Evaluation of run controls for cobas 6800 MPX and HEV assay

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Satellite Meeting before IPFA-PEI 25th Workshop on Surveillance and Screening of Blood Borne Pathogens, Tuesday, May 15th 2018, at the Royal Olympic Hotel in Athens

NSS: National Screening laboratory of Sanquin

- Sanquin is the Dutch organization responsible for the blood supply in the Netherlands
- ~340,000 donors (voluntary, non-renumerated donors)
- Annually ~720,000 donations (60% whole blood, 40% plasmaphereses)
- Since 2008 blood screening is centralized at NSS in Amsterdam
  - Blood grouping (AB0, rhD and many others)
  - Serology (HBV, HCV, HIV, HTLV, syphilis)
  - NAT (HBV, HCV, HIV, HEV)

NAT reactives in 2017 (absolute numbers)

- HBV DNA 9
- HCV RNA 1
- HIV RNA 3
- HEV RNA 104 (screening started July 3, 2017)

- NAT only's for HBV, HCV and HIV 0

Nucleic Acid Testing laboratory

Cobas 6800 kit controls

- cobas MPX test

Multi target control

- synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein
- synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein
- synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein

HIV-1 Group O

- synthetic (armored) HIV-1 Group O RNA encapsulated in MS2 bacteriophage coat protein

HIV-2

- synthetic (armored) HIV-2 RNA encapsulated in MS2 bacteriophage coat protein

Negative control

- negative control: normal human plasma

- cobas HEV test

HEV

- synthetic (armored) HEV RNA encapsulated in MS2 bacteriophage coat protein

Negative control

- negative control: normal human plasma

External quality controls (run controls)

- Specifications
  - Should contain the real viruses (life or inactivated)
  - Have relative low concentrations (challenging for the system)
  - Should be stable when stored below -20°C or -70°C
  - Minimal variation between different lot numbers
  - Calibrated in IU/mL

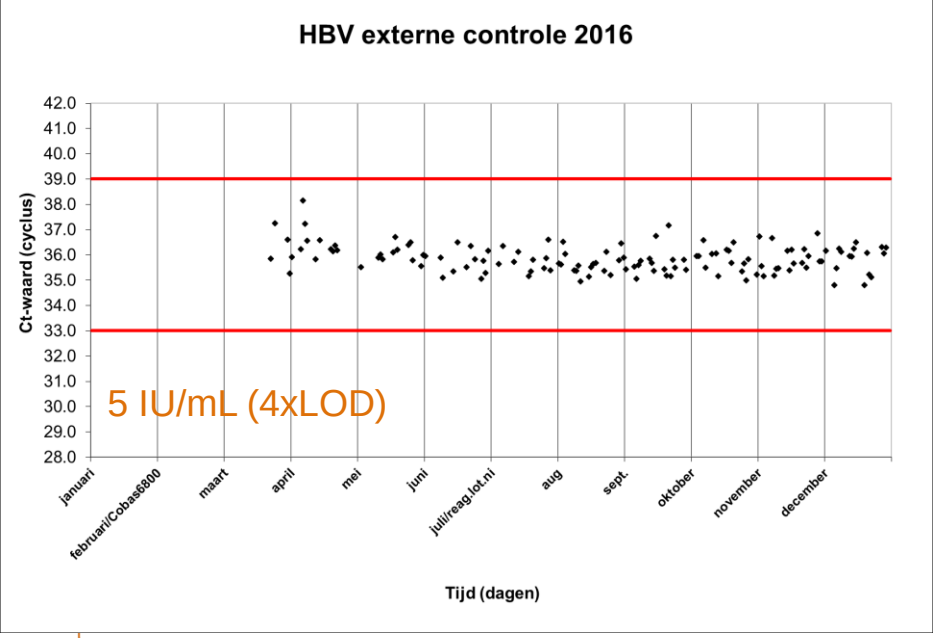
- To monitor performance of the cobas 6800 robot
  - Over time
  - Detect mechanical issues in an early stage

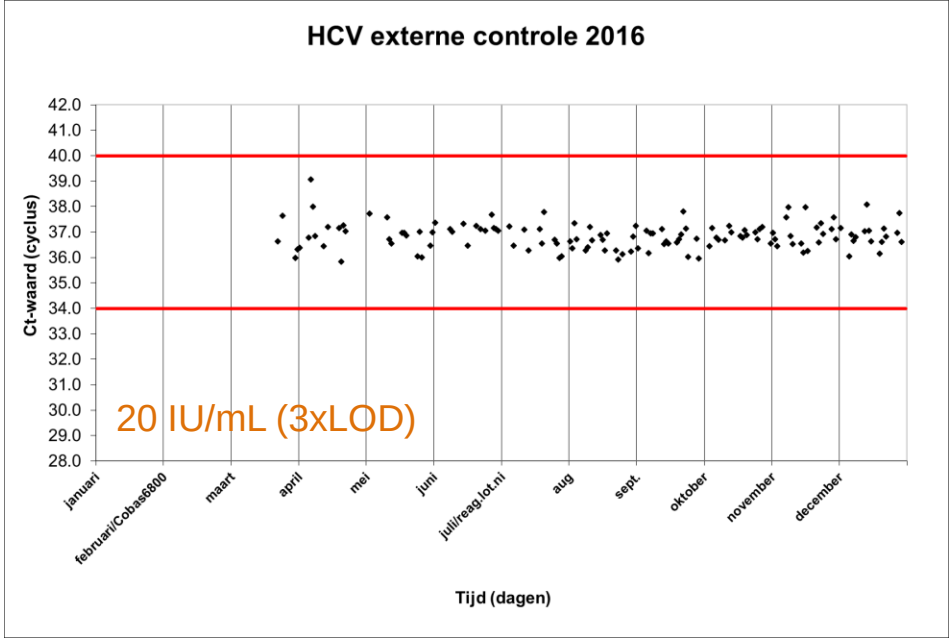
- To monitor performance of the assay
  - Over time
  - different batches of reagents

