UK NEQAS
International Quality Expertise

Impact of the introduction of the International Standards: evidence from EQA panels

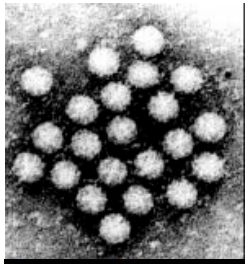
Dr Beatrix Kele
UK NEQAS for Microbiology

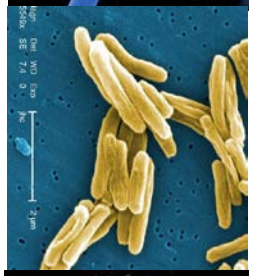
NCI Workshop on Harmonization of EBV Testing for Nasopharyngeal Cancer

5-6 November, 2015

Schemes overview

UK NEQAS
International Quality Expertise





44 SCHEMES

Identification
- Biochemistry
- Ag (IF / agglutination)
- Growth characteristics
- Molecular

Virology
- 22 schemes

Typing / subtyping
- Biochemistry
- Ag
- Molecular

Mycology
- 2 schemes

Quantification
- Microscopic
- Molecular

Bacteriology
- 12 schemes

Susceptibility
- phenotype
- genotype

Serology
- EIA
- Agglutination
- Line assays
- IF

Parasitology
- 8 schemes

UK NEQAS
International Quality Expertise

Introduction: EBV

- EBV infection
- Seroconversion: 90% at young adult
  100% by 40 years of age
- Transmission: body fluids, transplantation
- B-lymphocytes are the site of latency

UK NEQAS
International Quality Expertise

Introduction: Clinical significance

- Primary infection
- Infectious mononucleosis
- Chronic active EBV
- Associated with: Burkitt lymphoma and nasopharyngeal carcinoma (>95%), Hodgkin's disease (<5-70%), gastric adenocarcinomas (2-16%), T/NK cell lymphomas (40->90%)
- Immunocompromised patients
- Clinical diagnosis: EBV DNA detection

UK NEQAS
International Quality Expertise

QUESTIONNAIRE / April 2009

- Sent to CMV DNA quantification scheme participants (N=81)
- 78.8% use molecular methods to quantify EBV DNA (all real time PCR)
- Sample tested:
  - 62.5% use whole blood
  - 54.2% use plasma
  - 16.6% use both
- Median detection limit: 250 copies/mL (10-2,000)
- Median number of samples examined per week: 22.5 (0.5-200)
- Patient types:
  - 68.8% - HSCT
  - 36.8% - SOT
  - 7.9% - tumour
- Clinical cut-off: ‘viral load rise over a definite period of time’ & ‘confirm viral load above the cut-off’

Pilot /Specimen design

UK NEQAS
International Quality Expertise

	Namalwa/mL	Expected VL		Pre-distribution Artus		Homogeneity
		c/mL	Log	c/mL	Log	SD
A	78,000	156,000	5.19	102300	5.01	0.118
B	7,800	15,600	4.19	31600	4.50	0.149
		Log diff.	1.00	Log diff.	0.49	
				(Qiagen column)		(20 vials)

A total of 88 sets of specimens were distributed with 74 participants returning results, 13 participants did not examine the specimens

→61 sets of results

Pilot / Results by specimen

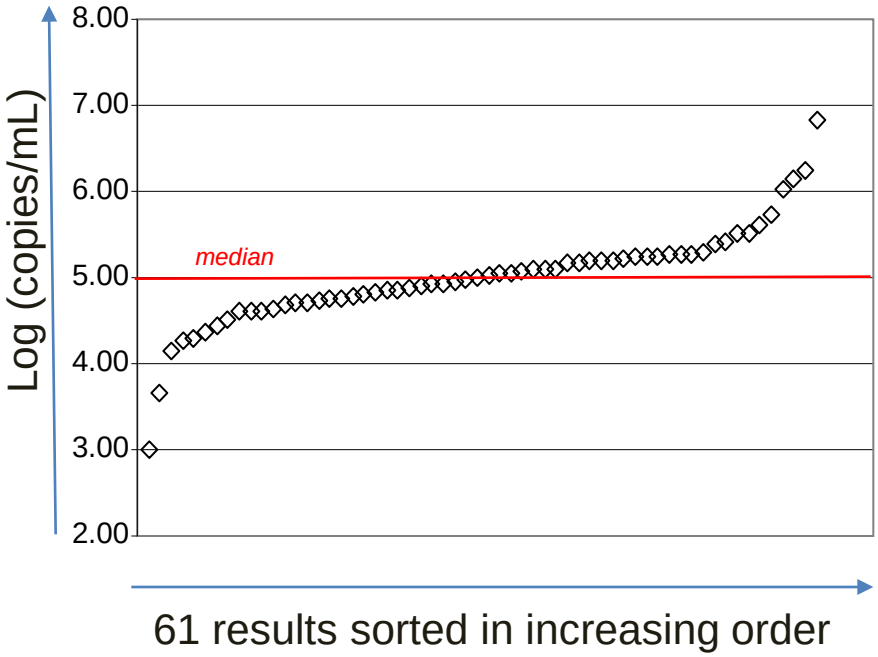
UK NEQAS
International Quality Expertise

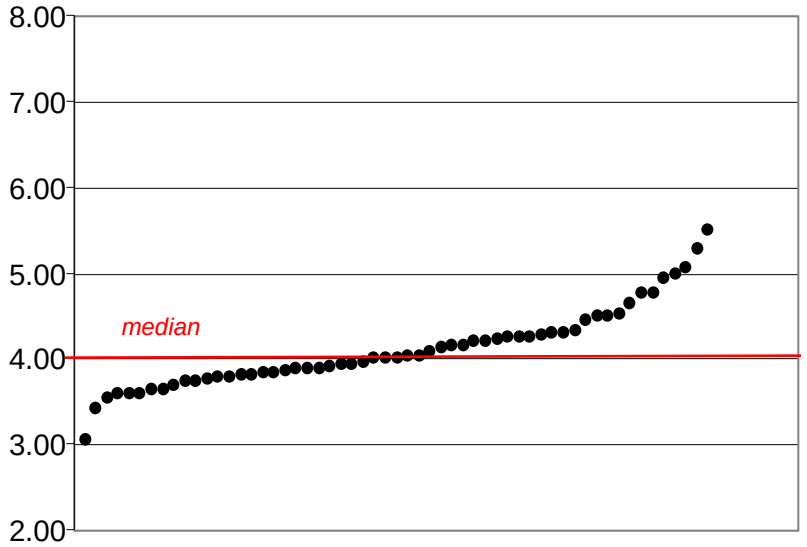
SPECIMEN A

- Expected copies/mL 5.19 log (156,000)
- median+/-SD 5.01 +/- 0.57
- range 3.00-6.84

