Monitoring the analytical sensitivity of Procleix reagent batches using ViraQ HEV Check controls and Trend controls

‘25 years standardisation &
quality control of NAT’

Fiona Boland, Irish Blood Transfusion Service

Athens, May 15th 2018

Agenda for this session

1. Our experience using the ViraQ HEV Check 125

2. Use of ViraQ Trend controls (HIV-1, HCV & HBV)

Note: Use of BioQControl reference panels – appendix data

ViraQ HEV Check 125 EQC (125 cps/mL ≡ 100 IU/mL)

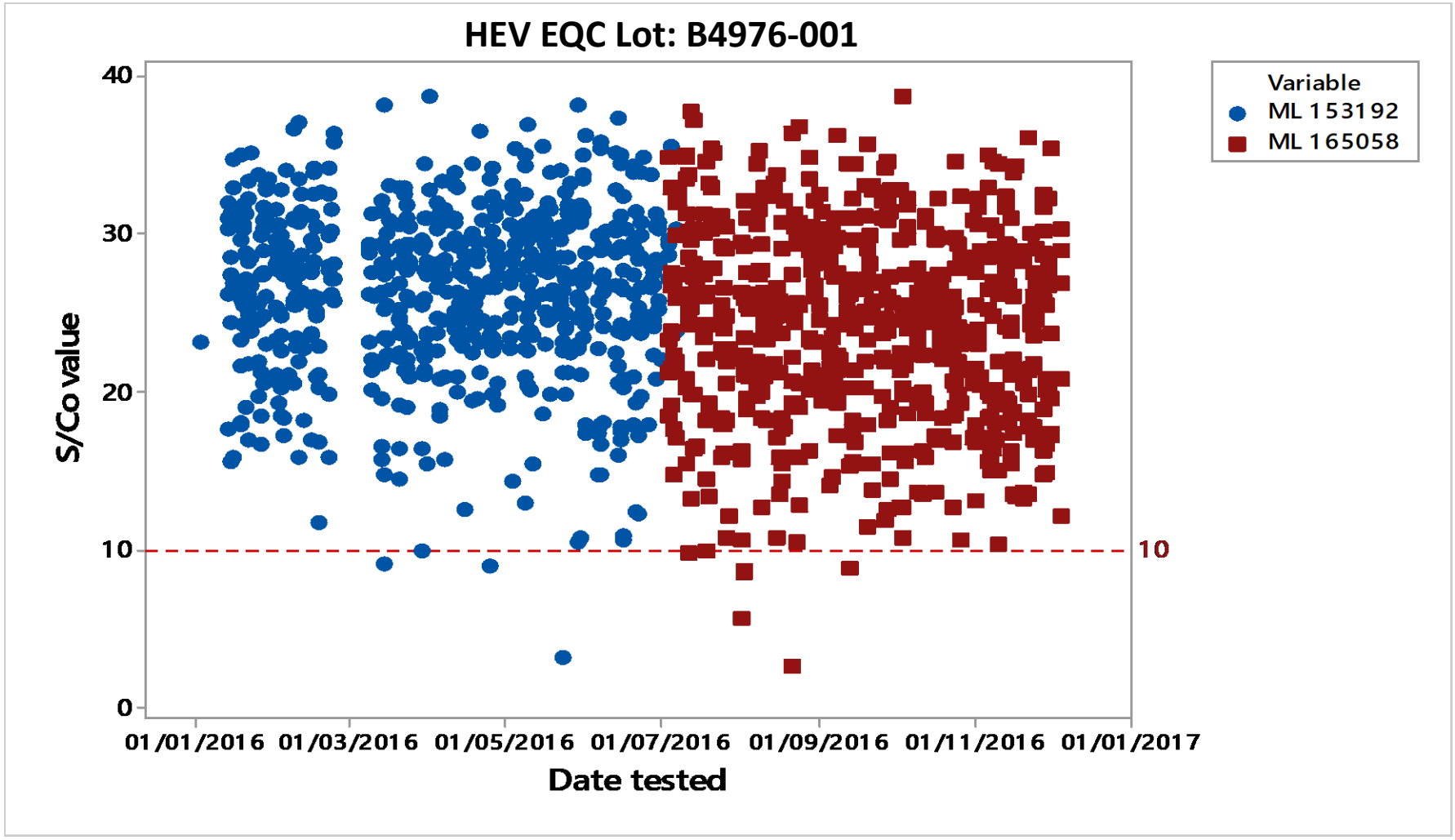
The story so far….