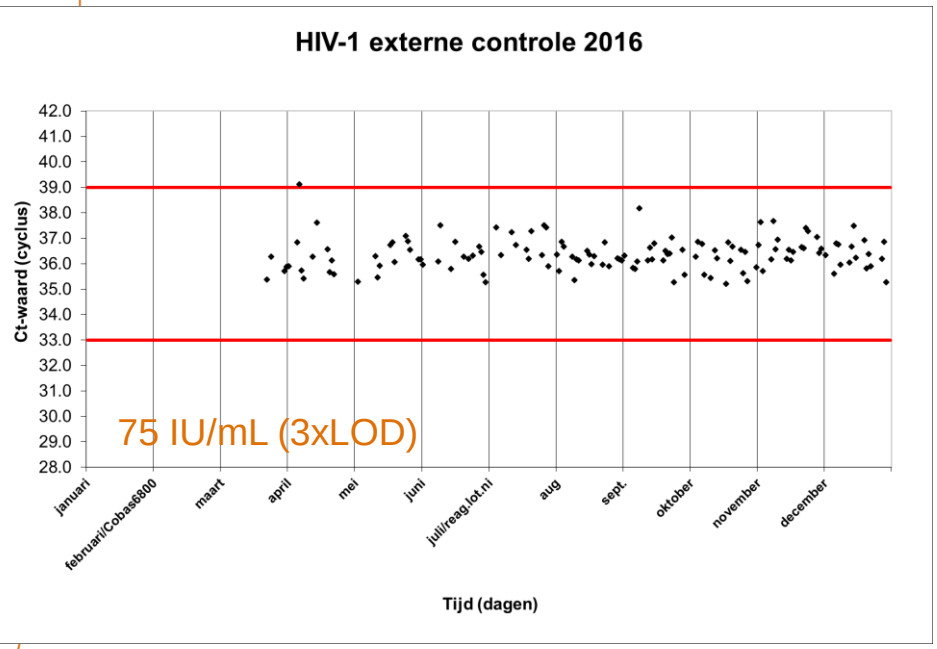
- To sleep well
  - Especially when the number numbers of reactive samples is low