SPECIMEN B

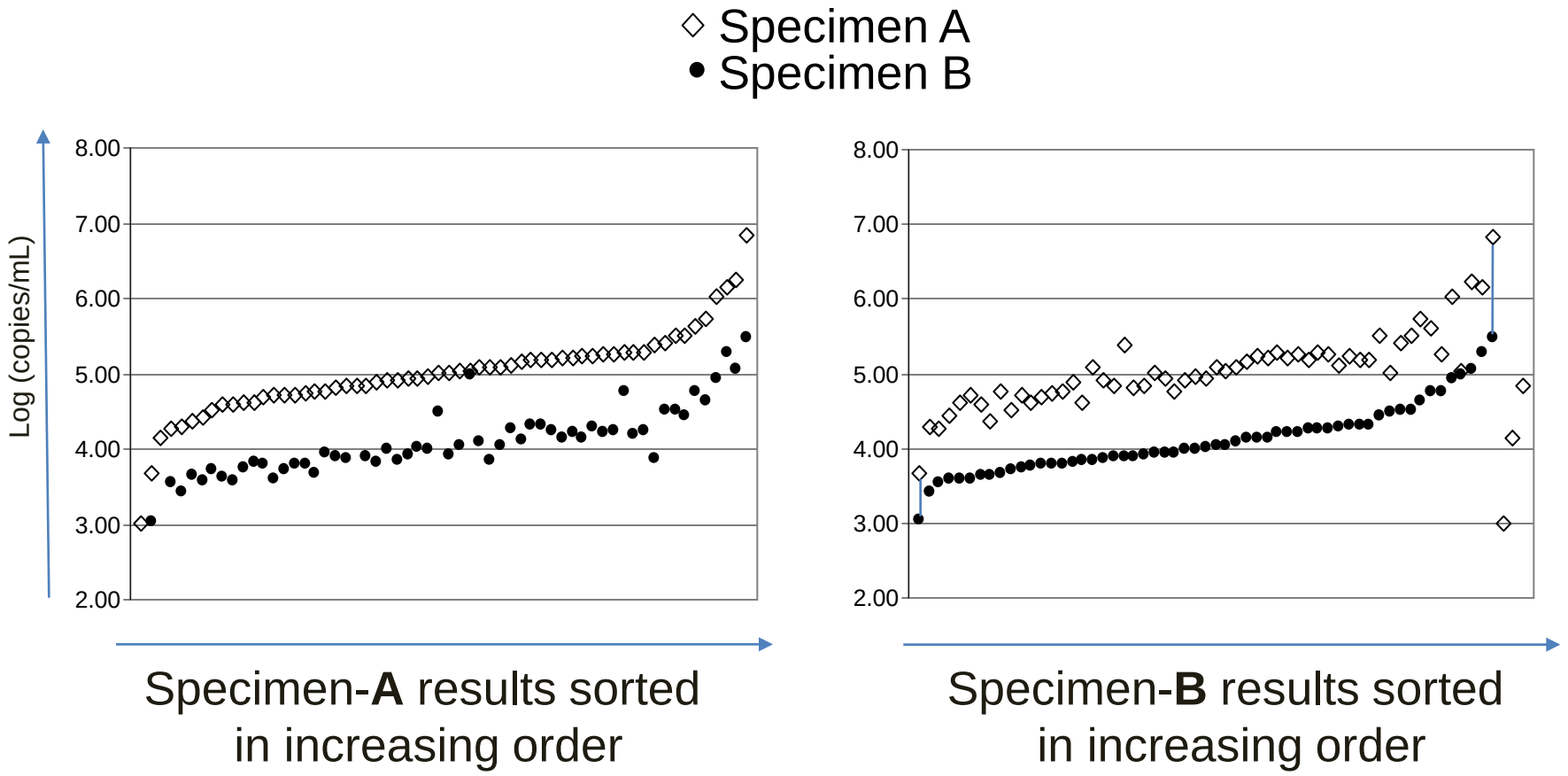
- Expected copies/mL 4.19 log (15,600)
- median+/-SD 4.02 +/- 0.47
- range 3.05-5.49





Pilot / Results by laboratory

UK NEQAS
International Quality Expertise



UK NEQAS
International Quality Expertise

Pilot / Results analysed as a log difference

98.4% (60/61) detected 5 log of EBV DNA (specimen A)

93.4% (57/61) detected 4 log of EBV DNA (specimen B)

-> 57 log differences calculated:	expected	1.00 log
	median+/-SD	0.96+/-0.21
	range	0.05 - 1.49

→ 96.5% (55/57) reported a 0.46 to 1.46 log difference

UK NEQAS
International Quality Expertise

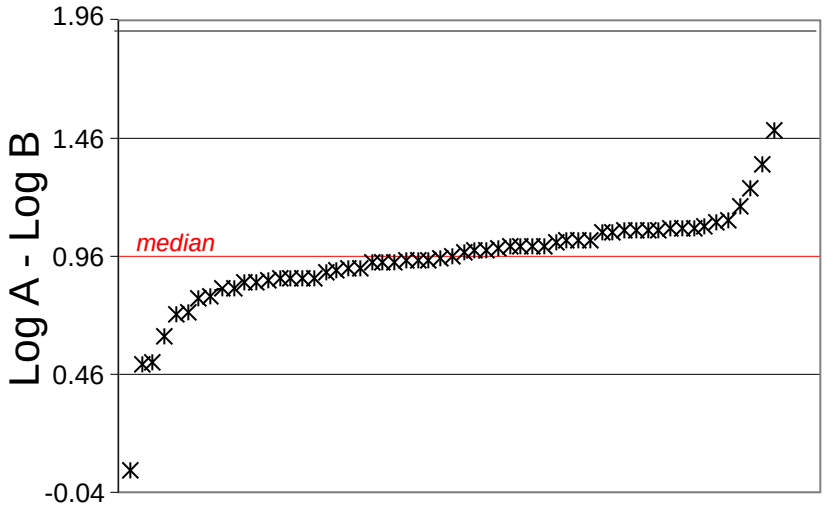
Pilot / Results analysed as a log difference

98.4% (60/61) detected 5 log of EBV DNA (specimen A)

93.4% (57/61) detected 4 log of EBV DNA (specimen B)

