2016 ESCMID Workshop Maastricht

Quality Control for Molecular Diagnostics

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Quality & standardisation have a long history: the Chinese, Greeks and Romans applied it

Qin (Ch'in) Dynasty– *Over 2000 years ago.*
One system of weights, measures, money, written language, laws
World's earliest vernier caliper around 9 AD

Roman Roads: *'Quality checks' a legionnaire inserting a knife into the gap between the paving.*
If the blade entered the joint freely the gap was too big

Coins: *Quality assurance comprised of marks to help regulate the consistency (weight, origin, etc) across the empire.*

'Building blocks': *Quality checks of raw materials, such as limestone. QC of the processes. Regulation was strict and failure to comply carried severe penalties.*

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Modern Standardisation & Quality in Measurements

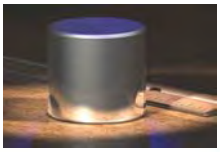
1799
Two platinum standards representing the meter and the kilogram

1960
The International System of Units (SI) established

Seven base units

Metre	- length
Kilogram	- mass
Second	- time
Ampere	- electric current
Kelvin	- temperature
Mole	- amount of substance
Candela	- luminous intensity

The New York Times
Headline news: the kilogram is loosing weight



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Why is quality management in clinical laboratories so important?

- Ability to reduce or eliminate error
- Ensures reliable patient test results
- Improves likelihood of meeting patient's and doctor's expectations
- More effective and efficient operations
- Provides demonstrable quality and compliance with regulatory requirements
- Is or will be mandatory

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Quality Concerns In Molecular Diagnostics

- Rapidly evolving field, new technologies, high expectations, great impact on patient management
- Sensitivity and specificity issues in early days
- Genotypic variation
- Contamination
- Clinical significance
- Large number of in-house assays
- Lack of robustness and standardisation
- Lack of international reference material

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False-Positivity in Molecular Diagnostics: the early years

Proficiency studies in the early years

Year	Pathogen	Reference	Data	False-positives
1992	HIV-1	Bootman and Kitchin (1991)	Not available	54% of labs
1993	Hepatitis C virus	Zaaijer et al. (1993)	31 data sets	29% of data sets
1994	*M. tuberculosis*	Noordhoek et al. (1994)	7 labs	Up to 77% of negative samples
1995	HBV	Quint et al. (1995)	43 data sets	35% of data sets
1996	HCV	Damen et al. (1996)	136 data sets	21% of data sets

Valenthine-Thon, JCV, 2002

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Improvements to molecular diagnostics 1995 - 2015

- Anti-contamination measures: physical separation, UNG system, real-time assays
- Technological developments: reagents, automation, real-time platforms
- Introduction of commercial assays
- WHO International Standards: few, mainly BBV's
- Use of universal internal controls
- External quality assessment programmes

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ISO 15189 :2012 Medical laboratories - Particular requirements for quality and competence

Quality Manual sections

A. Management requirements
- Organisation and management responsibilities, QMS
- Document control, service agreements, control of records
- Complaints, non-conformities, preventive/corrective actions
- Evaluation and audits: continual improvement

B. Technical requirements
- Personnel, accomodation, equipment, reagents and consumables
- **Examination processes**, including pre- and post examination
- **Ensuring quality of examination results: QC and EQA**
- Reporting and release of results

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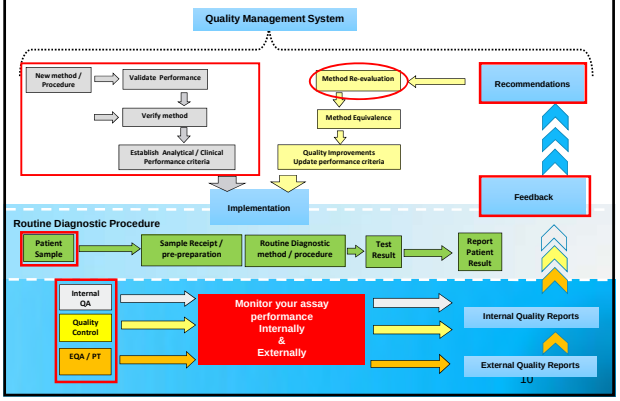
Managing & monitoring assay performance within the molecular laboratory