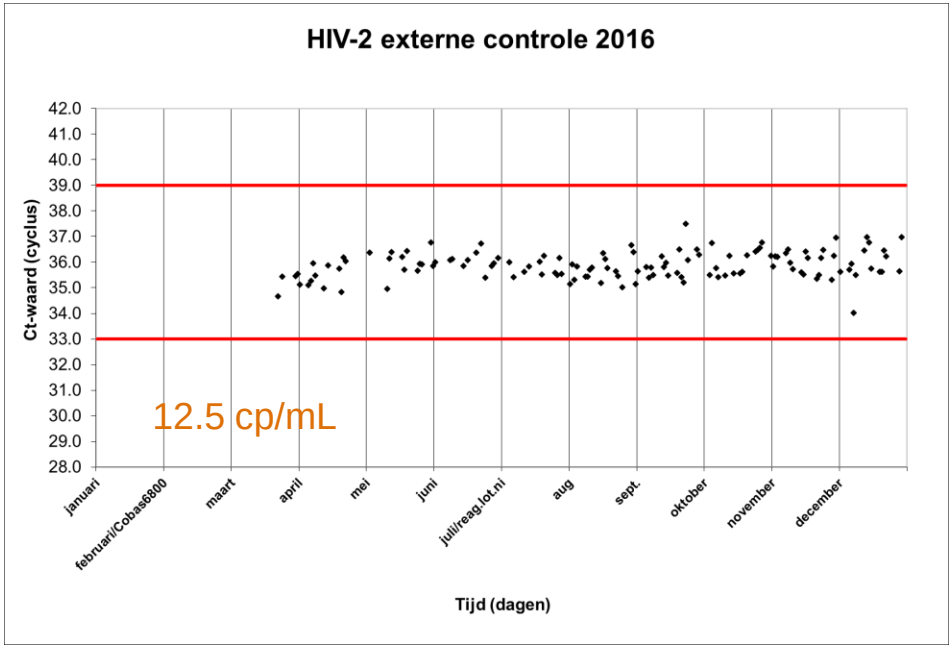
The use of run controls at Sanquin

ThermoFisher MPX controls

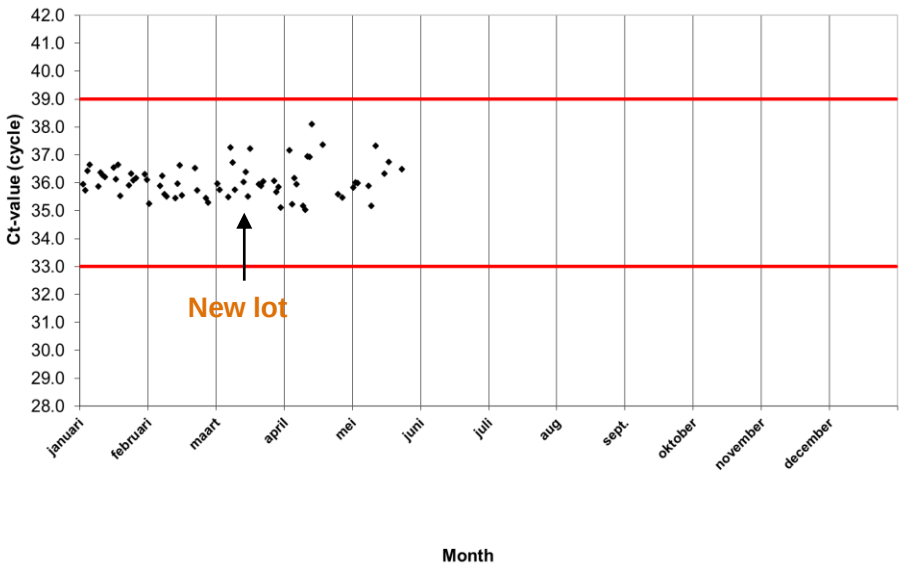




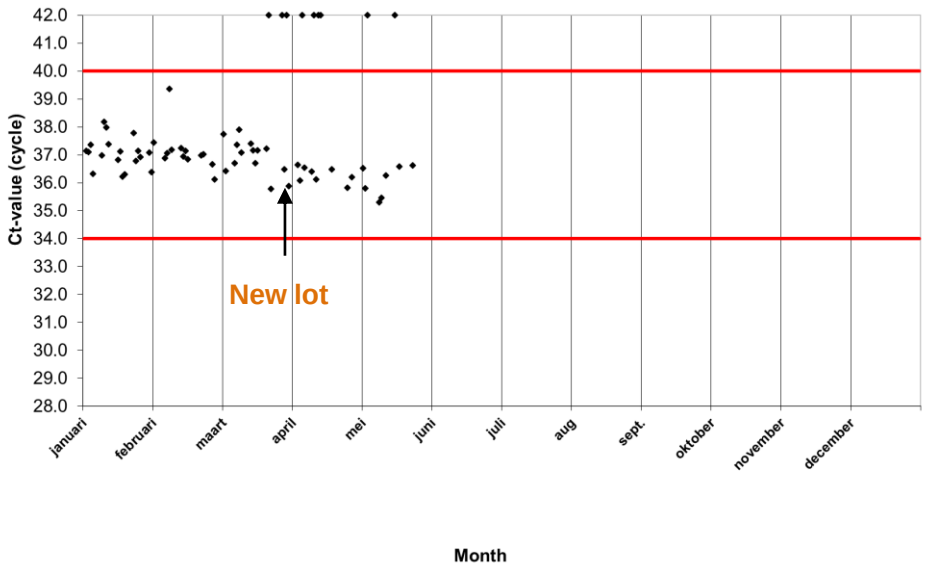