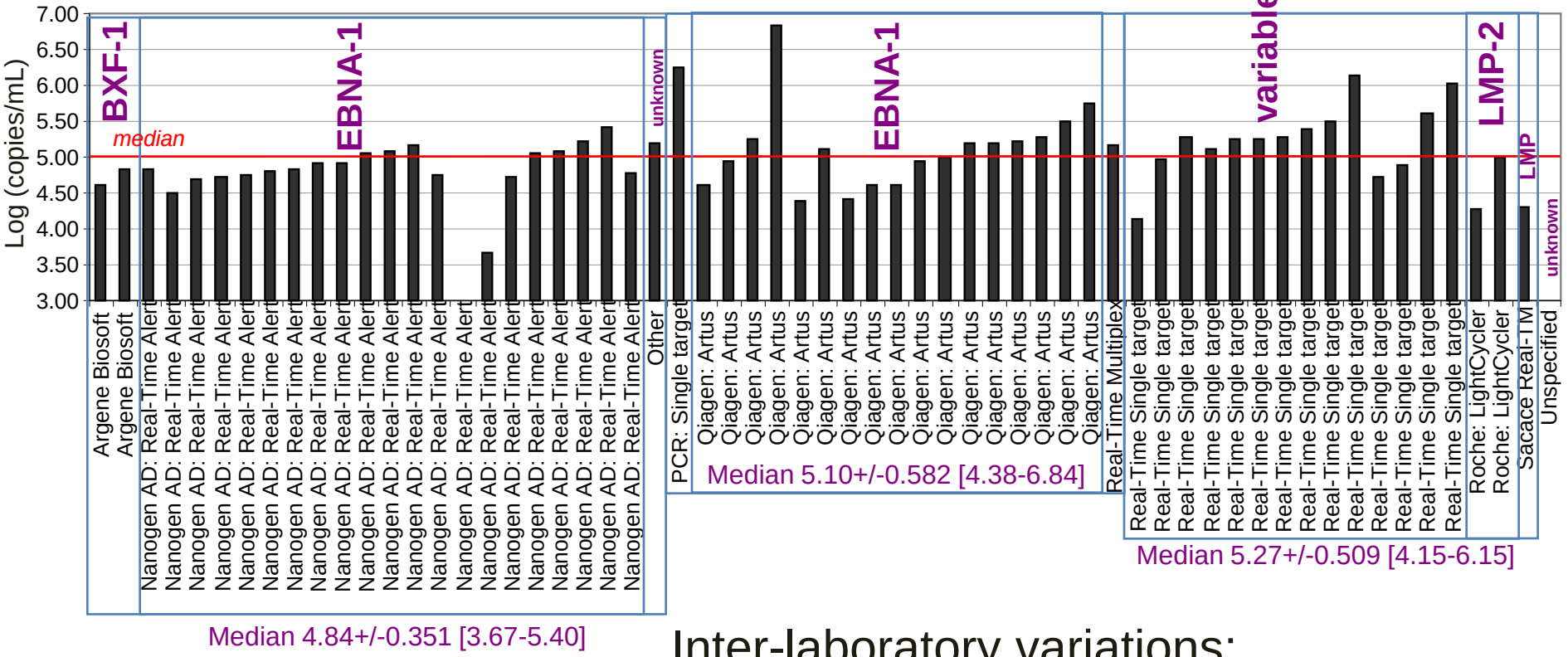
-> 57 log differences calculated:	expected	1.00 log
	median+/-SD	0.96+/-0.21
	range	0.05 - 1.49

→ 96.5% (55/57) reported a 0.46 to 1.46 log difference



UK NEQAS
International Quality Expertise

Pilot / Results by amplification methods



Inter-laboratory variations:

1) same amplification assay
2) different amplification platforms
3) different extraction assays

UK NEQAS
International Quality Expertise

Pilot / Extraction and amplification methods

Amplification method	n	%
Nanogen AD: Real-Time Alert	21	34.4%
Qiagen: Artus	17	27.9%
Real-Time Single target	14	23.0%
Roche: LightCycler	2	3.3%
Argene Biosoft	2	3.3%
PCR: Single target	1	1.6%
Real Time Multiplex	1	1.6%
Sacace Real-TM	1	1.6%
Unspecified	1	1.6%
Other	1	1.6%

- 26.2% in house assays
- 73.8% commercial assays
- All open systems:
  free choice of extraction methods:

Extraction method	n	%
Qiagen*	24	36.9%
NucliSENS easyMAG	19	29.2%
MagnaPure	9	13.8%
Magnetic beads	2	3.1%
Nucleo Spin	1	1.5%
Unspecified	3	4.6%
Other	3	4.6%

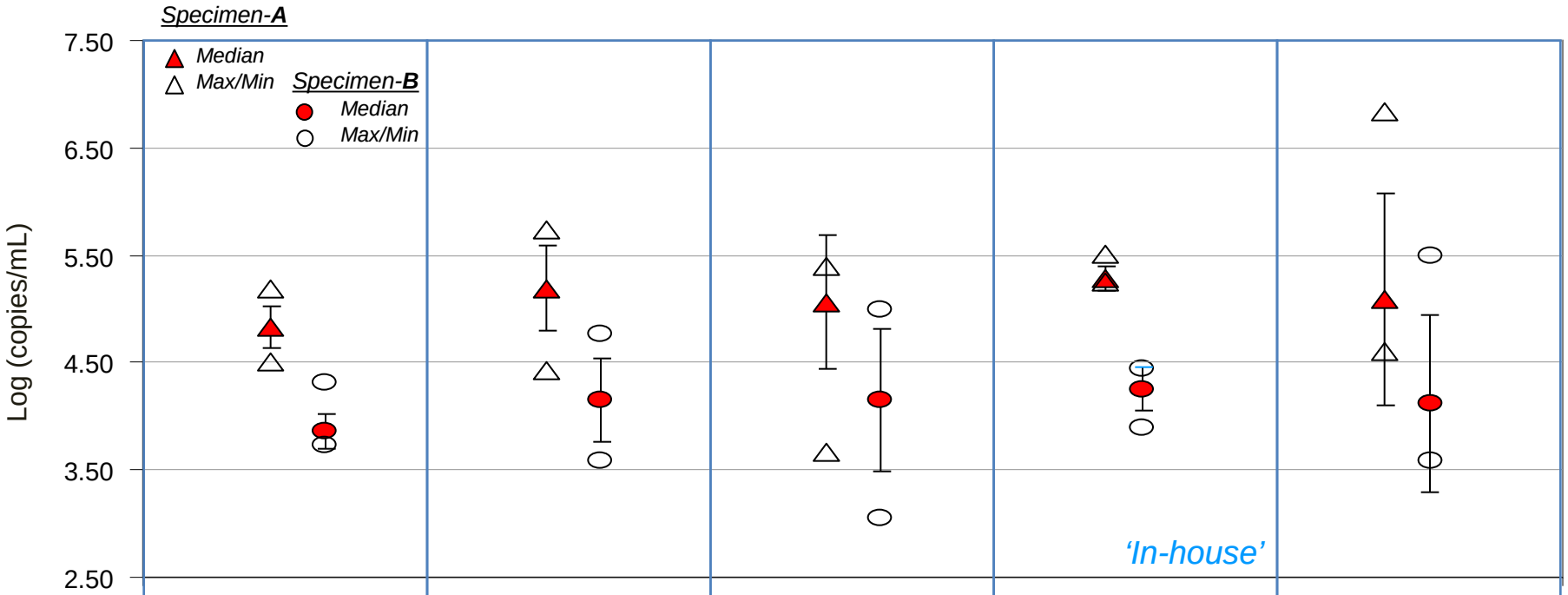
** BioRobot MDx QIAsymphony*

- >23 combinations of extraction and amplification assays
- 5 most frequent combinations:

Extraction method	Amplification method	n	%
NucliSENS easyMAG	Nanogen AD: Real-Time Alert	11	18.0
Qiagen*	Qiagen: Artus	11	18.0
Qiagen*	Nanogen AD: Real-Time Alert	6	9.8
NucliSENS easyMAG	Real-Time Single target	5	8.2
MagnaPure	Qiagen: Artus	4	6.6