ViraQ HEV Check 125 EQC

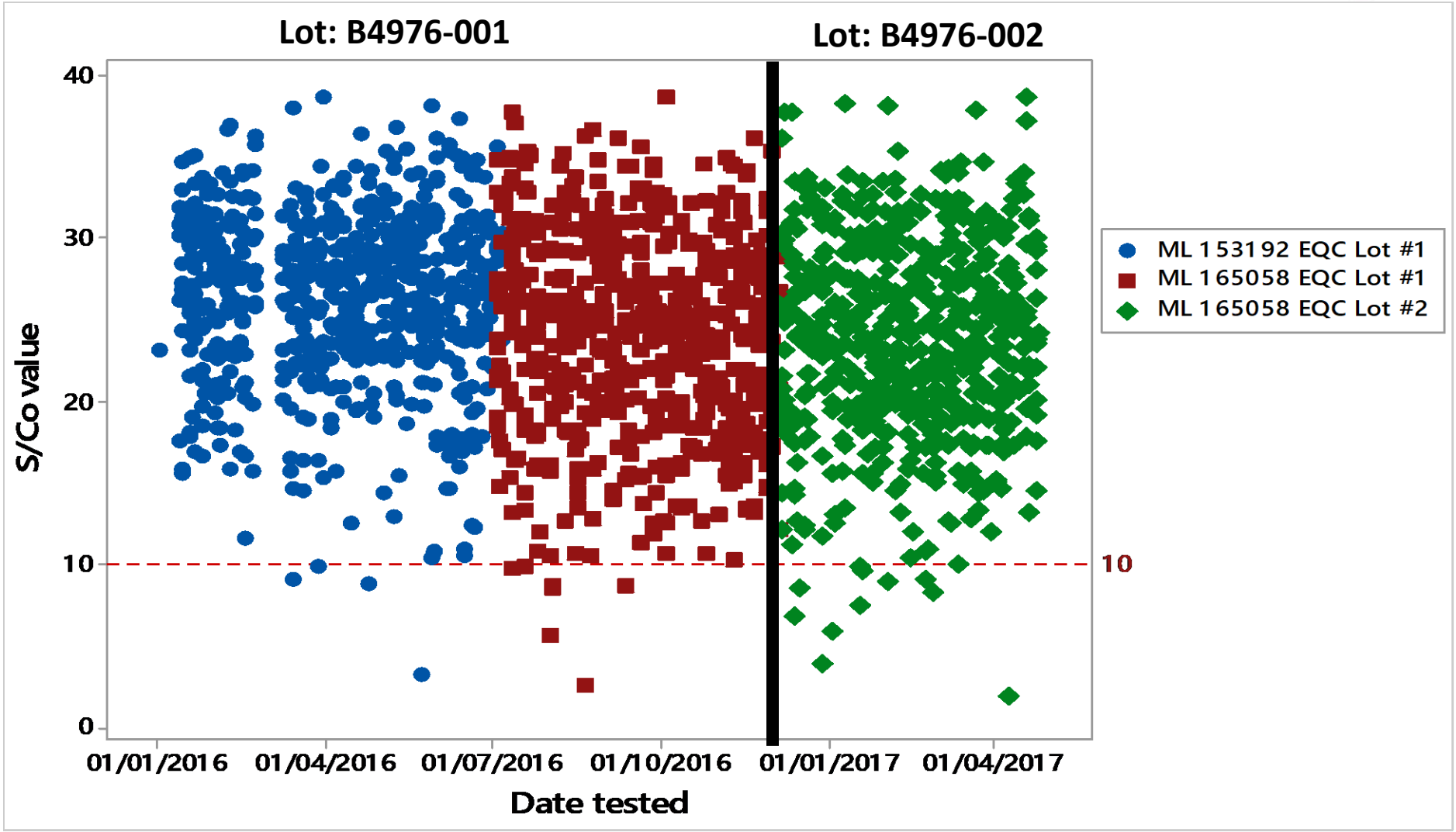
panel	Grifols	IBTS	BioQControl P0262
Lab	Grifols PI	IBTS	Grifols
IU/mL	proportion reactive on WHO standard 6329/10		
90	162/162 (100%)	74/74 (100%)	18/18 (100%)
30	162/162 (100%)	74/74 (100%)	18/18 (100%)
10	159/162 (98%)	73/74 (99%)	18/18 (100%)
3	109/162 (67%)	48/74 (65%)	9/18 (50%)
1	44/162 (27%)	22/74 (30%)	3/18 (17%)
50% LOD	2.0 (1.7-2.3)	1.8 (1.5-2.2)	2.5 (1.7-3.5)
95% LOD	7.9 (6.6-9.8)	8.2 (6.1-12.9)	8.6 (5.4-24.6)

- ID-NAT HEV screening from 1st January 2016
- Needed a HEV EQC: 100 IU/mL

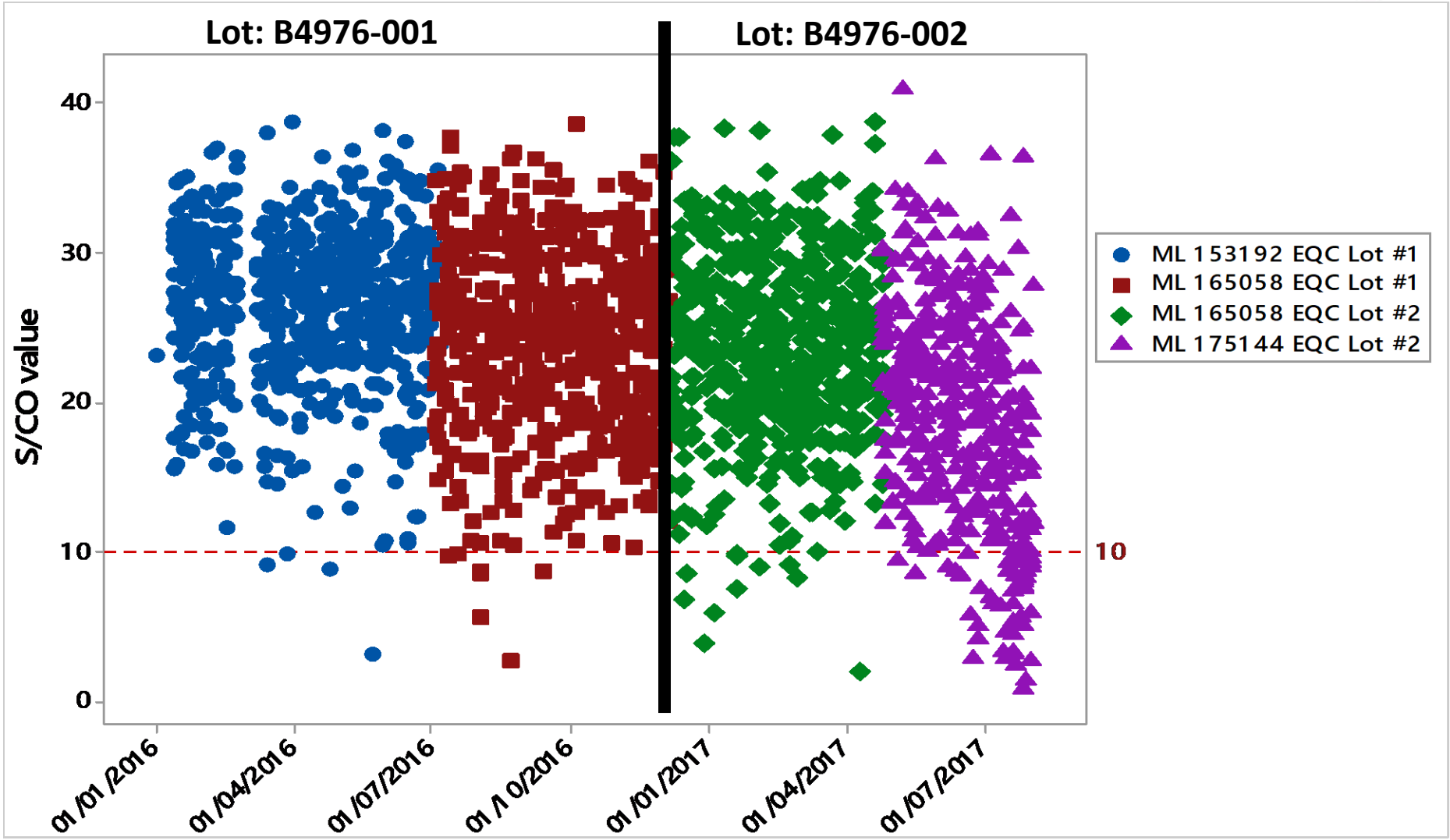
HEV EQC Lot # 01 performance by Master lot: Jan 2016 to Jan 2017



