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healthy all life long

QUALITY CONTROL RESULTS ON MOLECULAR MICROBIOLOGY

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Legal framework

. IPH (Sciensano) organizes EQA for more than 25 years

. Prof. J-C. Libeer

. Royal Decree of 3/12/1999 on the licensing of clinical biology laboratories including the mandatory participation to EQA organized by IPH (now Sciensano).

. RD of 18.04.2008. Creation of article 24bis (INAMI/RIZIV)

Obligation of accreditation ISO15189.

RD 29.05.2009 on Research of High Risk HPV by Molecular methods (Article 32)

RD 05.12.2011 on licensing of Pathological Anatomy labs

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What is proficiency testing?

Proficiency testing determines the performance of individual laboratories for specific tests or measurements and is used to monitor laboratories' continuing performance.

Proficiency testing is also called interlaboratory comparison or External Quality Assessment. As this term implies, proficiency testing compares the measuring results obtained by different laboratories.

In a proficiency test one or more samples are sent around between a number of participating laboratories. Each laboratory analyzes the sample according to a given set of instructions and reports its results to the PT organizer.

The results reported by each laboratory for a measurand are compared to the reference value for that measurand. The reference value can be determined in various ways. The two most common ways are to use a reference laboratory or use the average of the values reported by the participants.

The parameters

Article 24.

Chlamydia trachomatis and *Neisseria gonorrhoeae*

Article 24bis

HSV, VZV, EV

HBV, HCV, HCV genotyping

M. tuberculosis

T. gondii

Article 32

HrHPV

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The panels

Collaboration with QCMD since 2008

QCMD: Quality Control for Molecular Diagnostics-Scotland (www.qcmd.org)

Panels contain negative and positive samples

Sample type	Scoring
Negative	0 if success, 3 if failing
Frequently detected (>95%)	0 if success, 3 if failing
Detected (65≤X≤95%)	0 if success, 2 if failling
Infrequently detected (<65%)	0 if success, 1 if failling

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ISO17043

QCMD is accredited for PT organisation

Homogeneity: involves to test samples

Stability: involves logistic problems (dry ice shipments)

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Comparison with non Belgian participants

Sample code	Sample status	Total All participants n=290				Total BEIPH only n=23			
		0	1	2	3	0	1	2	3
EVRNA14-07	Detected	274	0	16	0	23	0	0	0
EVRNA14-09	Frequently detected	286	0	0	4	23	0	0	0
EVRNA14-03	Detected	274	0	16	0	23	0	0	0
EVRNA14-11	Frequently detected	284	0	0	6	23	0	0	0
EVRNA14-05	Detected	225	0	65	0	20	0	3	0
EVRNA14-12	Frequently detected	282	0	0	8	23	0	0	0
EVRNA14-06	Detected	229	0	61	0	22	0	1	0
EVRNA14-10	Detected	275	0	15	0	23	0	0	0
EVRNA14-01	Frequently detected	287	0	0	3	23	0	0	0
EVRNA14-04	Detected	224	0	66	0	21	0	2	0
EVRNA14-08	Negative	282	0	0	8	23	0	0	0
EVRNA14-02	Negative	284	0	0	6	23	0	0	0

☺ The Belgian laboratories performed well in general

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Disadvantages

No control on samples or on the timing for a survey

No possibility to be accredited for these parameters

Expensive samples

Since 2015: We organize the PT for *C. trachomatis*, *N. gonorrhoeae* and *T. gondii*.

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Chlamydia: the samples

sample	*Matrix**	*C. trachomatis*	*N. gonorrheae*
BMM 2016-1	*urine*	*Frequently detected*	*Negative*
BMM2016-2	*urine*	*Negative*	*Negative*
BMM2016-3	*urine*	*Frequently detected*	*Frequently detected*
BMM2016-4	*Urine*	*Negative*	*Frequently detected*
BMM2016-5	*Urine*	*Negative*	*Frequently detected*
BMM2016-6	*Swabs*	*Negative*	*Negative*
BMM2016-7	*Swabs*	*Frequently detected*	*Negative*
BMM 2016-8	*Swabs*	*Negative*	*Frequently detected*
BMM2016-9	*Swabs*	*Frequently detected*	*Frequently detected*
BMM2016-10	*Swabs*	*Negative*	*Frequently detected*