Analytic process: QA requirements for implementation of a new test

- **Development (in-house) or selection of assay (commercial/CE)**
- **Validation or verification of the assay**
- **Implementation of the assay**
  SOP's, training, equipment, reagents, supplies, reference materials, run controls (QC), alert criteria, IT, reporting, responsibilities
- **Continual monitoring and evaluation of performance**
  - Daily practice:suspicious results
  - Application of statistical procedures to QC results, such as Shewhart/ Levey-Jennings charts, Westgard rules
  - Participation in External Quality Assessment (EQA) schemes
- **Remedial or corrective action, if needed**
- **All to be appropriately registered and documented**

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Validation and Verification (ISO 15189:2012 – 5.5.1)

- **Validation:** evaluation of an assay's performance in order to determine its fitness for use, including accuracy, precision, analytical sensitivity and specificity, clinical relevance (LDT and CE-IVD assays)
- **Verification**: evaluation of an assay's performance in relation to its known or reported performance, for instance against a manufacturer's specifications (f.e. CE - marked IVD assays): accuracy, precision (intra/interassay), linearity.

References: Rabenau et al. J Clin Virol 2007, Bustin et al. Clin Chem 2009, Raymaekers et al J Clin Lab Anal 2009

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Verification and Validation : number of samples required

		Verification (IVD/CE)		Validation (in-house)	
Characteristic	Sample type	Qualitative	Quantitative	Qualitative	Quantitative
Accuracy	Positive	3	3	3	3
	Low positive*	3	3	3	3
	Negatieve	3	3	3	3
Sensitivity	Positive	-	-	10	10
	Low positive*	-	-	10	10
Specificity	Negative	-	-	20	20
Precision (intra)	Positive	1	3	1	6
	Low positive*	1	3	1	3
Precision (inter)	Positive	1	1	1	2
	Low positive*	1	1	1	1
Linearity	Positive	0	1	0	2

* Up to 1 log 10 over the LOD

Rabenau et al, 2007

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Quality Control (ISO 15189: 2012 - 5.6.2.2)

- ✓" The laboratory shall use QC materials that react to the examining system in a manner as close as possible to a patient's specimen "
  - Laboratory should choose concentrations **close to clinical decision levels**
  - Use of **independent 3rd party controls** should be considered
- ✓" When control rules are violated ..... results shall be rejected and patient's samples re-examined ..... "
  - QC data shall be reviewed regularly using **statistical and non-statistical techniques** for process control
- ✓Type of QC materials
  - Positive & negative internal controls: assess validity of results of **every run** (LDT, CE-IVD assays)
  - Universal intern control: assess validity of results of **every sample** (LDT)
  - External run controls: independent 3rd party control to monitor daily test variation, lot-to-lot performance, operator variation, identifies **increased random and systemic variation** (CE-IVD assays)

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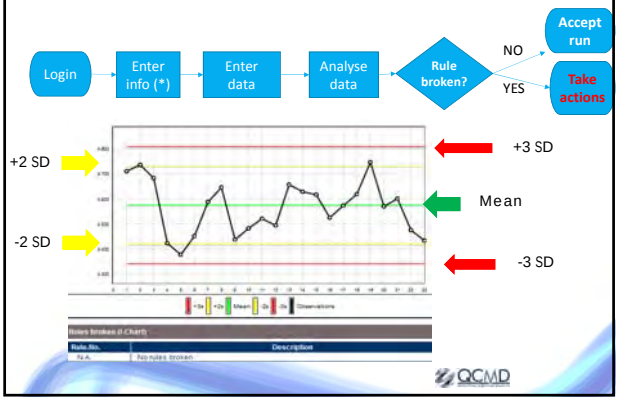
Daily assay monitoring: Shewhart plot



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Westgard rules

- 1_{2SD} : IQC value exceeds the mean by ±2SD: usually warning; probably random result
- 2_{2SD} : two consecutive IQC values exceed the mean on the same side of the mean by ±2SD; possibly systemic error
- 4_{1SD} : four consecutive IQC values exceed the same limit (mean ± 1SD), may indicate the need to perform instrument maintenance or reagent calibration
- 1_{3SD} : IQC value exceeds mean + 3SD : reject result, examine and correct
- Others:

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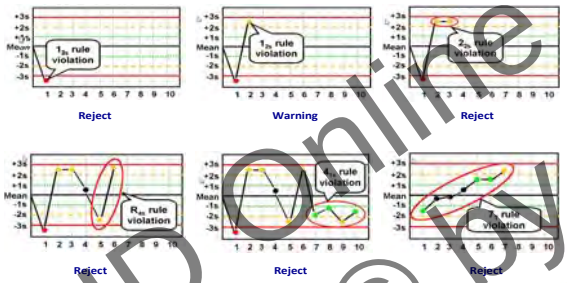
Examples of Westgard rules



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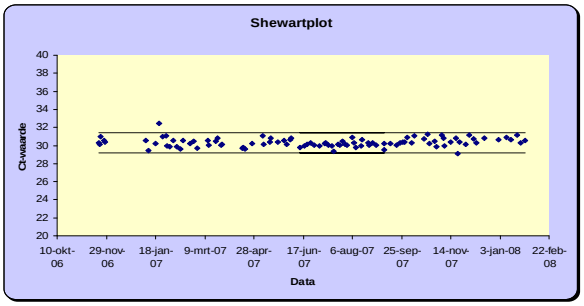
Positive run control TOX-PCR: Shewhart plot



Mean Ct value: 30.3 ± 0.55

Acceptable range Ct value (2SD): 29.2 – 31.4

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Universal Internal Controls

DNA viruses

Phocine HerpesVirus 1 (PhHV)

RNA viruses

Encephalomyocarditis virus (EMCV)

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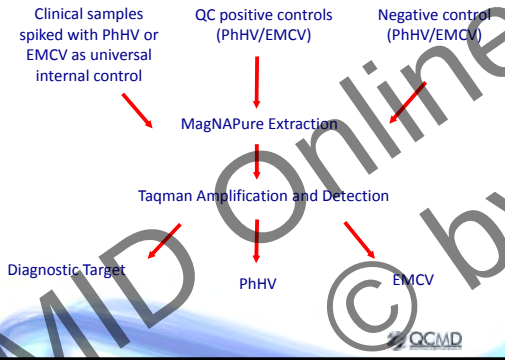
Molecular diagnostic assay setup: universal internal controls



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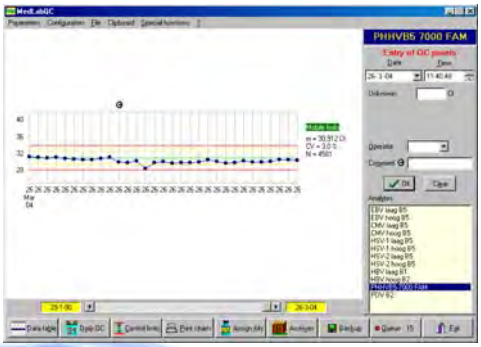
Ct values Universal Internal Controls



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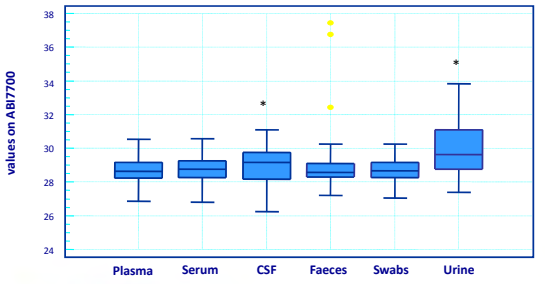
Comparison universal internal control on various clinical samples



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The importance of clinical relevance in MDx assay design…

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Clinical relevance of laboratory testing: any information that contributes to patient management

- ✓Testing purpose: diagnosis, monitoring, screening
- ✓Viral load: threshold level for disease, relation to severity/outcome?
- ✓Type of patient: immunocompetent/-compromised; neonate vs adult, etc
- ✓Duration of illness, antiviral treatment?
- ✓Type (and quality) of material: CSF, vesicle fluid, BAL, stool, swab, etc
- ✓Type of virus and genotype; are all genotypes equal, or some more than others? HPV, EV, etc

→ Interpretation of MDx results requires clinical information and microbiological expertise

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Clinical relevance : viruses detected in LRTI patients visiting their GP, and their matched controls (GRACE study)