Pilot / Results for the 5 most frequent E&A assay combinations

UK NEQAS
International Quality Expertise



Extraction Amplification	NucliSENS easyMAG NanogenAD: Real-Time Alert	Qiagen* Qiagen: Artus	Qiagen* NanogenAD: Real-Time Alert	NucliSENS easyMAG Real-Time Single target	MagnaPure Qiagen: Artus
n (%)	11 (18%)	11 (18%)	6 (9.8%)	5 (8.2%)	4 (6.6%)
Median A +/- SD	**4.83** +/- 0.194	5.19 +/- 0.397	5.06 +/- 0.624	**5.28** +/- 0.111	5.09 +/- 0.991
Median B +/- SD	**3.86** +/- 0.168	4.15 +/- 0.383	4.15 +/- 0.660	**4.25** +/- 0.202	4.12 +/- 0.827

**BioRobot MDx, QIAsymphony*

Conclusion

UK NEQAS
International Quality Expertise

- This pilot study illustrates the lack of standardisation, with participants results ranging 3.84 log for specimen A (5 log) and 2.44 log for specimen B (4 log) using different commercial and in house assays.
- Standardisation:
  - Matrix
  - Extraction assay
  - Amplification platform
  - Amplification/detection assay
- Needed to establish guidelines for the surveillance of HSCT and SOT at risk of developing PTLD:
  - Clinical cut-off for pre-emptive therapy
  - Frequency of assessment (+)
  - Treatment efficacy

UK NEQAS
International Quality Expertise

EBV DNA quantification scheme

- To assess testing laboratories performance in the measurement of the EBV viral load
- Sample format: Freeze-dried plasma
- No. of distributions / year = 3
- No. of samples / distribution = 2
- Scoring: reported log difference in viral load between specimen pair
- Absolute quantitation data by method is also presented in the scheme distribution reports

UK NEQAS
International Quality Expertise

EBV DNA quantification scheme

- Virus source: Namalwa cells
- Plasma EBV VCA IgG and EBNA G antibody status tested
- Number data sets 60
- No. method groups 10
- No. commercial 7
- No. commercial with >5 participants

  Qiagen Artus, Artus Biosoft, ELITech: ELITe MGB and Nanogen AD RT Alert
- 3 participants stated their method was standardised against the IS
  - 1 gave a conversion factor of 13 (in house RT single target)

UK NEQAS
International Quality Expertise

EBV: Current number of users by method

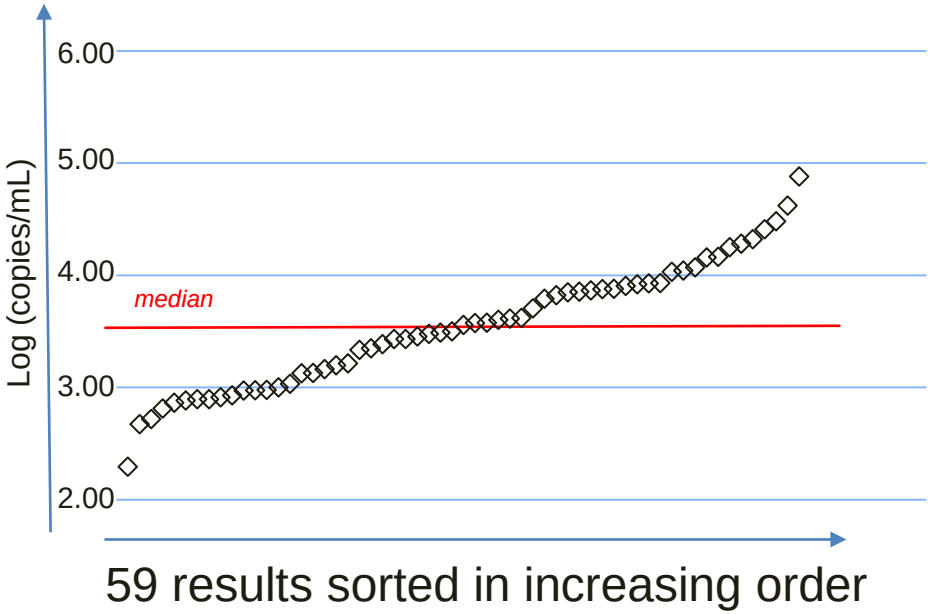
Unspecified	1
Focus: Simplexa	1
FTD	1
Altona: RealStar	2
Nanogen AD: R-T Alert	5
Argene Biosoft	6
ELITech: ELITe MGB	7
Real-Time Multiplex	8
Real-Time Single target	11
Qiagen: Artus	16

Distribution number: 3683
Results by specimen

UK NEQAS
International Quality Expertise

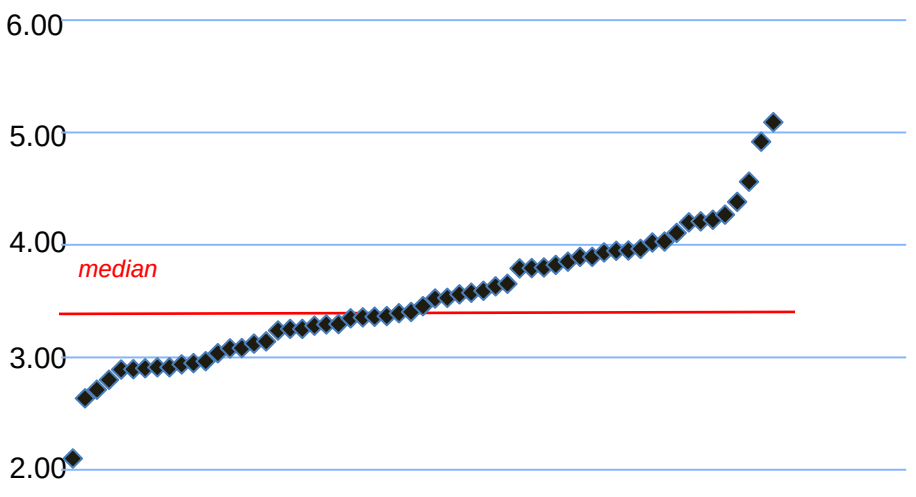
SPECIMEN 2620

- Expected copies/mL 3.47 log (2,900)
- median+/-SD 3.56 +/- 0.54
- range 2.29-4.88



SPECIMEN 2621

- Expected copies/mL 3.47 log (2,900)
- median+/-SD 3.43 +/- 0.57
- range 2.09-5.09

