 7: VQA HIV Nucleic Acid Control Requirements - Abbott HIV QUANTITATIVE assay - 24 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	kit control - high positive	kit control - high positive	kit control - high positive
4	1	1	VQA extraction Control #1 (blinded) in matrix #1
5	2	2	1
6	3	3	2
7	4	4	3
8	5	5	4
9	6	6	5
10	7	7	6
11	8	8	7
12	9	9	8
13	10	10	9
14	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
15	11	11	10
16	12	12	11
17	13	13	12
18	14	14	13
19	15	15	14
20	16	16	15
21	17	17	16
22	18	18	17
23	19	19	18
24	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 8: Abbott HIV Quant – 48 samples

Template 8: VQA HIV Nucleic Acid Control Requirements - Abbott HIV QUANTITATIVE assay - 48 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	kit control - high positive	kit control - high positive	kit control - high positive
4	1	1	VQA extraction Control #1 (blinded) in matrix #1
5	2	2	1
6	3	3	2
7	4	4	3
8	5	5	4
9	6	6	5
10	7	7	6
11	8	8	7
12	9	9	8
13	10	10	9
14	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
15	11	11	10
16	12	12	11
17	13	13	12
18	14	14	13
19	15	15	14
20	16	16	15
21	17	17	16
22	18	18	17
23	19	19	18
24	20	20	19
25	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3
26	21	21	20
27	22	22	21
28	23	23	22
29	24	24	23
30	25	25	24
31	26	26	25
32	27	27	26
33	28	28	27
34	29	29	28
35	30	30	29
36	VQA extraction Control #3 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #3 (blinded) in matrix #1	VQA extraction Control #4 (blinded) in matrix #1, 2 or 3
37	31	31	30
38	32	32	31
39	33	33	32
40	34	34	33
41	35	35	34
42	36	36	35
43	37	37	36
44	38	38	37
45	39	39	38
46	40	40	39
47	VQA extraction Control #4 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #4 (blinded) in matrix #2	VQA extraction Control #5 (blinded) in matrix #1, 2, or 3
48	41	41	40

Labs running multiple matrices can mix and match blinded extraction controls to ensure even use of control sets. A minimum of one control for each matrix must be included in each run.

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 9: Abbott HIV Quant – 72 samples

Template 9: VQA HIV Nucleic Acid Control Requirements -Abbott HIV QUANTITATIVE assay - 72 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	kit control - high positive	kit control - high positive	kit control - high positive
4	1	1	VQA extraction Control #1 (blinded) in matrix #1
5	2	2	1
6	3	3	2
7	4	4	3
8	5	5	4
9	6	6	5
10	7	7	6
11	8	8	7
12	9	9	8
13	10	10	9
14	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
15	11	11	10
16	12	12	11
17	13	13	12
18	14	14	13
19	15	15	14
20	16	16	15
21	17	17	16
22	18	18	17
23	19	19	18
24	20	20	19
25	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3
26	21	21	20
27	22	22	21
28	23	23	22
29	24	24	23
30	25	25	24
31	26	26	25
32	27	27	26
33	28	28	27
34	29	29	28
35	30	30	29
36	VQA extraction Control #3 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #3 (blinded) in matrix #1	VQA extraction Control #4 (blinded) in matrix #1
37	31	31	30
38	32	32	31
39	33	33	32
40	34	34	33
41	35	35	34
42	36	36	35
43	37	37	36
44	38	38	37
45	39	39	38
46	40	40	39
47	VQA extraction Control #4 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #4 (blinded) in matrix #2	VQA extraction Control #5 (blinded) in matrix #2
48	41	41	40
49	42	42	41
50	43	43	42
51	44	44	43
52	45	45	44
53	46	46	45
54	47	47	46
55	48	48	47
56	49	49	48
57	50	50	49
58	VQA extraction Control #5 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #5 (blinded) in matrix #1	VQA extraction Control #6 (blinded) in matrix #3
59	51	51	50
60	52	52	51
61	53	53	52
62	54	54	53
63	55	55	54
64	56	56	55
65	57	57	56
66	58	58	57
67	59	59	58
68	60	60	59
69	VQA extraction Control #6 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #6 (blinded) in matrix #2	VQA extraction Control #7 (blinded) in matrix #1, 2, or 3
70	61	61	60
71	62	62	61
72	63	63	62

Labs running multiple matrices can mix and match blinded extraction controls to ensure even use of control sets. A minimum of one control for each matrix must be included in each run.

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 10: Abbott HIV Quant – 96 samples