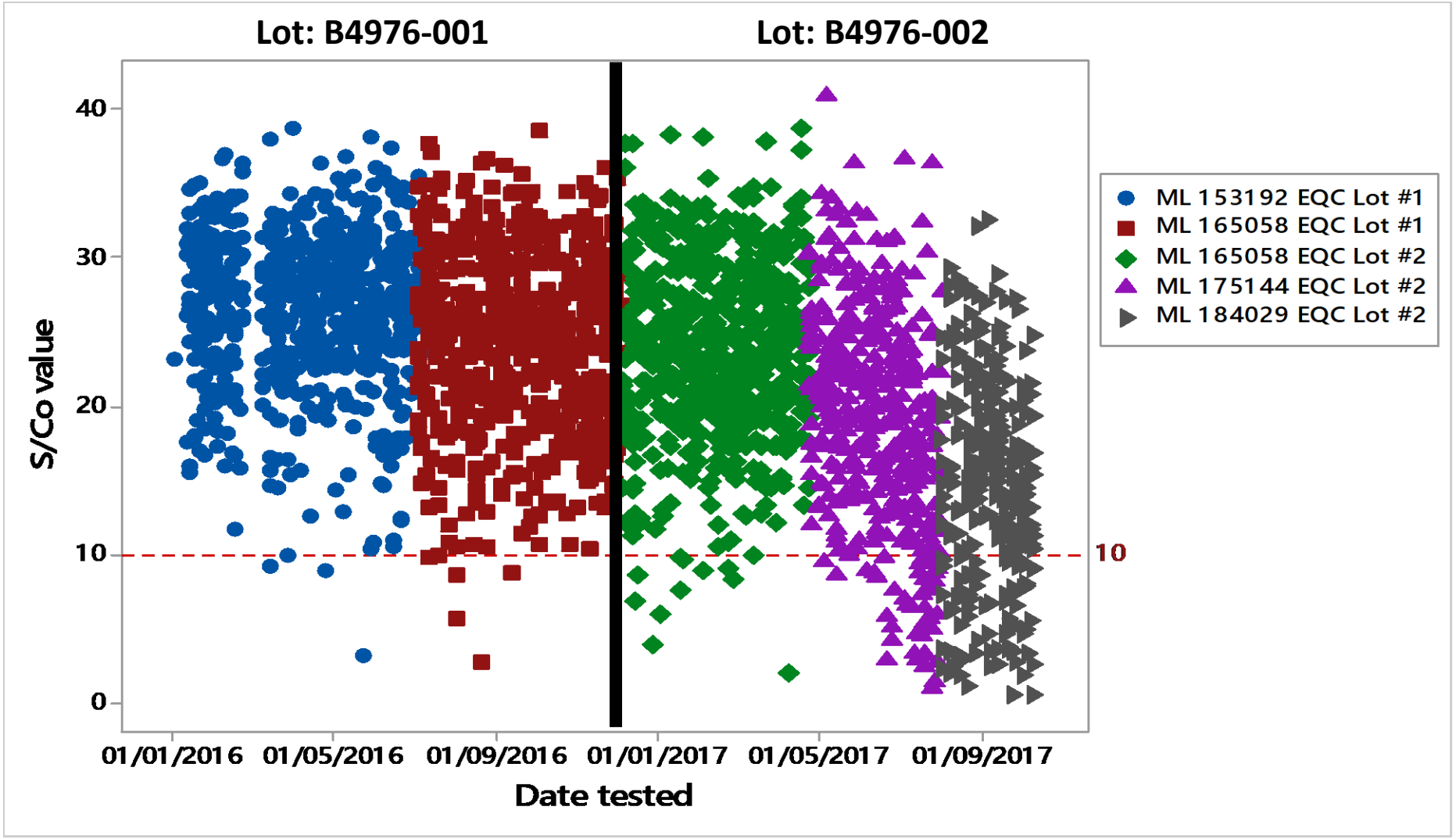
HEV EQC Lot # 01 & 02 performance by Master lot Jan 2016 to April 2017



HEV EQC Lot # 01 & 02 performance by Master lot Jan 2016 to July 2017



HEV EQC Lot # 01 & 02 performance by Master lot Jan 2016 to Oct 2017



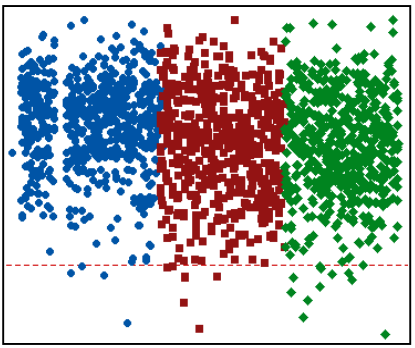
ViraQ HEV Check 125 EQC

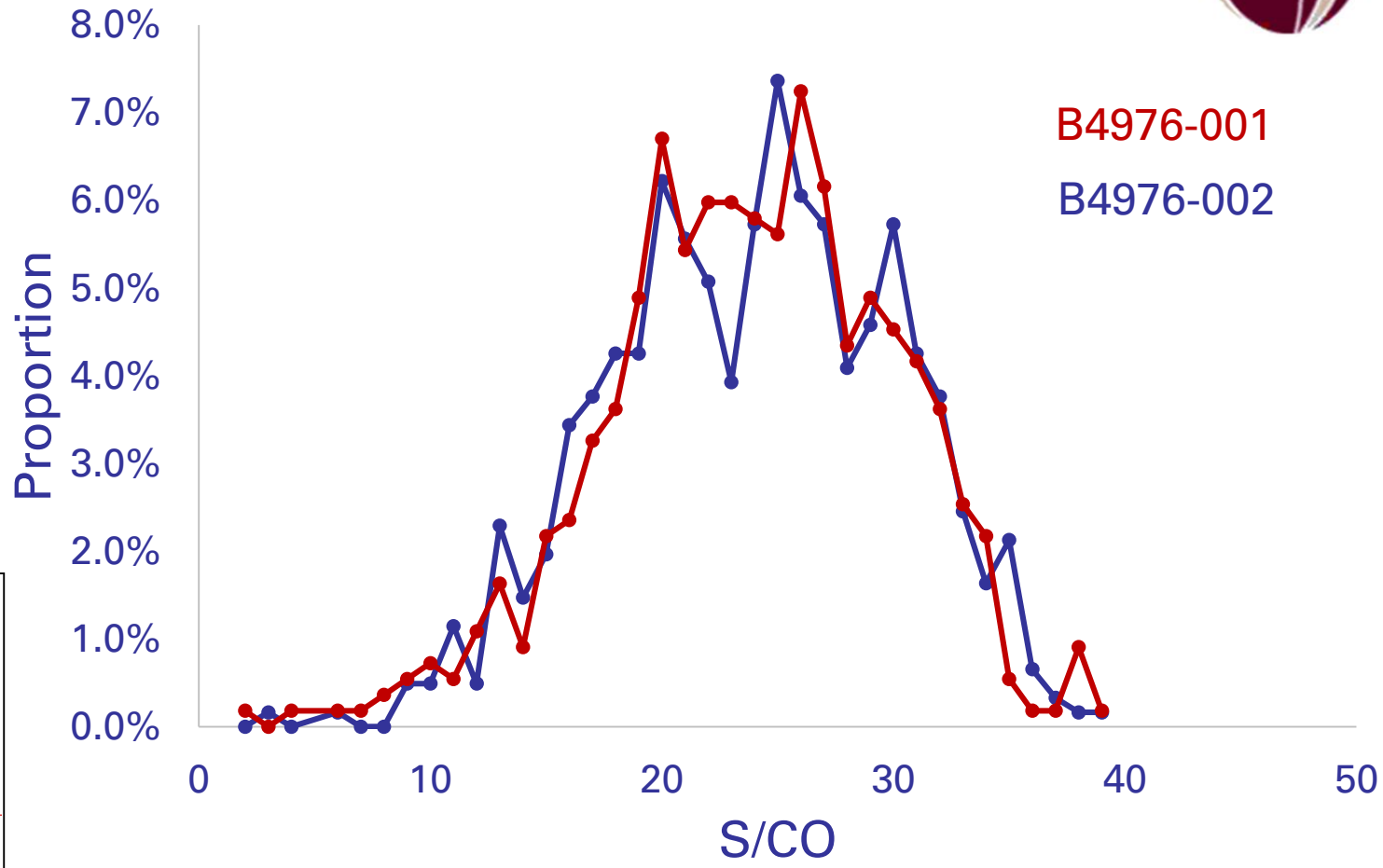
Date tested	HEV EQC S/Co	HEV EQC Lot	Panther	HEV assay ML	HEV EQC S/Co (retested in same W/L)
27/07/2017	0.87	B4976-002-HEV	4	175144	8.09
23/08/2017	0.80	B4976-002-HEV	5	184029	22.98
27/09/2017	0.42	B4976-002-HEV	5	184029	14.55
10/10/2017	0.56	B4976-002-HEV	1	184029	15.32
23/10/2017	0.90	B4976-003-HEV	3	184029	N/T
26/10/2017	0.65	B4976-003-HEV	1	184029	13.40