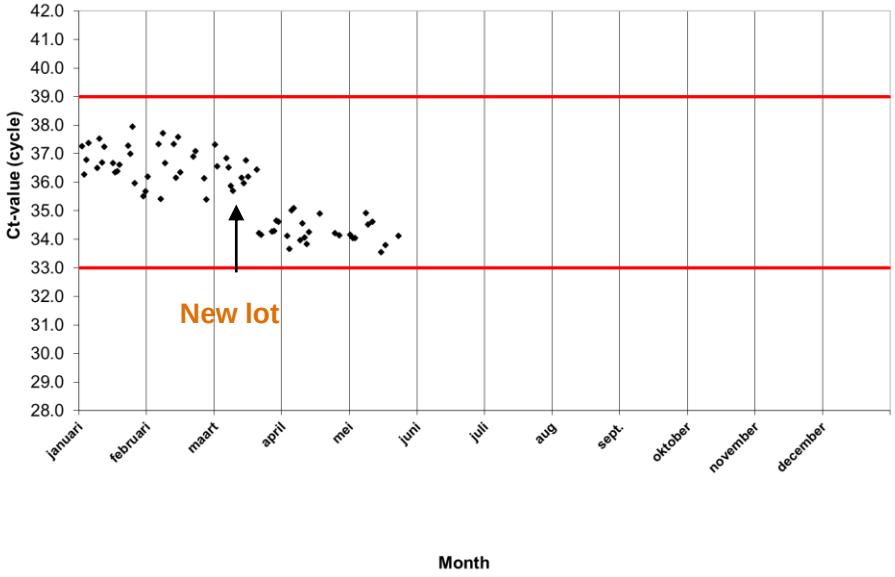
5 IU/mL



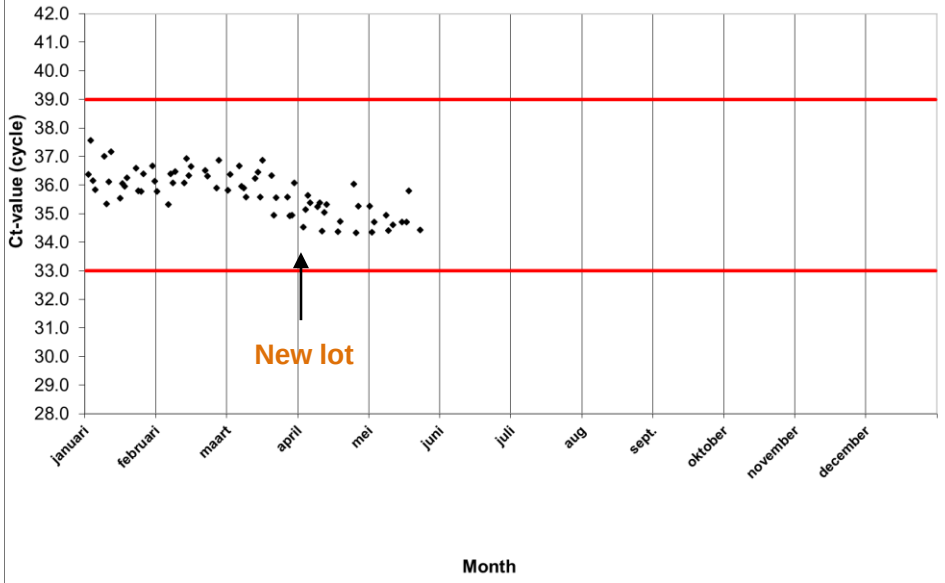
20 IU/mL



75 IU/mL



12.5 cp/mL



Evaluation of two run controls (BioQControl)