Template 10: VQA HIV Nucleic Acid Control Requirements -Abbott HIV QUANTITATIVE assay - 96 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	kit control - high positive	kit control - high positive	kit control - high positive
4	1	1	VQA extraction Control #1 (blinded) in matrix #1
5	2	2	1
6	3	3	2
7	4	4	3
8	5	5	4
9	6	6	5
10	7	7	6
11	8	8	7
12	9	9	8
13	10	10	9
14	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
15	11	11	10
16	12	12	11
17	13	13	12
18	14	14	13
19	15	15	14
20	16	16	15
21	17	17	16
22	18	18	17
23	19	19	18
24	20	20	19
25	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3
26	21	21	20
27	22	22	21
28	23	23	22
29	24	24	23
30	25	25	24
31	26	26	25
32	27	27	26
33	28	28	27
34	29	29	28
35	30	30	29
36	VQA extraction Control #3 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #3 (blinded) in matrix #1	VQA extraction Control #4 (blinded) in matrix #1
37	31	31	30
38	32	32	31
39	33	33	32
40	34	34	33
41	35	35	34
42	36	36	35
43	37	37	36
44	38	38	37
45	39	39	38
46	40	40	39
47	VQA extraction Control #4 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #4 (blinded) in matrix #2	VQA extraction Control #5 (blinded) in matrix #2
48	41	41	40
49	42	42	41
50	43	43	42
51	44	44	43
52	45	45	44
53	46	46	45
54	47	47	46
55	48	48	47
56	49	49	48
57	50	50	49
58	VQA extraction Control #5 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #5 (blinded) in matrix #1	VQA extraction Control #6 (blinded) in matrix #3
59	51	51	50
60	52	52	51
61	53	53	52
62	54	54	53
63	55	55	54
64	56	56	55
65	57	57	56
66	58	58	57
67	59	59	58
68	60	60	59
69	VQA extraction Control #6 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #6 (blinded) in matrix #2	VQA extraction Control #7 (blinded) in matrix #1, 2, or 3
70	61	61	60
71	62	62	61
72	63	63	62
73	64	64	63
74	65	65	64
75	66	66	65
76	67	67	66
77	68	68	67
78	69	69	68
79	70	70	69
80	VQA extraction Control #7 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #7 (blinded) in matrix #1	VQA extraction Control #8 (blinded) in matrix #2
81	71	71	70
82	72	72	71
83	73	73	72
84	74	74	73
85	75	75	74
86	76	76	75
87	77	77	76
88	78	78	77
89	79	79	78
90	80	80	79
91	VQA extraction Control #8 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #8 (blinded) in matrix #2	VQA extraction Control #9 (blinded) in matrix #3
92	81	81	80
93	82	82	81
94	83	83	82
95	84	84	83
96	85	85	84

Labs running multiple matrices can mix and match blinded extraction controls to ensure even use of control sets. A minimum of one control for each matrix must be included in each run.

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 11: Roche TaqMan Quant – 24 samples

Template 11: VQA HIV Nucleic Acid Control Requirements - Roche TaqMan HIV QUANTITATIVE assay - 24 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	kit control - high positive	kit control - high positive	kit control - high positive
4	1	1	VQA extraction Control #1 (blinded) in matrix #1
5	2	2	1
6	3	3	2
7	4	4	3
8	5	5	4
9	6	6	5
10	7	7	6
11	8	8	7
12	9	9	8
13	10	10	9
14	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
15	11	11	10
16	12	12	11
17	13	13	12
18	14	14	13
19	15	15	14
20	16	16	15
21	17	17	16
22	18	18	17
23	19	19	18
24	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.

Template 12: Roche TaqMan Quant – 48 samples

Template 12: VQA HIV Nucleic Acid Control Requirements - Roche TaqMan HIV QUANTITATIVE assay - 48 Sample Template

n=	One Matrix (e.g. PEL, FWB or DBS)	Two Matrices (e.g. PEL/DBS, FWB/DBS or PEL/FWB)	Three Matrices (e.g. PEL, DBS, and FWB)
1	kit control - negative	kit control - negative	kit control - negative
2	kit control - low positive	kit control - low positive	kit control - low positive
3	kit control - high positive	kit control - high positive	kit control - high positive
4	1	1	VQA extraction Control #1 (blinded) in matrix #1
5	2	2	1
6	3	3	2
7	4	4	3
8	5	5	4
9	6	6	5
10	7	7	6
11	8	8	7
12	9	9	8
13	10	10	9
14	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
15	11	11	10
16	12	12	11
17	13	13	12
18	14	14	13
19	15	15	14
20	16	16	15
21	17	17	16
22	18	18	17
23	19	19	18
24	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3
25	kit control - negative	kit control - negative	kit control - negative
26	kit control - low positive	kit control - low positive	kit control - low positive
27	kit control - high positive	kit control - high positive	kit control - high positive
28	20	20	VQA extraction Control #1 (blinded) in matrix #1
29	21	21	19
30	22	22	20
31	23	23	21
32	24	24	22
33	25	25	23
34	26	26	24
35	27	27	25
36	28	28	26
37	29	29	27
38	VQA extraction Control #1 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #1 (blinded) in matrix #1	VQA extraction Control #2 (blinded) in matrix #2
39	30	30	28
40	31	31	29
41	32	32	30
42	33	33	31
43	34	34	32
44	35	35	33
45	36	36	34
46	37	37	35
47	38	38	36
48	VQA extraction Control #2 (blinded) in the same matrix used for clinical sample testing)	VQA extraction Control #2 (blinded) in matrix #2	VQA extraction Control #3 (blinded) in matrix #3

Labs running multiple matrices can mix and match blinded extraction controls to ensure even use of control sets. A minimum of one control for each matrix must be included in each run.

All controls must be valid for the samples within the run to be valid. If the control of one matrix fails in a run where multiple matrices are included, only the unknown samples of the same matrix as the failed control will be deemed invalid; the samples consisting of the second or third matrix included in the same run would still be valid as long as those matrix-specific controls were valid.