Root Cause analysis

1. HEV EQC material (BioQControl): ?stability issue may indicate deterioration of performance or batch-to-batch variation.
2. Procleix HEV assay (Grifols): ? Shift to lower HEV EQC S/CO ratios may indicate decreased analytical sensitivity of reagent lot?
3. A combination of both assay & EQC

Distribution of S/CO on 2 lots of HEV Control (1,163 runs) with ML 165058





No significant difference in S/CO value distribution (unpaired t-test) when comparing 611 runs on B4976-001 and 552 runs on B04976-002

[difference was 0,13 (95% CI: -0,58 to 0,81) assuming parametric distribution]

Proportion of low S/CO values on HEV EQC in consecutive reagent batches

S/CO ratio	153192	165058	175144	184029
<1	0.00%	0.00%	0.25%^	0.17#
<2	0.00%	0.09%	0.50%	0.69%
<3	0.00%	0.17%	1.51%	1.39%
<4	0.16%	0.26%	2.26%	2.26%
<5	0.16%	0.26%	3.27%	1.73%

^ 1 nonreactive result in 398 test runs

1 nonreactive result in 576 test runs

HEV EQC Viral Load Testing

NVRL results

- Qualitative HEV RNA PCR (Altona)
- HEV Check 125 EQC vial that tested Neg in July '17 (Lot 02)
- 10 additional vials of stock material (Lot 02)
- All samples detectable HEV RNA (95% LoD=140 IU/mL; Ct values 35-39).
- Detectable viral load in EQC consistent with assay sensitivity.

PHE results

- Quantitative VL test (in-house Taqman assay)
- Three HEV EQC vials (stock Lot 02) (LLQ = 100 IU/mL; LoD 22 IU/mL [1,2])
- VL were reported as: 37 IU/mL, 33 IU/mL and < 10 IU/mL.
- HEV EQC does contain HEV RNA (? How much)

1. *Ijaz et al, J Infect Dis 2005;192:1166-72*
2. *Garson et al, J Virol Methods 2012;186:157-60*

Clinical HEV donation samples

Donation	PHE VL IU/mL	Initial results (S/CO)	ML used	Repeat testing	ML used
072681 *	210	22.06, 31.09, 30.99	165058	5 / 5 Reactive	184029
076298 N	66	12.11, 10.96, 9.01	175144	5 / 5 Reactive	184029
037671 V	64	28.58, 24.94, 13.35	153192	11/11 Reactive	184029
038174 C	54	12.16, 14.58, 10.18	153192	10/10 reactive	184029
122455 L	20	1.26, 1.90, 2.74	153192	2 / 9 Reactive	184029
059568 R	14	34.58, 24.98 & 11.46	165058	4 / 5 Reactive	184029
088434 T	5	19.68, 7.10, 5.58, 5.58, 4.96, 2.64, 0.59, 0.49, 0.19, 0.07, 0.00	153192	6 /13 Reactive	184029
025542 2	4	26.77, 26.76, 21.15, 19.53, 16.76, 15.81, 7.55, 2.83, 2.87, 0.47	153192	10 /10 reactive	184029

Repeat LoD testing (Nov '17) with ML 184029 across 3 Panthers

Reference panel	Member-ID	IU/mL (95% CI)	No. Tested	No. Reactive
P0274 HEV-RNA genotype 3a panel	B4266-01	300 (230-391)	36	36
	B4266-02	100 (77-130)	36	36
	B4266-03	30 (23-39)	36	33 (92%)
	B4266-04	10 (7.7-13)	36	18
	B4266-05	3 (2.3-3.9)	36	6
	B4266-06	1 (0.77-1.30)	36	2
	B4266-07	0.3 (0.23-0.39)	36	0
	B4266-08	0.1 (0.08-0.13)	36	0
P0262 Grifols WHO HEV-RNA reference panel 6329/10	B4262-01	90	48	47 (98%)
	B4262-02	30	48	44 (92%)
	B4262-03	10	48	25
	B4262-04	3	48	10
	B4262-05	1	48	1
	B4262-06	negative	48	0

Repeat LoD testing (Nov '17) with ML 184029 across 3 Panthers

Panel	50% LoD (IU/mL)	95% LoD (IU/mL)
	September 2015	
Dilutions WHO IS (IBTS validation)	1.80 (1.43 – 2.21)	8.85 (6.38 – 14.63)
Procleix HEV assay Product Insert (Grifols)	2.02 (1.71 – 2.32)	7.89 (6.63 – 9.83)
	November 2017 with ML 184029	
WHO Panel P0262 Genotype 3a	8.18 (6.42 – 10.40)	49.14 (33.96 – 82.84)
BioQControl HEV Panel P0274 Genotype 3a	8.07 (6.13 – 10.69)	47.36 (31.20 – 87.68)