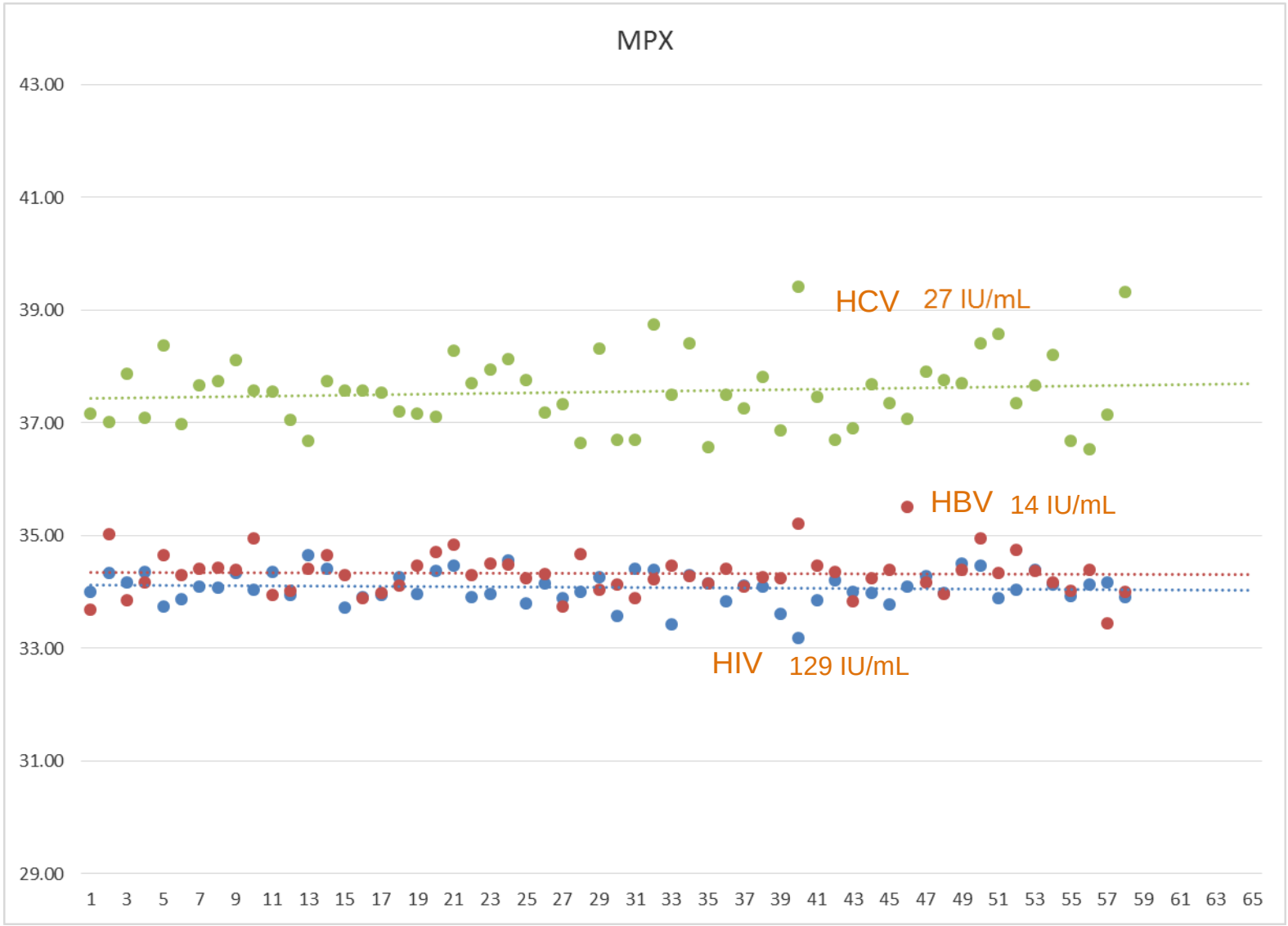
ViraQ Multi-Marker Check 75

- mixture of inactivated HBV, HCV and HIV-1 plasma standards in plasma
- contains 75 copies /mL HBV DNA (gt A), HCV RNA (gt 3a) and HIV RNA (subtype B)
- calibrated against secondary WHO standards: 14 IU/mL HBV, 27 IU/mL HCV, 129 IU/mL HIV
- approx. 5-10 x LOD cobas 6800 MPX test

ViraQ HEV Check 125

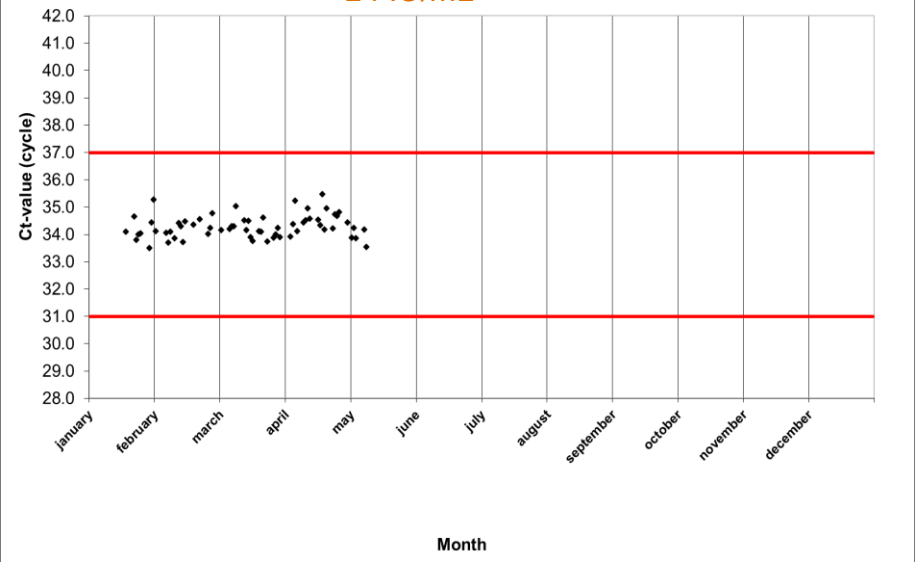
- plasma from HEV RNA (gt 3) positive blood donation
- calibrated against secondary WHO standards: 100 IU/mL
- approx. 5x LOD cobas 6800 HEV test