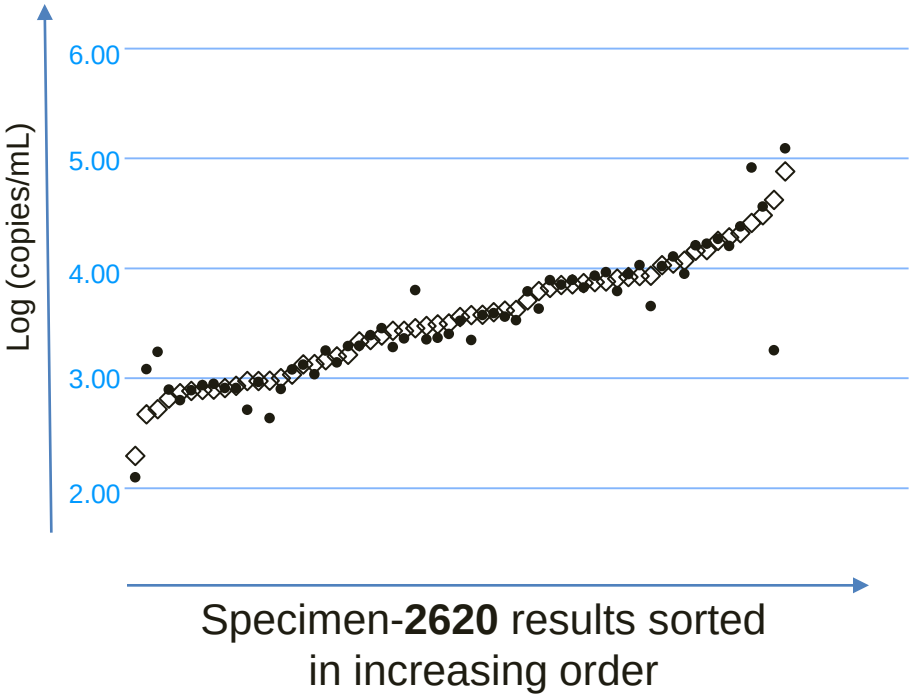


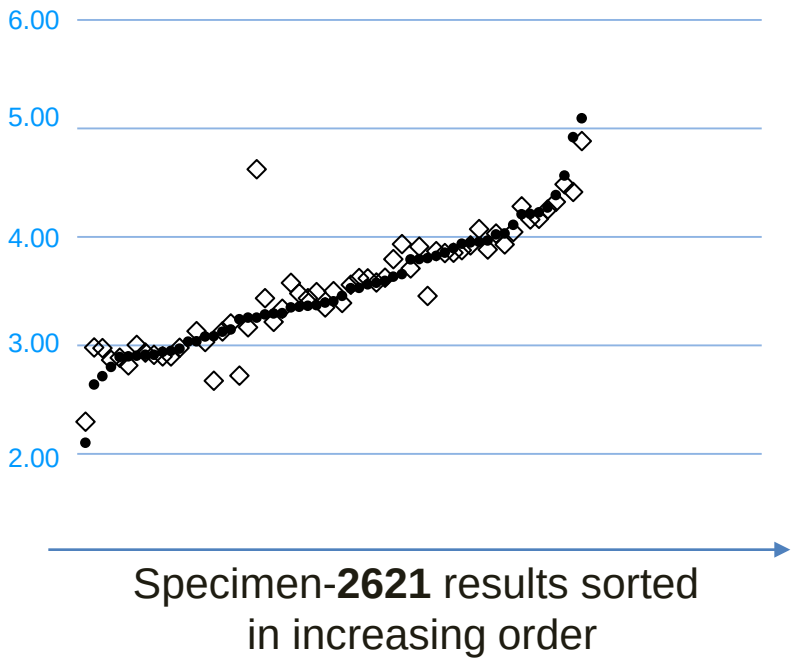
Distribution number: 3683
Results by laboratory

UK NEQAS
International Quality Expertise

◇ Specimen 2620

• Specimen 2621





Distribution number 3683
Results analysed as a log difference

UK NEQAS
International Quality Expertise

96.7% (59/61) detected 3.5 log of EBV DNA (specimen 2620)

96.7% (59/61) detected 3.5 log of EBV DNA (specimen 2621)

-> 59 log differences calculated:	expected	0.00 log
	median+/-SD	0.00+/-0.28
	range	-0.52 - 1.37

→ 96.6% (57/59) reported a -0.51 to 0.49 log difference

Distribution number 3683
Results analysed as a log difference

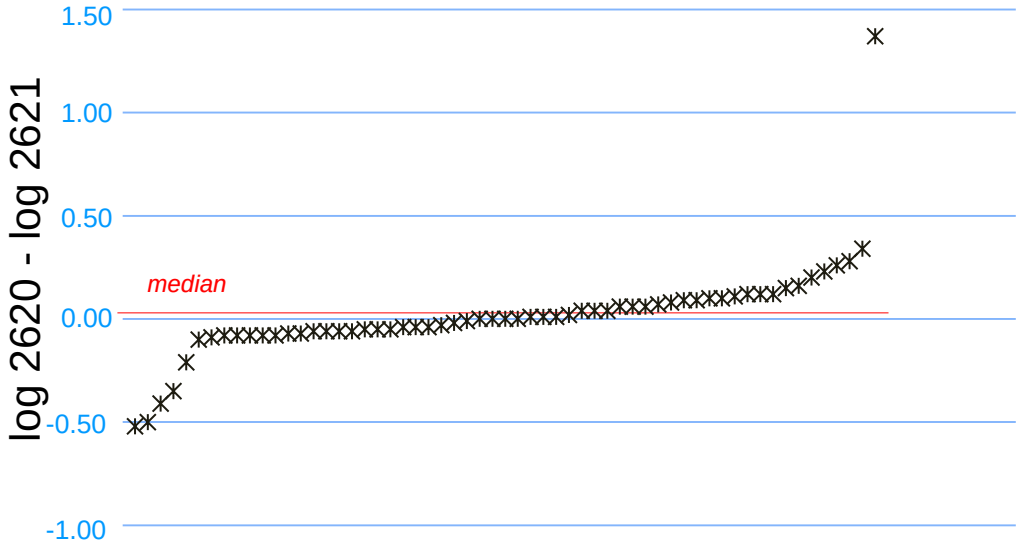
UK NEQAS
International Quality Expertise

96.7% (59/61) detected 3.5 log of EBV DNA (specimen 2620)

96.7% (59/61) detected 3.5 log of EBV DNA (specimen 2621)

-> 59 log differences calculated:	expected	0.00 log
	median+/-SD	0.00+/-0.28
	range	-0.52 - 1.37

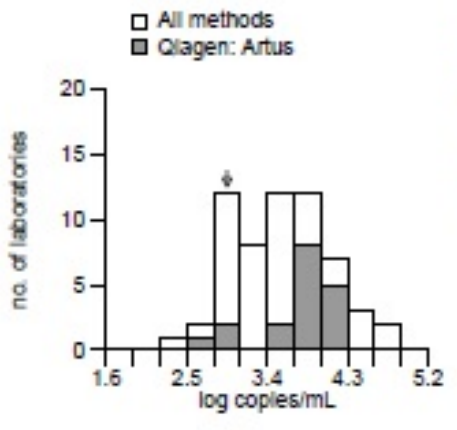
→ 96.6% (57/59) reported a -0.51 to 0.49 log difference