Grifols LoD testing with BioQControl Reference panel P0274

Results for BioQControl P0274 panel testing at Grifols and IBTS

		ML 184029 (n=7) tested at Grifols	ML 219329 (n=7) tested at Grifols	ML 184029 (n=24) tested at IBTS
Panel	**Concentration (IU/mL)**	**% Reactive**	**% Reactive**	**% Reactive**
BioQControl P0274 HEV-RNA genotype 3a	300	100	100	100
	100	**100**	**100**	**100**
	30	85.71	100	87.5
	10	42.86	57.14	58.33
	3	14.28	28.57	16.67
	1	0	14.28	4.17
	0.3	0	14.28	0
	0.1	0	0	0

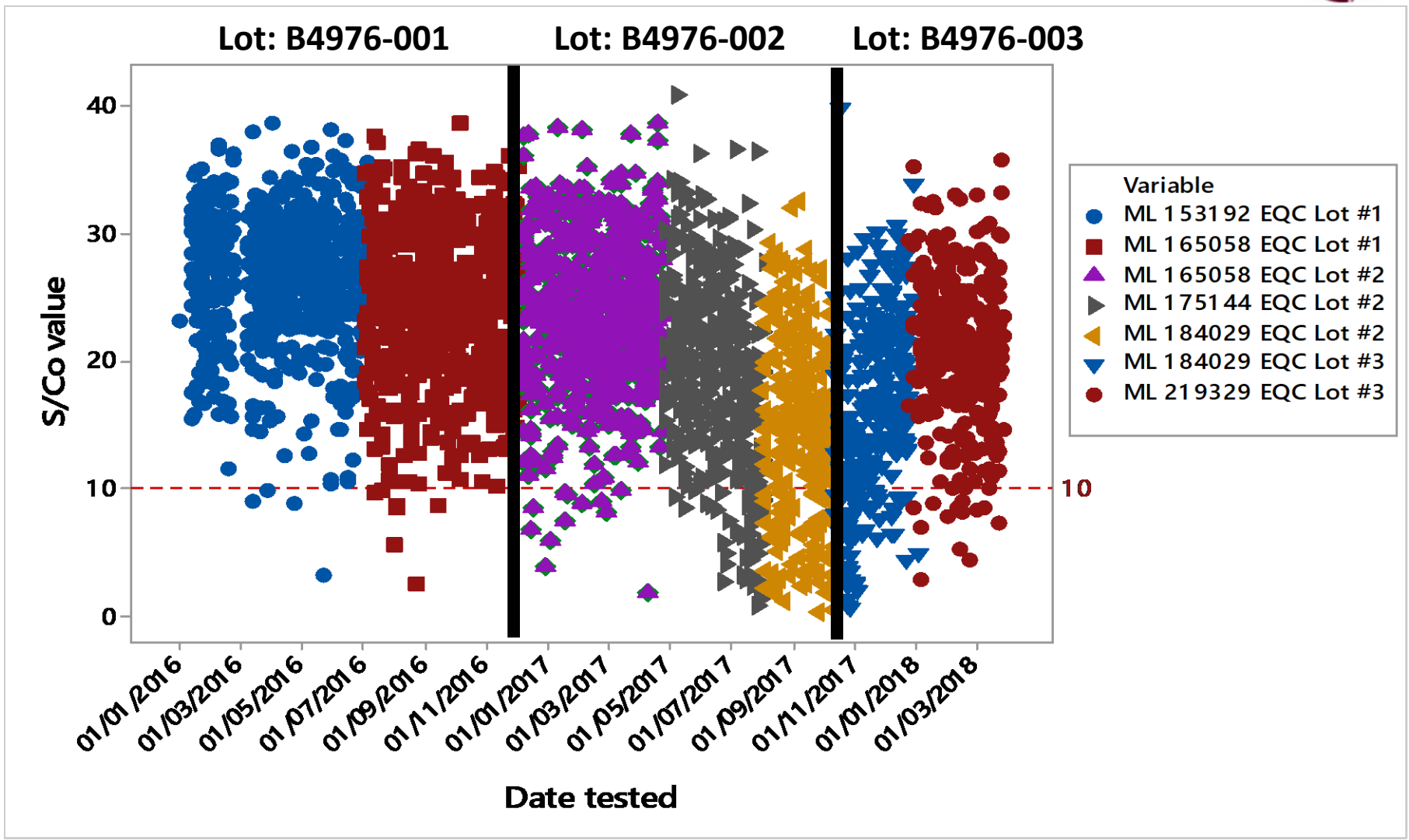
LoD Testing by Grifols: ML 175144 & 184029

Probit Analysis for HEV WHO International Standard 6329/10

Source	Reagent ML	Number tested	Concentrations (IU/mL)	
			50% Detection Probability (95% Fiducial Limits)	95% Detection Probability (95% Fiducial Limits)
Procleix HEV PI	-	162	2.02 (1.71 – 2.32)	7.89 (6.63 – 9.83)
Investigation August 2017	175144	27	2.12 (1.83-2.42)	8.22 (6.99-10.09)
	184029	27	1.99 (1.21-2.77)	7.85 (5.43-15.01)

- 95% LOD values obtained with Master Lots 175144 & 184029 were similar to the 95% LOD from the package insert