Virus	Patients (n=3104)	Controls (n= 2063)	P-value
Rhinovirus	623 (20.1%)	72 (3.5%)	P < 0.0001
Influenzavirus A/B	307 (9.9%)	7 (0.3%)	P < 0.0001
Coronavirus	231 (7.4%)	19 (1.4%)	P < 0.0001
RSV	144 (4.6%)	10 (0.5%)	P < 0.0001
hMPV	138 (4.4%)	3 (0.1%)	P < 0.0001
Parainfluenzavirus	81 (2.6%)	7 (0.3%)	P < 0.0001
Adenovirus	41 (1.3%)	23 (1.1%)	0.831
Polyomavirus	69 (2.2%)	52 (2.5%)	N.S.
Polyomavirus WU	44 (1.4%)	36 (1.7%)	N.S.
Polyomavirus KI	27 (0.9%)	17 (0.8%)	N.S.
Bocavirus	18 (0.6%)	16 (0.8%)	N.S.

G leven et al 2014

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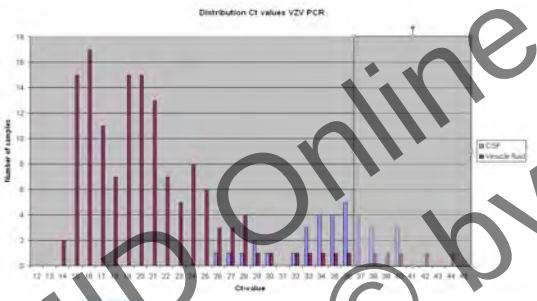
VZV Ct value distribution: CSF vs vesicle fluid



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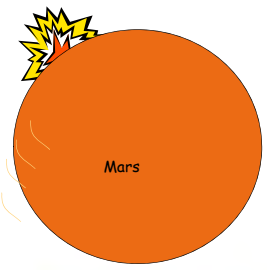
Need for Standardisation: the case of the Mars Orbiter



The likely cause.....

Lack of standardization when determining thruster impulse

One team used Pound force (Imperial unit)
Other team used Newton (Metric measurement).

One pound force = 4.45 Newton .....

Therefore the spacecraft's trajectory was 4.4 times greater than NASA navigation team believed.

Source: Report NASA mission failure investigation board November 1999

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Observed variation in molecular testing across different EQAs.

Less variation,

Quantitation: more clinical relevant

Virus Target (EQA programme)	International Standard	SD range of geometric mean (log_{10})
HIV	Y	0.17 – 0.27
HCV	Y	0.20 – 0.30
HBV	Y	0.30 – 0.45
CMV	Y	0.40 – 0.53
EBV	Y	0.46 – 0.63
HAV	Y	>1.0
BKV	N	0.50 – 0.60
HSV	N	0.60 – 0.70
EV	N	>1.0

More variation,

Quantitation: less clinical relevant?

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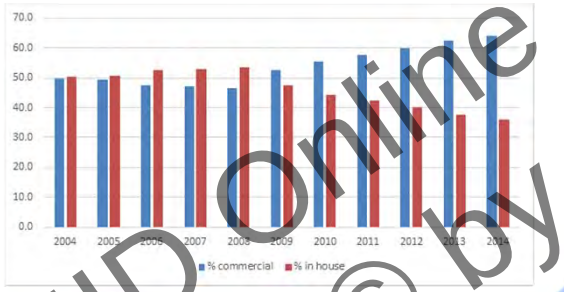
In house versus commercial assay reported across QCMD EQA programmes



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ISO 15189:2012 Medical Laboratories - Requirements for quality and competence

Section 5.6.3 Interlaboratory comparisons

" The laboratory shall participate in an interlaboratory comparison programme(s) (such as an **external assessment programme or proficiency testing programme**) appropriate to the examination and interpretations of examination results. "

" Interlaboratory comparison programme(s) chosen by the laboratory shall, as far as possible, provide **clinically relevant challenges** that mimic patients samples and have the effect of checking the entire examination process ."

" The laboratory should participate in interlaboratory programmes that substantially fulfil the relevant **requirements of ISO/IEC 17043**. "

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External Quality Assessment (EQA/PT)

- ✓ Integral part of a laboratory quality assurance procedures; essential requirement for accreditation
- ✓ Helps identify weak spots in a laboratory's general performance
- ✓ Helps improves reliability of results, and gives confidence in reporting results
- ✓ Allows comparison of performance with other laboratories & methods
- ✓ Provides a reference level in the absence of International Standards.
- ✓ Mandatory requirement in many countries.