UK NEQAS
International Quality Expertise

Distribution number: 3683

Specimen: 2620

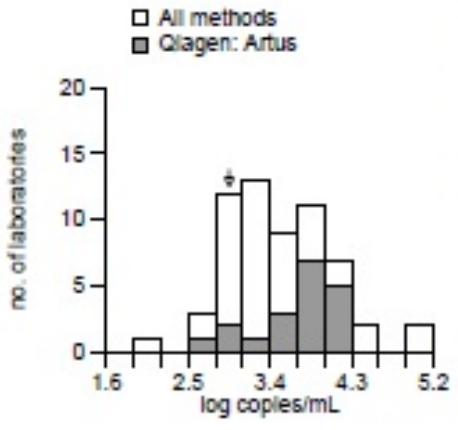


Your result :
2.91 log copies/mL

Method median concentration :
3.87 log copies/mL

Median concentration :
3.56 log copies/mL

Specimen: 2621

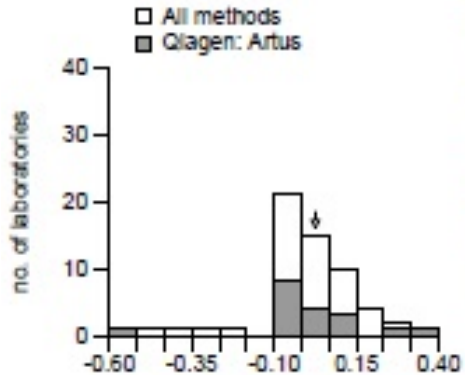


Your result :
2.91 log copies/mL

Method median concentration :
3.82 log copies/mL

Median concentration :
3.43 log copies/mL

Intended result: -0.50-0.50 log copies/mL



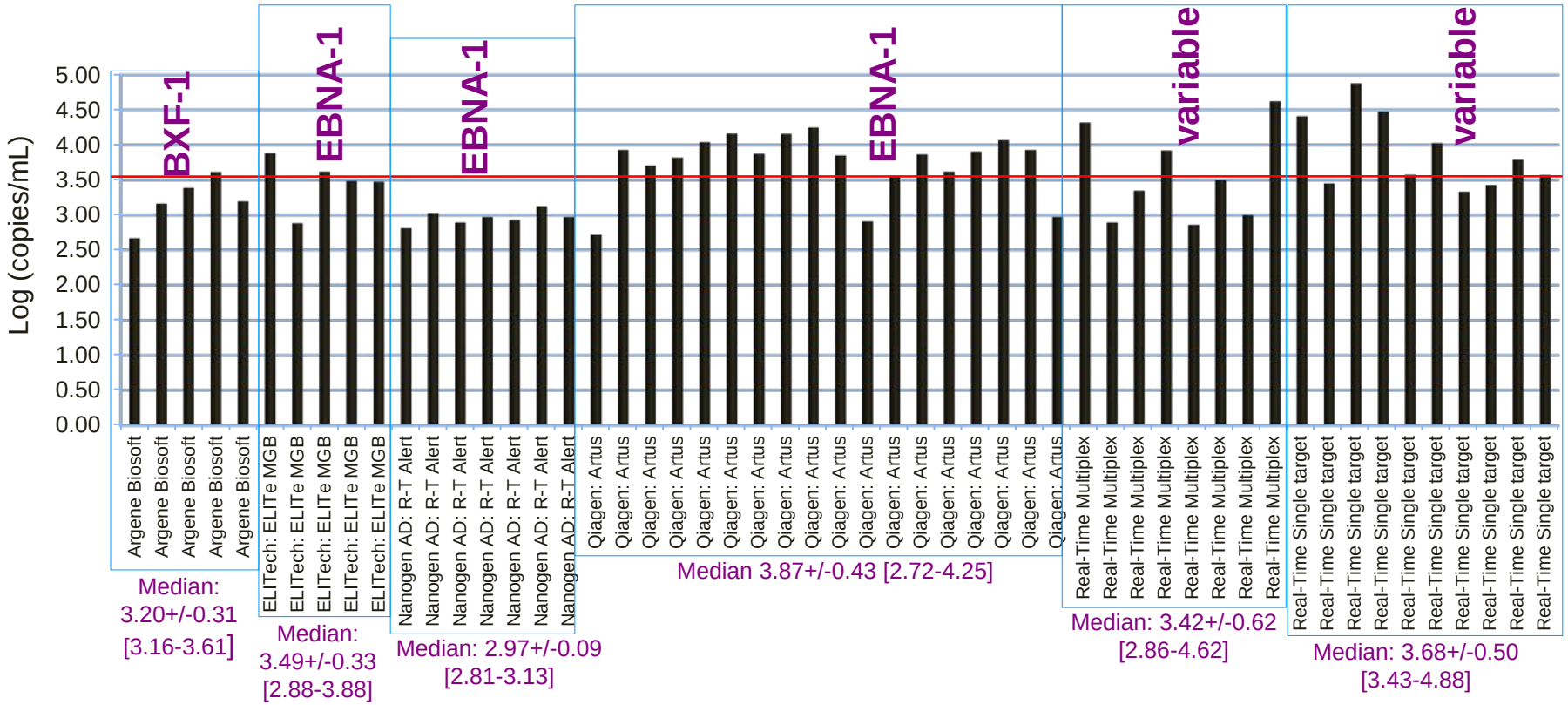
Your result :
Difference in conc. of 0.00 log copies/mL

Your score : 2

Overall results	UK	All	Score
Median			
+/- 0.5 log	25	57	2
+/- >0.5 to 0.75 log	0	1	1
+/- >0.75 log	1	1	-1
One Incorrect	1	1	0
Two Incorrect	0	1	-2
Total	27	61	
%Correct	92.6	93.4	