2017: Validation period testing ViraQ Multi-Marker Check 75



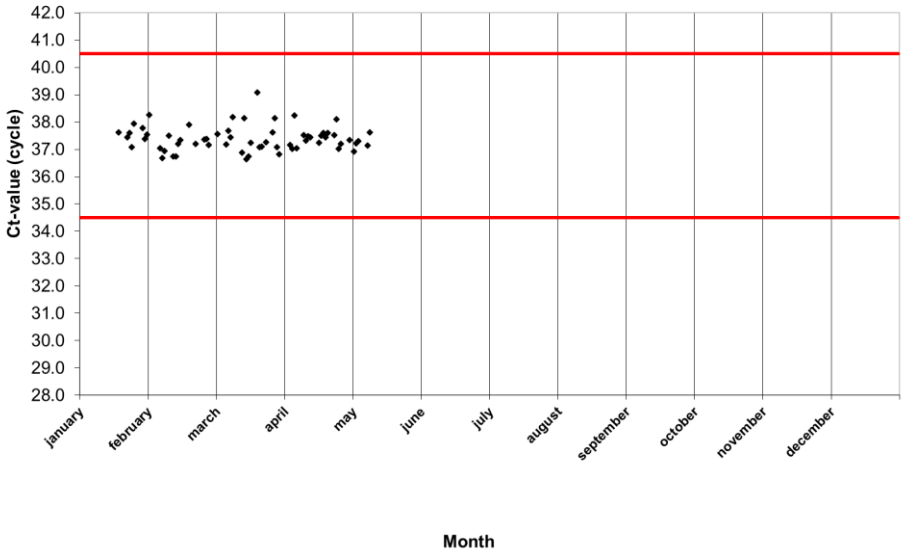
HBV run control 2018

14 IU/mL



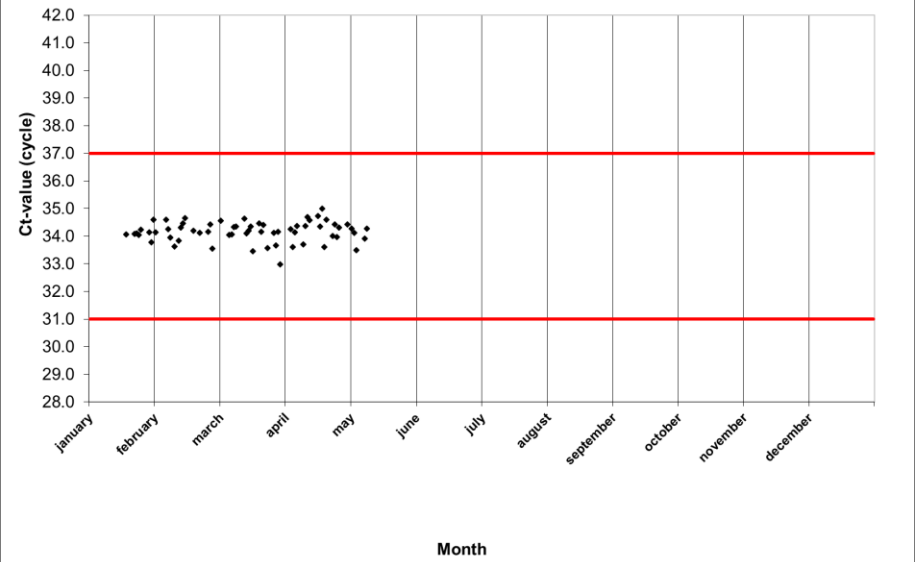
HCV run control 2018

27 IU/mL



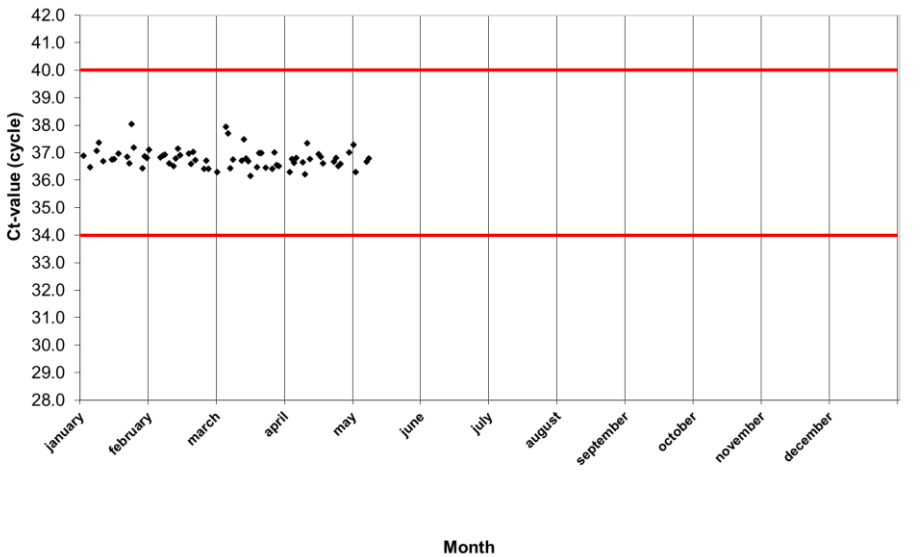
HIV-1 run control 2018

129 IU/mL

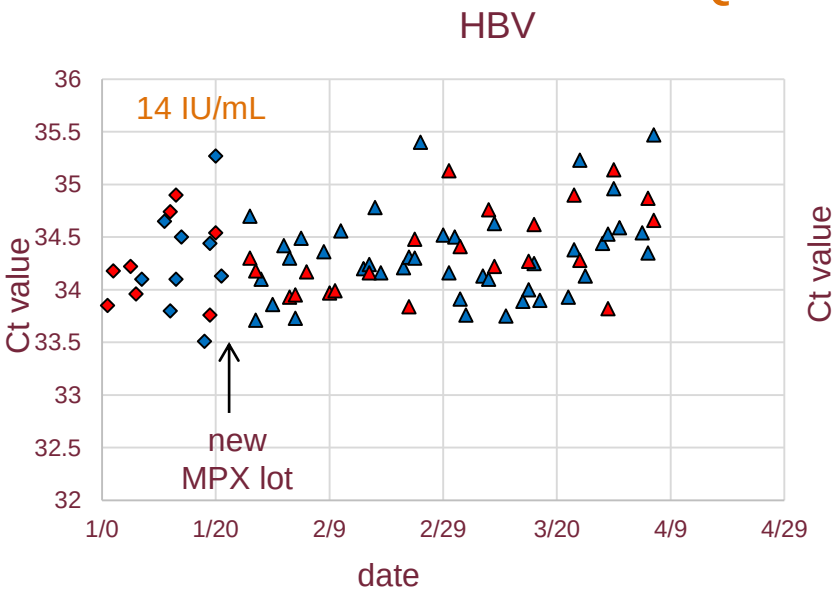


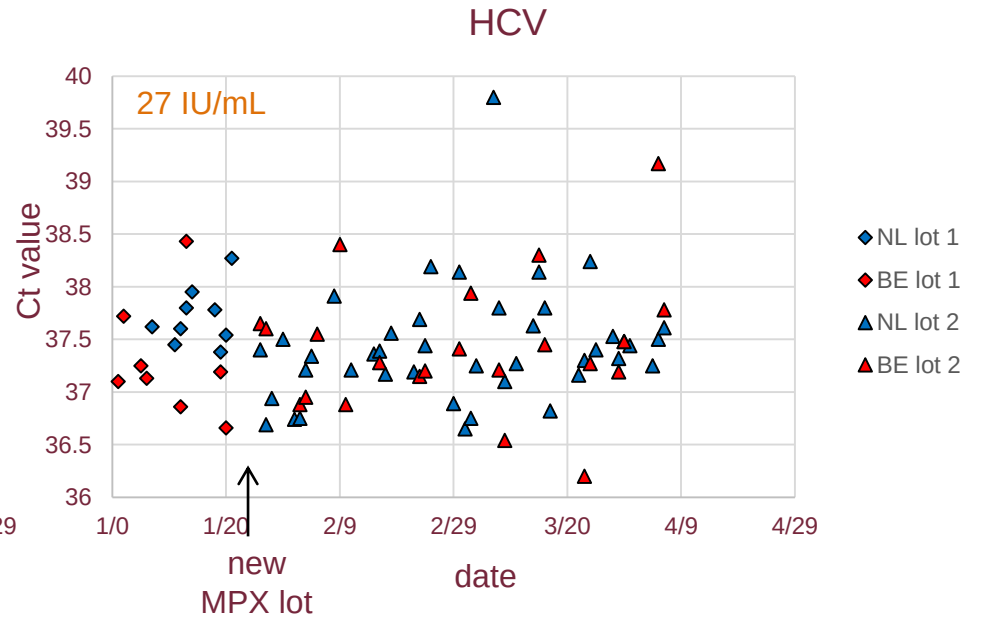
HEV run control 2018

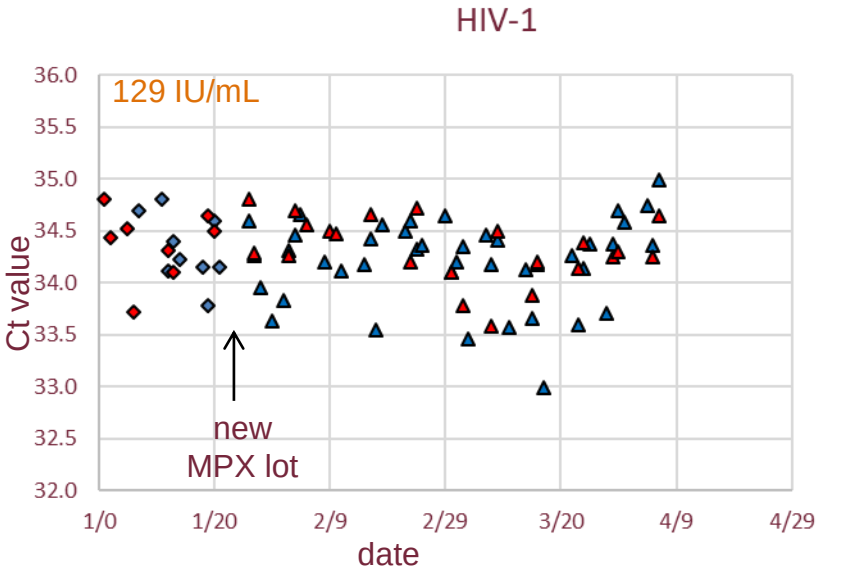
100 IU/mL



Comparable Dutch and Belgian# data on two reagent lots of cobas MPX on ViraQ Multi-marker Check 75 Control







marker	MPX lot	n NL	n BE	Avg (Std) NL	Avg (Std) BE
HBV	1	9	8	34.28 (0.51)	34.27 (0.42)
	2	42	22	34.33 (0.42)	34.33 (0.31)
HCV	1	9	8	37.71 (0.28)	37.29 (0.55)
	2	42	22	37.43 (0.55)	37.43 (0.64)
HIV	1	9	8	34.32 (0.33)	34.38 (0.34)
	2	42	22	34.21 (0.40)	34.33 (0.31)

data Flemish Red Cross Blood Center, Mechelen, Belgium

Conclusions

- Since 2010 Sanquin has used run controls for the S201 system and cobas 6800 system
  - always very stable results indicating stable test systems and stable run controls
  - problem with a new lot of external controls from ThermoFisher in March 2017

- After 4 months of testing on a daily basis, ViraQ Multi-Marker check 75 and ViraQ HEV check 125 show stable test results (one lot of external controls and two lots MPX/HEV reagents) on the cobas 6800 system