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External Quality Control MDx in Europe : 2001

- No uniform regulatory system for medical laboratories across Europe
- QC organisations for external proficiency testing in many countries (NEQAS, INSTAND, EQUALIS, SKML, Labquality, etc.), mainly focusing on (virus)culture and serology.
- Lack of International Standards or reference reagents for molecular testing
- Molecular methods: high rate of false-positives (> 40 %); sensitivity often unclear
- QCMD : Quality Control *for* Molecular Diagnostics was established as an independent, not-for-profit organization run and served by experts from the field (www.qcmd.org)

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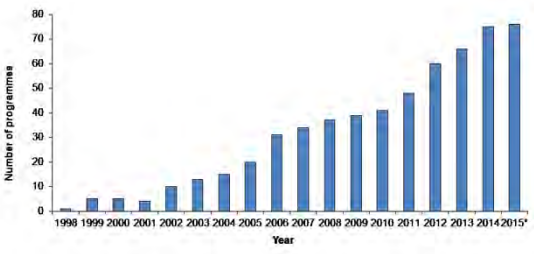
Number of QCMD programmes per year



* Programme year currently in progress.

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Distribution of EQA participation in 2015

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Differences in sensitivity in MDx of enteroviruses EU-QCCA panel 1998

Virus	Viral load (cps/ml)	All datasets (n = 70)	Commercial test X (n = 16)
CA9	10^7	63	15
CA9	10^6	64	13
CA9	10^5	55	9
CA9	10^4	30	2
CA9	10^3	10	1

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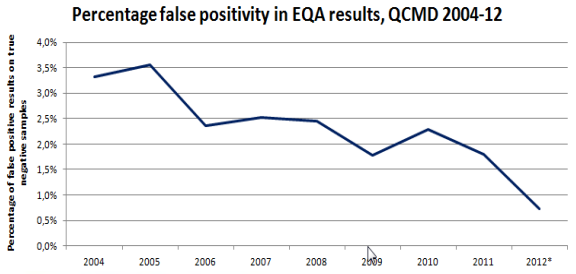
False-positivity rate in QCMD proficiency panels : 2004 - 2012



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QCMD 2010 Aspergillus distribution

Sample	Sample content	Sample conc.	Total datasets n=27	
			n	%
ASPDNA10-01	*A. fumigatus* DNA	30,000 G.eq/ml	24	88.9
ASPDNA10-04	*A. fumigatus* DNA	3000 G.eq/ml	19	70.4
ASPDNA10-02	*A. fumigatus* DNA	300 G.eq/ml	7	25.9
ASPDNA10-03	Clinical negative		22	81.5
ASPDNA10-05	*A. fumigatus* DNA	30,000 G.eq/ml	24	88.9
ASPDNA10-07	*A. fumigatus* DNA	3000 G.eq/ml	13	48.1
ASPDNA10-09	*A. fumigatus* DNA	300 G.eq/ml	8	29.6
ASPDNA10-06	Analytical negative		21	77.8
ASPDNA10-13	*A. fumigatus* conidia	1000 Con/ml	21	77.8
ASPDNA10-10	*A. fumigatus* conidia	100 Con/ml	16	59.3
ASPDNA10-11	*A. fumigatus* conidia	10 Con/ml	11	40.7
ASPDNA10-12	Clinical negative		23	85.2

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QCMD 2010 Legionella distribution: sensitivity and conyamination issues