Specimen number: 2620
Results by amplification methods

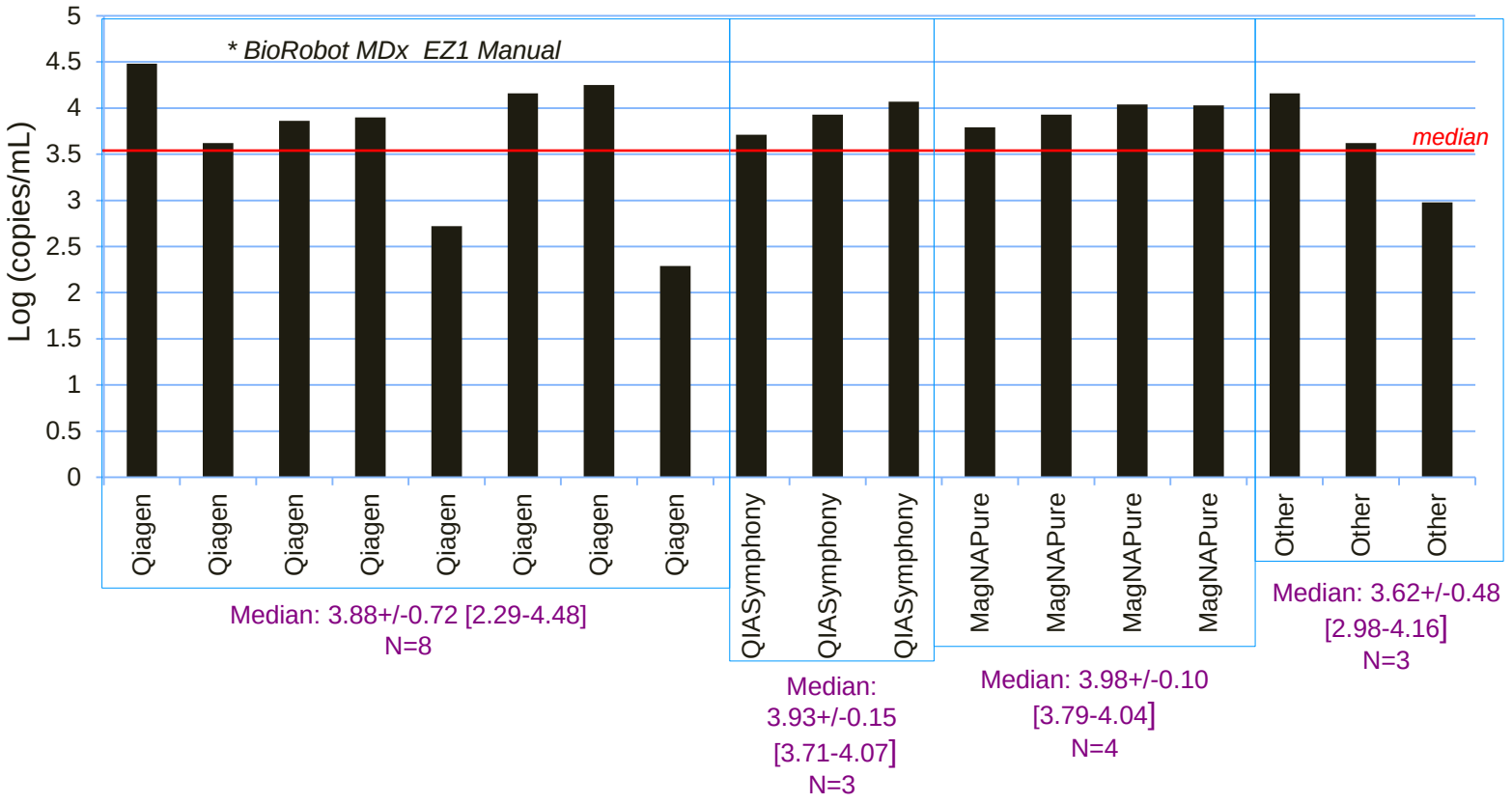
UK NEQAS
International Quality Expertise



Specimen number: 2620

UK NEQAS
International Quality Expertise

Qiagen: Artus results by extraction methods



Extraction and amplification methods

UK NEQAS
International Quality Expertise

Amplification method	N pilot	% pilot	N now	% now
Nanogen AD: Real-Time Alert	21	34.4	8	11.8
Qiagen: Artus	17	27.9	20	29.4
Real-Time Single target	14	23.0	11	16.2
Roche: LightCycler	2	3.3	0	0
Argene Biosoft	2	3.3	5	7.4
PCR: Single target	1	1.6	0	0
Real Time Multiplex	1	1.6	9	13.2
Sacace Real-TM	1	1.6	0	0
ELITech: ELITe MGB	0	0	9	13.2
Unspecified	1	1.6	1	1.5
Other	1	1.6	5	7.4

** BioRobot MDx QIAsymphony EZ1*

- 29.4% in house assays (in pilot 26.2%)
- 70.6% commercial assays: all use IC

- Free choice of extraction methods:

Extraction method	N Pilot	% Pilot	N now	% now
Qiagen*	24	36.9	31	51.7
NucliSENS easyMAG	19	29.2	18	30
MagnaPure	9	13.8	9	15
Magnetic beads	2	3.1	0	0
Nucleo Spin	1	1.5	1	1.7
Abbott: m2000sp	0	0	1	1.7
Unspecified	3	4.6	0	0
Other	3	4.6	0	0

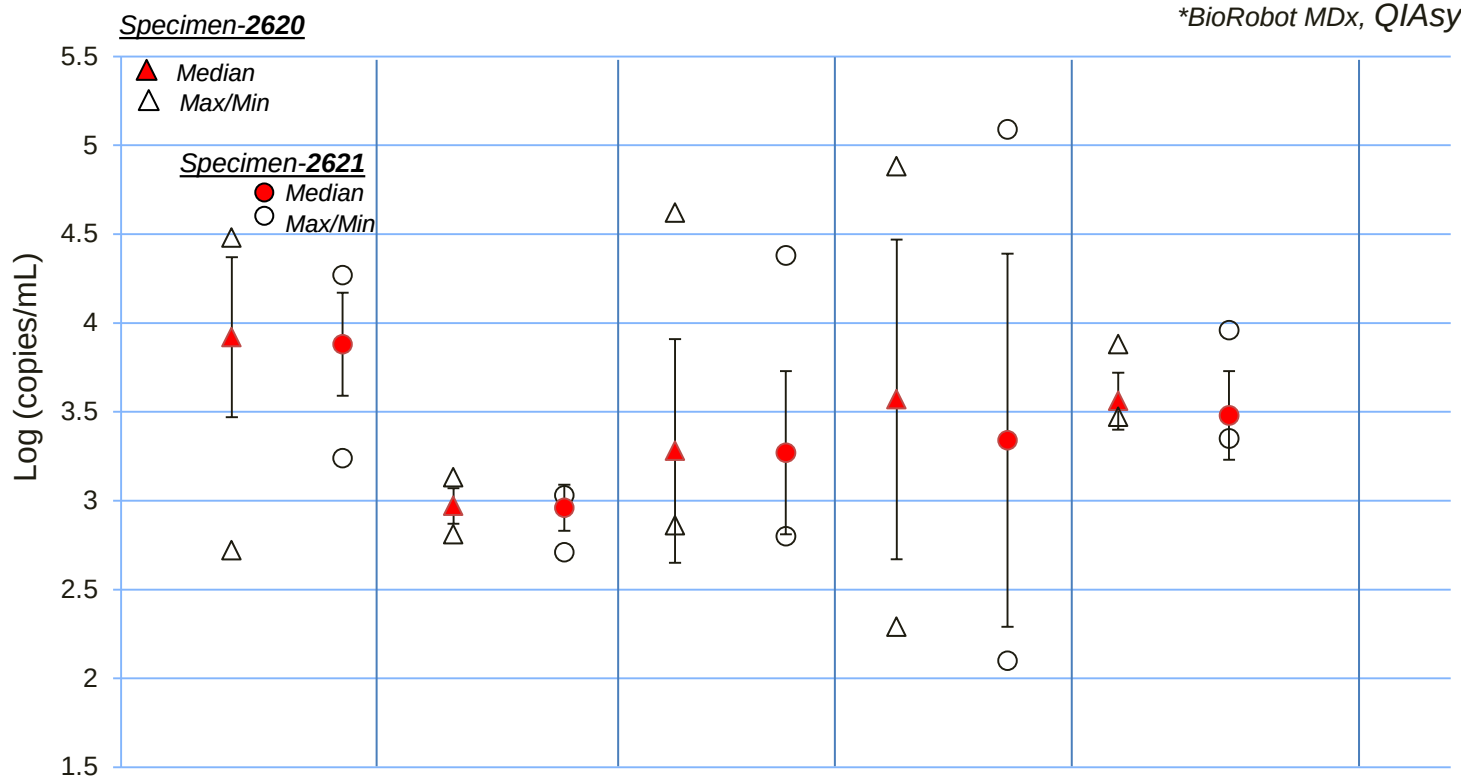
- >22 combinations of extraction and amplification assays
- 5 most frequent combinations