HEV EQC Lot # 01, 02 & 03 by Master lot Jan 2016 to Mar 2018

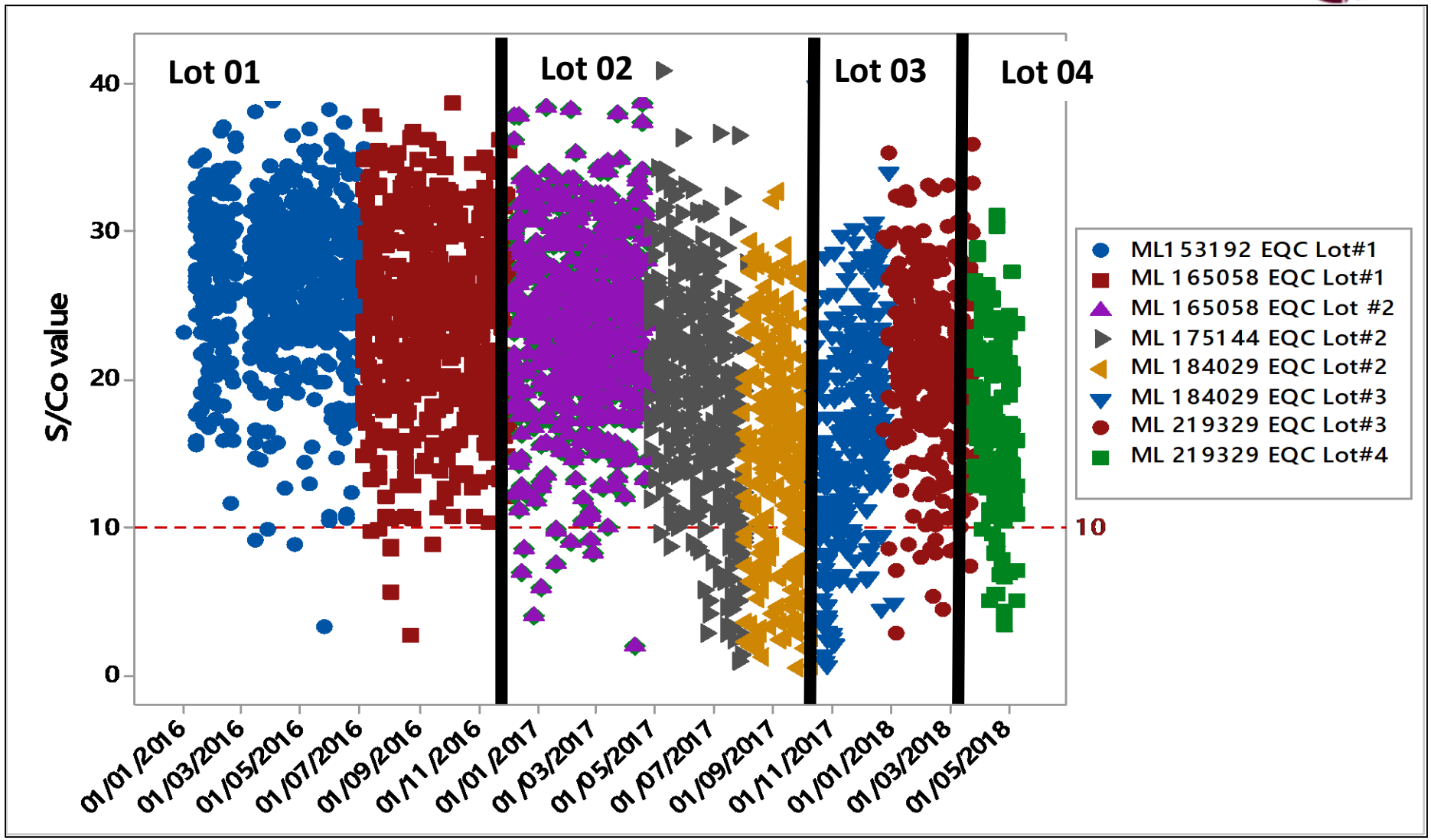


Specificity data on Procleix HEV assay

No further NonReactive HEV EQCs since October 2017

Master Lot	In Use	No. tested	No. False Reactives	No. True Reactives	PHE VL IU/mL
153192	04/01/16 - 08/07/16	72,327	21 (99.97%)	27	17 – 17,000
165058	22/06/16 - 03/05/17	114,478	56 (99.95%)	16	14 –260,000
175144	24/04/17 - 02/08/17	40,233	11 (99.97%)	4	20 - 66
184029	02/08/17 - 27/12/17	56,138	11 (99.97%)	12	4 – 66,000
219329	22/12/17 - 31/03/18	37,593	6 (99.98%)	6	Not Av.

HEV EQC Lot # 01 to 04 by Master lot Jan 2016 to May 2018



Repeat LoD testing again (April '18) with ML 219329 on 1 Panther

Panel	50% LoD (IU/mL)	95% LoD (IU/mL)
	September 2015	
IBTS WHO Dilutions	1.80 (1.43 – 2.21)	8.85 (6.38 – 14.63)
Procleix HEV PI (Grifols)	2.02 (1.71 – 2.32)	7.89 (6.63 – 9.83)
	November 2017 (ML 184029)	
WHO P262 Genotype 3a	8.18 (6.42 – 10.40)	49.14 (33.96 – 82.84)
BioQControl P274 Genotype 3a	8.07 (6.13 – 10.69)	47.36 (31.20 – 87.68)
	April 2018 (ML 219329; n=24 reps)	
WHO P262 Genotype 3a	5.97 (4.31 – 8.26)	28.18 (17.97 – 59.81)

HEV: the next chapter…

- No negative HEV EQCs since October 2017
- No clinical concerns with donor sample sensitivity
- Grifols & BioQControl batch release criteria met
- Continue to VL test all EQCs
- Continue to build & analyse data

? Stability & degradation studies of HEV EQC

? Stability & degradation of HEV reagents

? Why does matrix effect occur

? More BE users of HEV EQC for comparison purposes

? How do manufacturers track shifts in their products

ViraQ Trend controls

PANTHER

RESULTS BY WORKLIST REPORT

Serial Number:	00479	**System SW Version:**	5.3.2.9	**Date Range:**	3/29/2018 22:52:36 to 3/30/2018 19:56:33
Assay:	**Ultrio Elite**				
Worklist IDs:	00479-20180329-10 Version: 2.4.5 (For In Vitro Diagnostic Use) ML: 186731 Exp: 3/15/2019 TCR: 02298 ICCutoff: 103616 AnalyteCutoff: 90751				
Operators:	youngf, bonieckad, ciarar				
Specimen Tested:	213	Reactive: 7 | Invalid: 1			
Status Flag Legend:	p - Assay processing error. PCL1 - ProcessControl check failed for AutoDetect1.				

Reviewed By: _____ **Date:** _____ **Verified By:** _____ **Date:** _____

Ultrio Elite

Version: 2.4.5 (For In Vitro Diagnostic Use)

Worklist ID: 00479-20180329-10

Time	Rack-Tube #	Sample ID	IC RLU	IC Result	RLU	S/CO	Result	Flags
3/29/2018 22:52:36	677-01	Negative Calibrator - Lot 187143	194943	Valid	9438	0.10	Valid	
3/29/2018 22:52:36	677-01	Negative Calibrator - Lot 187143	213817	Valid	8876	0.09	Valid	
3/29/2018 22:52:36	677-01	Negative Calibrator - Lot 187143	212936	Valid	4516	0.04	Valid	
3/29/2018 22:52:36	677-02	HIV Calibrator - Lot 187144	238352	Valid	1260259	13.88	Valid	
3/29/2018 22:52:36	677-02	HIV Calibrator - Lot 187144	238224	Valid	1263792	13.92	Valid	
3/29/2018 22:57:36	677-03	HCV Calibrator - Lot 187146	263954	Valid	775655	8.54	Valid	
3/29/2018 22:57:36	677-03	HCV Calibrator - Lot 187146	278227	Valid	820675	9.04	Valid	
3/29/2018 22:57:36	677-04	HBV Calibrator - Lot 187145	183108	Valid	1286490	14.17	Valid	
3/29/2018 22:57:36	677-04	HBV Calibrator - Lot 187145	170372	Valid	1310927	14.44	Valid	
3/29/2018 22:57:36	677-05	HIVRNA160812010318	207609	Valid	1053220	11.60	Reactive	
3/29/2018 23:02:37	677-06	HCVRNA160817010318	230102	Valid	765501	8.43	Reactive	
3/29/2018 23:02:37	677-07	HBVDNA160812050318	192889	Valid	1225649	13.50	Reactive	
3/29/2018 23:02:37	677-08	B4063x010xHIV	208115	Valid	866186	9.54	Reactive	
3/29/2018 23:02:37	677-09	B4062x010xHCV	256284	Valid	746921	8.23	Reactive	
3/29/2018 23:02:37	677-10	B4154x008xHBV	180625	Valid	1267503	13.96	Reactive	