Sample	Sample content	Sample conc.	Total datasets n=87		PCR						Other[a]	
					Conventional				Real time			
					Commercial[a] n=7		In-house[b] n=3		Commercial[c] n=15		In-house[d] n=61		n=1	
			n	%	n	%	n	%	n	%	n	%	n	%
LP10-01	*L. pneumophila* sg1 (DNA)	1.0 x 10^4 gen.eq/ml	87	100.0	7	100.0	3	100.0	15	100.0	61	100.0	1	100
LP10-03	*L. pneumophila* sg1 (DNA)	1.0 x 10^3 gen.eq/ml	82	94.3	6	85.7	2	66.7	15	100.0	58	95.1	1	100
LP10-02	*L. longbeachae* (DNA)	1.0 x 10^3 gen.eq/ml	77	88.5	7	100.0	2	66.7	13	86.7	54	88.5	1	100
LP10-05	*L. pneumophila* sg1	1.8 x 10^5 cfu/ml	87	100.0	7	100.0	3	100.0	15	100.0	61	100.0	1	100
LP10-09	*L. pneumophila* sg1	1.8 x 10^4 cfu/ml	87	100.0	7	100.0	3	100.0	15	100.0	61	100.0	1	100
LP10-07	*L. pneumophila* sg1	1.8 x 10^3 cfu/ml	83	95.4	5	71.4	2	66.7	15	100.0	60	98.4	1	100
LP10-04	*L. pneumophila* sg3	1.3 x 10^5 cfu/ml	59	67.8	1	14.3	0	0.0	9	60.0	48	78.7	1	100
LP10-08	*L. pneumophila* sg3	1.3 x 10^3 cfu/ml	29	33.3	1	14.3	0	0.0	5	33.3	22	36.1	1	100
LP10-10	*L. micdadei*	1.0 x 10^4 cfu/ml	76	87.4	7	100.0	3	100.0	13	86.7	53	86.9	0	0
LP10-06	*L. pneumophila* negative		75	86.2	7	100.0	3	100.0	13	86.7	52	85.2	0	0

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Effect of serotype variation : results QCMD EVRNA11 panel

Sample	Sample content	Stock titre TCID50/0.05ml	Dilution factor	Total datasets n=245	
				n	%
EVRNA11-01	Coxsackievirus A16	$4.0x10^5$	$1x10^{-5}$	242	98.8
EVRNA11-09	Coxsackievirus A21	$2.3x10^3$	$1x10^{-5}$	225	91.8
EVRNA11-02	Coxsackievirus A24	$1.5x10^4$	$1x10^{-5}$	225	91.8
EVRNA11-07	Echovirus 11	$2.5x10^7$	$1x10^{-5}$	232	94.7
EVRNA11-11	Echovirus 11	$2.5x10^7$	$1x10^{-7}$	134	54.7
EVRNA11-12	Echovirus 30	$2.7x10^5$	$4x10^{-6}$	239	97.6
EVRNA11-05	Enterovirus 68	$1.6x10^4$	$1x10^{-3}$	202	82.4
EVRNA11-03	Enterovirus 68	$1.6x10^4$	$1x10^{-5}$	117	47.8
EVRNA11-08	Enterovirus 71	$1.0x10^5$	$1x10^{-5}$	240	98.0
EVRNA11-06	Enterovirus 71	$1.0x10^5$	$1x10^{-4}$	212	86.5
EVRNA11-04	Human rhinovirus 16	$2.5x10^4$	$1x10^{-3}$	218	89.0
EVRNA11-10	Negative (VTM)			235	95.9

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QCMD Pilot EQA's for 2016

Programme Code	Programme Description
BSEPSIS16	Bacterial Sepsis
B16SrRNA16	Bacterial 16S Ribosomal RNA
EBOLA16	Ebola Virus
HSVDR16	HSV drug resistance
EVTP16	EV typing
DERMA16	Dermatophilosis
RESPI	Multi-Respiratory I - Targeted for multiplex systems - Potentially include Inf A and B, RSV, MPV
RESPII	Multi-Respiratory II - Targeted for multiplex based systems - Potentially include RV, EV, CV, PINF, ADV

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Summary and Conclusions (1)

- Establishing and maintaining an accredited QMS under ISO 15189:2012 requires a major effort from most laboratories, but is or will be mandatory in most countries in the near future
- The introduction of MDx is rapidly and fundamentally changing the laboratory diagosis of infectious diseases
- Much progress has been achieved in improving the quality of MDx methods since the early days
- However, continuous monitoring of quality remains essential

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Summary and Conclusions (2)

- Quality assurance measures should include the use of:
  - Run controls (internal/external): validation of every run
  - Universal internal controls: validation of every sample
  - Participation in external quality assessment programmes
- Issues still to be adressed:
  - False-positivity rate (contamination, cross-reactivity)
  - International reference materials
  - Clinical relevance
  - Costs
- New QC challenges
  - Multiplex assays
  - Point-of-care testing
  - NGS

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