- Comparable Dutch (NSS) and Belgian (Flemish Red Cross Blood Center) results on two reagent lots of cobas MPX on ViraQ Multi-marker Check 75 Control

Acknowledgements

Colleagues from the National Screening Lab Sanquin & Virus Diagnostic Services and colleagues from the Flemish Red Cross Blood Center, Mechelen, Belgium

Analytical sensitivity of Roche PCR assay versions on HBV-DNA standards

HBV genotype A standard	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
S0011 VQC-Sanquin	PeliCheck	Multiprep Ampliscr.	24	5.9 (3.9-9.3)	72.4 (37.3-211.4)
WHO 97/746#	Sanquin	Multiprep Ampliscr.	24	3.7 (2.7-5.3)	27.7 (17.6-53.3)
S0011 VQC-Sanquin	PeliCheck	Ampliprep Ampliscr.	24	8.6 (5.9-12.9)	88.2 (48.7-215.7)
WHO 97/746#	Sanquin	Ampliprep Ampliscr.	24	5.3 (3.7-6.9)	35.7 (22.9-69.3)
S0011 VQC-Sanquin	PeliCheck	TaqScreen 1.0	12	2.9 (1.6-4.9)	26.9 (13.9-71.0)
WHO 97/746#	EFS	TaqScreen 1.0	24	3.7 (2.8-5.0)	22.0 (15.5-34.6)
S0010 Eurohep	P0001	TaqScreen 1.0	12	2.3 (1.3-3.8)	14.1 (7.2-56.6)
S0043 BioQ inact.	P0251	TaqScreen 2.0	12	2.8 (1.5-4.3)	23.8 (12.4-99.3)
S0043 BioQ inact.	P0031	cobas MPX	12	2.4 (1.4-4.2)	18.6 (9.1-75.9)
WHO 97/750#	P0023	cobas MPX	12	1.8 (0.93-2.8)	8.0 (4.4-37.4)
S0011 VQC-Sanquin	P0007	cobas MPX	24	1.9 (1.3-2.7)	13.0 (7.7-29.6)

1 IU = 5.33 copies

Analytical sensitivity of Roche PCR assay versions on HCV-RNA standards

HCV genotype 1 standard	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
WHO 96/790#	Roche	Multiprep-Ampliscr.	24	19.9 (6.0-27.3)	79.2 (57.3-234)
S0009 VQC-Sanquin	PeliCheck	Ampliprep Ampliscr.	24	16.5 (11.5-22.3)	90.6 (60.4-168)
WHO 96/790#	Sanquin	Ampliprep Ampliscr.	24	7.9 (4.6-15.0)	62.8 (30.0-289)
S0009 VQC-Sanquin	PeliCheck	TaqScreen 1.0	12	4.9 (3.2-7.5)	19.7 (12.0-43.2)
WHO 96/798#	EFS	TaqScreen 1.0	24	6.1 (4.9-8.2)	48.9 (13.4-83.8)
S0109 BioQ gt 3a inact.	P0020	TaqScreen 2.0	12	5.2 (3.3-7.8)	35.2 (19.3-114)
S0109 BioQ gt 3a inact.	P0020	cobas MPX	10	2.5 (1.3-4.4)	15.6 (7.6-77.7)
S0009 VQC-Sanquin	P0019	cobas MPX	60	2.9 (2.3-3.6)	20.4 (14.3-33.3)

1 IU = 2.73 copies

Analytical sensitivity of Roche PCR assay versions on HIV-1 RNA standards

HIV-1 subtype B standard	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
WHO 97/656#	Roche	Multiprep-Ampliscr.	24	8.6 (5.1-11.3)	30.4 (23.4-53.4)
S0012 VQC-Sanquin	PeliCheck	Ampliprep Ampliscr.	24	11.8 (8.4-17.2)	76.9 (45.4-182)
WHO 97/656#	Sanquin	Ampliprep Ampliscr.	24	7.0 (5.1-10.1)	72.9 (42.1-164.6)
S0012 VQC-Sanquin	PeliCheck	TaqScreen 1.0	12	4.2 (2.6-6.7)	22.8 (13.0-52.9)
WHO 97/650^	EFS	TaqScreen 1.0	24	4.5 (3.4-5.8)	27.6 (19.1-44.1)
S0041 BioQ inact.	P0251	TaqScreen 2.0	12	2.0 (1.3-2.8)	7.6 (4.9-21.4)
S0041 BioQ inact.	P0026	cobas MPX	12	1.0 (0.6-1.60)	5.8 (3.0-23.2)
WHO 97/650^	P0022	cobas MPX	12	2.7 (1.7-3.9)	5.8 (3.9-24.9)

1 IU = 0.39 copies
^ 1 IU = 0.58 copies

Analytical sensitivity of MPX and HEV test on cobas 6800

Target	95% LOD (IU/mL)	95% CI
HBV	1.4	1.2-1.7
HCV	7.0	5.9-8.6
HIV-1	25.7	21.1-32.8
HIV-2	4.0	3.3-5.2
HEV	18.6	15.9-22.6