Trend information

- HIV-1 Trend 25 Subtype B (P0068)
- HCV Trend 25 Genotype 3a (P0067)
- HBV Trend 50 Genotype A2 (P0154)

Purpose:

External run control

Monitor consistent detection of low viral load samples

Monitor analytical sensitivity of TMA assay

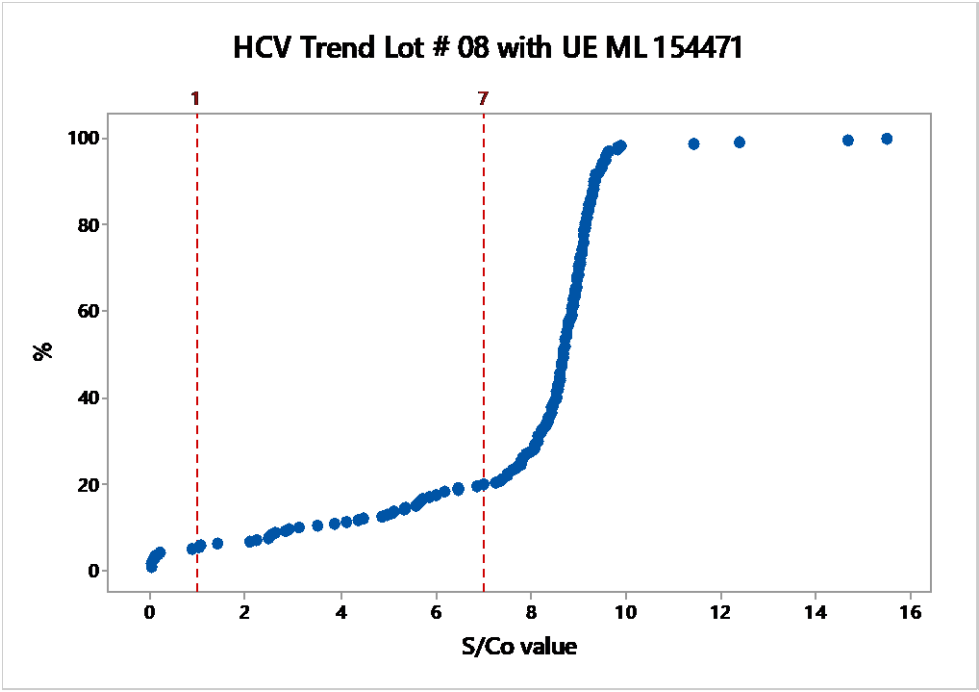
ViraQ HCV Trend 25 Reactivity rates

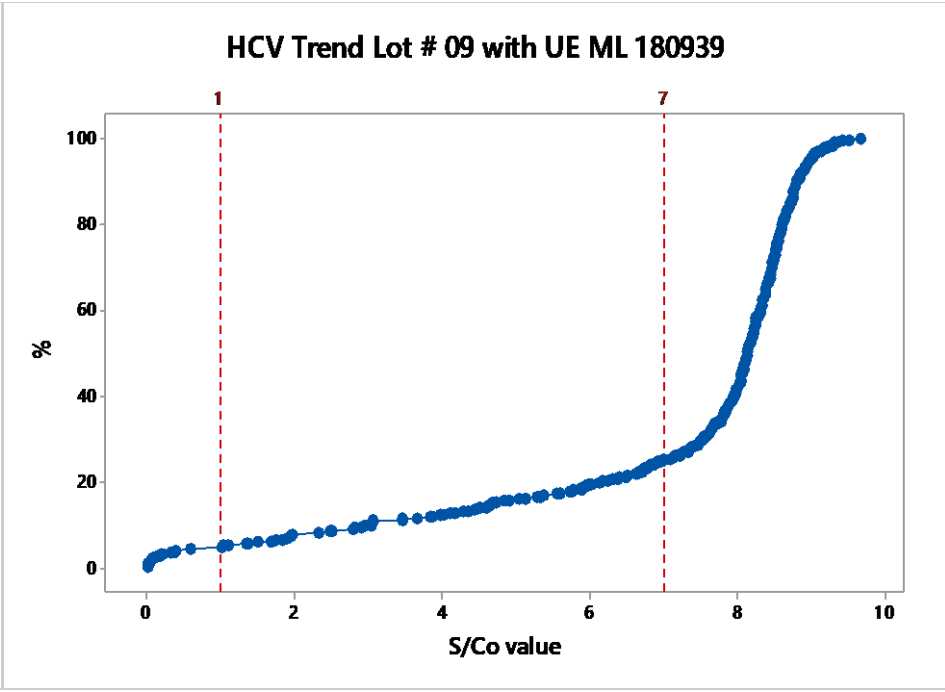
UElite ML	Dates in use	No. Tested	No. Neg	Reactivity rate	P1	P2	P3	P4	P5
180939	Sept '17 - Jan '18	445	19	96%	94%	92.5% 7 Neg	95%	99% 1 Neg	98%
186731*	Jan '18 - Mar '18	128	14*	89%*	80% 8 Neg	92% *	95% * 1 Neg	84% * 3 Neg	100% *

* Need > 30 points per instrument for 'real' analysis

HCV Trend Lot no.	No. Tested	No. Neg	Reactivity rate	P1	P2	P3	P4	P5
B4062-009	568	33	94%	91% 13 Neg	92.5% 9 Neg	95% 9 Neg	95.5% 4 Neg	98% 2 Neg
B4062-008	564	29	94.5%	95% 5 Neg	97% 3 Neg	91% 10 Neg	95% 6 Neg	95% 5 Neg
B4062-007	1083	85	92%	95% 11 Neg	91% 20 Neg	89% 24 Neg	94% 12 Neg	91% 18 Neg
TOTAL/ AVG	2,215	147	93%	93%	93%	92%	95%	95%