Extraction method	Amplification method	N Pilot	% Pilot	N Now	% Now
NucliSENS easyMAG	Nanogen AD: Real-Time Alert	11	18.0	6	11.3
Qiagen*	Qiagen: Artus	11	18.0	12	22.6
Qiagen*	Nanogen AD: Real-Time Alert	6	9.8	0	0
NucliSENS easyMAG	Real-Time Single target	5	8.2	3	5.7
MagnaPure	Qiagen: Artus	4	6.6	4	7.5
Qiagen*	Real-Time Multiplex	0	0	8	15
Qiagen*	Real-Time Single target	0	0	5	9.4
NucliSENS easyMAG	ELITech: ELITe MGB	0	0	5	9.4

Distribution number 3683
Results for the 5 most frequent E&A assay combinations

UK NEQAS
International Quality Expertise

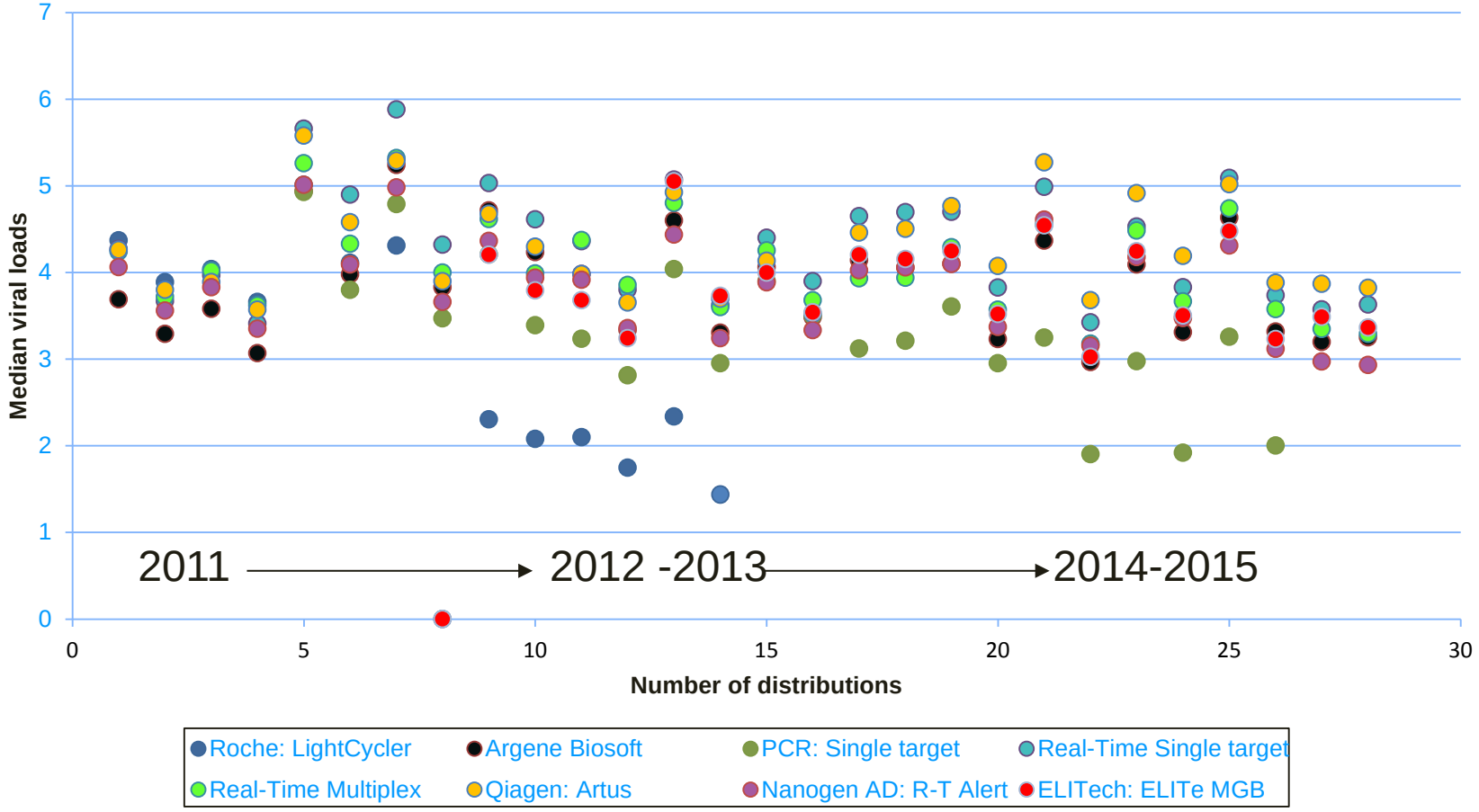
**BioRobot MDx, QIAsymphony, EZ1*



Extraction	**Qiagen***	**NucliSENS easyMAG**	**Qiagen***	**Qiagen***	**NucliSENS easyMAG**
Amplification	**Qiagen: Artus**	**NanogenAD: Real-Time Alert**	**Real-Time Multiplex**	**Real-Time Single target**	**ELITech: ELITe MGB**
n (%)	12 (20%)	6 (10%)	8 (13.3%)	5 (8.3%)	5 (8.3%)
Median 2620 +/- SD	3.92 +/- 0.45	**2.97** +/- 0.10	3.28 +/- 0.63	3.57 +/- 0.90	**3.56** +/- 0.16
Median 2621 +/- SD	3.88 +/- 0.29	**2.96** +/- 0.13	3.27 +/- 0.46	3.34 +/- 1.05	**3.48** +/- 0.25

UK NEQAS
International Quality Expertise

EBV: Median viral load by method



UK NEQAS
International Quality Expertise

Summary

- The introduction of the EBV WHO International Standard is relatively recent: use has not been widely adopted
- Several participants indicated they are in the process of introducing the Standard
- It is too soon to see any improvement in the comparability of results with different kits
- Reportable ranges
- Sample type
- Converting viral load values from c/mL to IU

UK NEQAS
International Quality Expertise

Thank you for listening

Many thanks to:

- Colleagues from the Microbiology Laboratory at the Northern General Hospital, Sheffield for their kind assistance with pre-distribution testing
- Participants for their data
- Great team at UK NEQAS

Brigitte Senechal

Vivienne James

and Maila Mutso