Distribution of S/Co ratios for UElite Master Lot & HCV Trend lot





HCV Trend control performance: Feb 2016 to March 2018

Trend Lot	Timeframe	ML	Total N	Mean	Median	Delta
B4062-009	Sept ’17 - Mar ‘18	Overall	568	6.955	8.095	1.14
	Sept ’17 - Jan ‘18	180939	445	7.187	8.14	0.953
	Jan ’18 - Mar ‘18	186731	123	6.114	7.61	1.496
B4062-008	Feb ‘17 - Sept ‘17	Overall	564	7.458	8.395	0.937
	Feb ’17 - Mar ‘17	146182	31	8.556	8.78	0.224
	Feb ‘17 - May ‘17	154471	235	7.793	8.69	0.897
	May ’17 - Aug ‘17	159260	223	7.076	8.27	1.181
	Aug ’17 - Sept ‘17	180939	75	7.089	8.01	0.921
B4062-007	Feb ’16 - Feb ‘17	Overall	1083	7.1749	8.28	1.105
	Feb ’16 - May ‘16	119400	285	6.236	7.53	1.294
	May ‘16 - Sept ‘16	131154	315	7.72	8.64	0.92
	Sept ’16 - Mar ‘17	146182	483	7.373	8.47	1.097

Trend controls

Benefits

1. Constant monitoring of analytical sensitivity of UElite assay close to limit of detection of assay
2. Consistent detection of low viral load samples; a trigger in the event of change/drift
3. TTI cases: Trend control of relevant viral marker is reactive in run/worklist with false negative donation
4. Assists with root cause investigation when the performance of other controls or EQAS is poor e.g. QConnect EQCs (Go/No-go)

Thank you

- NAT Team in Dublin
- Dr. Niamh O'Flaherty, Consultant Microbiologist

- Nico & Harry & BioQControl team
- Jeff Linnen & Grifols US team
- Boris Hogema, Sanquin

Appendix data

Use of Reference panels for Procleix reagent batch release testing

- HIV-1 RNA Group M Subtype B panel (P0026)
- HIV-2 RNA Subtype A panel (P0034)
- HCV RNA Genotype 3a panel (P0020)
- HBV DNA Genotype A panel (P0031)

- HEV RNA Genotype 3a panel (P0274)

UElite Master Lot batch release testing per kit delivery: HIV-1

Panel member	HIV-1 Sub B IU/mL	HIV-1 Sub B cps/mL	UElite Master lot number by delivery					
			217450	186731	180939 (D2)	180939 (D1)	159260	154471
1	4,217	2,460	R	R	R	R	R	R
2	1,406	819	R	R	R	R	R	R
3	422	246	R	R	R	R	R	R
4	141	81.9	R	R	R	R	R	R
5	42.2	24.6	R	R	R	R	R	R
6	14.1	8.19	R	R	R	NR	R	NR
7	4.2	2.46	NR	NR	NR	NR	NR	NR
8	1.4	0.819	NR	R	NR	NR	NR	NR
9	0.42	0.246	NR	NR	NR	NR	NR	NR
10	0.14	0.08	NR	NR	NR	NR	NR	NR

Yellow = Minimum sensitivity must be met

Blue = Close to 95% LoD

	Assay	UElite 95% LoD IBTS	UElite 95% LoD Product Insert
HIV-1 WHO (10/152) Sub. B	Multiplex	22.75 (20.11–26.47)	18.0 (15.0 – 23.5)
	dHIV	26.33 (20.94 – 34.78)	17.3 (14.4 – 22.6)

Panel member	HIV-2 Sub A Conc IU/mL	HIV-2 Sub A Conc cps/mL	217450	186731	180939 (D2)	180939 (D1)	159260	154471
1	8,658	5,014	R	R	R	R	R	R
2	2,909	1,686	R	R	R	R	R	R
3	866	501	R	R	R	R	R	R
4	291	169	R	R	R	R	R	R
5	87	50.1	R	R	R	R	R	R
6	29	16.9	R	R	R	R	R	R
7	8.7	5.01	R	R	R	R	R	R
8	2.9	1.69	R	NR	NR	R	NR	R
9	0.87	0.5	R	NR	R	NR	R	NR
10	0.29	0.17	NR	NR	NR	NR	R	NR

Panel member	HCV Gt 3a Conc IU/mL	HCV Gt 3a Conc cps/mL	217450	186731	180939 (D2)	180939 (D1)	159260	154471
1	1,262	3,450	R	R	R	R	R	R
2	424	1,160	R	R	R	R	R	R
3	126	345	R	R	R	R	R	R
4	42.4	116	R	R	R	R	R	R
5	12.6	34.5	R	R	R	R	R	R
6	4.24	11.6	R	R	R	R	R	R
7	1.26	3.45	NR	NR	NR	R	R	R
8	0.42	1.16	NR	R	NR	NR	R	NR
9	0.13	0.345	NR	R	NR	NR	NR	NR
10	0.042	0.116	NR	NR	NR	NR	NR	R

Panel member	HBV Gt A Conc IU/mL	HBV Gt A Conc cps/mL	217450	186731	180939 (D2)	180939 (D1)	159260	154471
1	664	3,540	R	R	R	R	R	R
2	222	1,180	R	R	R	R	R	R
3	66.4	354	R	R	R	R	R	R
4	22.2	118	R	R	R	R	R	R
5	6.64	35.4	R	R	NR	R	R	R
6	2.22	11.8	NR	R	NR	R	NR	NR
7	0.66	3.54	R	R	R	R	R	NR
8	0.22	1.18	NR	NR	NR	NR	NR	NR
9	0.07	0.35	NR	NR	NR	NR	NR	NR
10	0.02	0.118	NR	NR	NR	NR	NR	NR

HEV Master Lot batch release testing per kit delivery

Panel member	HEV Gt 3a IU/mL	HEV Gt 3a cps/mL	219329 (D1)	219329 (D2)
1	300	350	R	R
2	100	118	R	R
3	30	35	R	R
4	10	11.8	R	R
5	3	3.57	NR	NR
6	1	1.18	NR	NR
7	0.3	0.357	NR	NR
8	0.1	0.118	NR	NR

	HEV 95% LoD IBTS	HEV 95% LoD Product Insert
HEV WHO IS Gt 3a	8.85 (6.38 – 14.63)	7.89 (6.63 – 9.83)

BioQControl Reference panels

Benefits

1. Monitoring the analytical sensitivity of each UElite assay by master lot & delivery (batch release testing)
2. Evaluate LoD of any viral marker independently e.g. instrument, assay, TTI cases
3. Health & Safety of scientists (Exposure) and human error during preparation of WHO IS dilutions
4. Building assay ML performance data over time