C. trachomatis Proficiency per sample

sample	Matrix	Status	Negative	Positive	ND	comment
BMM2016-1	urine	Frequently detected	0	78	0	Ok
BMM2016-2	urine	Negative	72	0	6	5 inhibitions 1 ND
BMM2016-3	urine	Frequently detected	1	77	0	1 false negative
BMM2016-4	Urine	Negative	75	3	0	3 false positives
BMM2016-5	Urine	Negative	76	2	0	2 false positive
BMM2016-6	swabs	Negative	71	2	5	4 inhibitions 1 Not determined 2 false positives
BMM2016-7	swabs	Frequently detected	0	78	0	OK
BMM2016-8	swabs	Negative	75	2	1	2 false positives 1 inhibition
BMM2016-9	swabs	Frequently detected	0	78	0	OK
BMM2016-10	swabs	Negative	78	0	0	OK

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Summary

78 participants and 10 samples = 780 results

Result	N (%)
Total number	780 (100%)
Correct answer	758 (97.2%)
Wrong answer	22 (1.8%)
False negative	1 (4.5%)
False positive	9 (40.9%)
Not determined	12 (54.5%)

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Scores

The minimum score was 0 in case of 100% of good results and the maximum score was 30 in case of 100% of wrong answers.

70 laboratories (89.7%) obtained a score of 0.
6 laboratories (7.7%) obtained a score of 3.
2 laboratories (2.6%) obtained a score of 6.

Comparison of methods

Method	N	total	Good Results	%	FP	FN	ND	Ranking
CT/NG Presto	1	10	10	100	0	0	0	1
Roche cobas 4800 CT/NG Test	14	140	140	100	0	0	0	1
Home made Realtime	4	40	40	100	0	0	0	1
Diagenode CT/NG Real time PCR	3	30	30	100	0	0	0	1
BD Probetec ET CT/GC/AC Amplified DNA Assay	1	10	10	100	0	0	0	1
BD MAX CT/NG/TV	1	10	10	100	0	0	0	1
TIB Mol Biol CT	1	10	10	100	0	0	0	1
Abbott RealTime CT/NG	20	200	199	99.5	1	0	0	2
COBAS TaqMan CT Test v2.0	8	80	78	97.5	1	1	0	3
Artus CT/NG	2	20	19	95	0	0	1	4
GenXpert CT/NG	15	150	139	92.7	0	0	11	5
Meridien Illumigen CT	6	60	55	91.7	5	0	0	6
Bio-Rad Dx CT/NG/MG assay	2	20	18	90	2	0	0	7
total	78	780	758	97.2	9	1	12	

FP: false positive-FN: false negative-ND: Not determined

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Example of qualitative and quantitative parameter
HBV in plasma

Samples	*Survey*	*Matrix*	*Content*	*status*
HBV16C1-01	2016/2	Plasma	HBV type A	Frequently Detected
HBV16C1-02	2016/2	Plasma	HBV type D	Frequently Detected
HBV16C1-03	2016/2	Plasma	HBV type A	Frequently detected
HBV16C1-04	2016/2	Plasma	No HBV	Negative
HBV16C3-01	2016/5	Plasma	HBV type A	Frequently Detected
HBV16C3-02	2016/5	Plasma	HBV type A	Frequently Detected
HBV16C3-03	2016/5	Plasma	HBV type A	Frequently Detected
HBV16C3-04	2016/5	Plasma	HBV type D	Frequently Detected

Proficiency per sample: qualitative data

Sample	Status	Negative	Positive	Comment
HBV16C101	Frequently Detected	0	22	Ok
HBV16C102	Frequently Detected	0	22	Ok
HBV16C103	Frequently detected	0	22	Ok
HBV16C104	Negative	21	1	1 false positive
HBV16C301	Frequently Detected	0	21	Ok
HBV16C302	Frequently Detected	0	21	Ok
HBV16C303	Frequently Detected	0	21	Ok
HBV16C304	Frequently Detected	0	21	Ok

Summary methods

Method	N (survey 2016/2)	N (Survey 2016/5)
Abbott Realtime	10	9
Altona Diagnostics RealStar	1	1
Qiagen Artus Real Time	3	3
Real-time In-House PCR	2	2
Roche Cobas Taqman	6*	6

*: false positive sample

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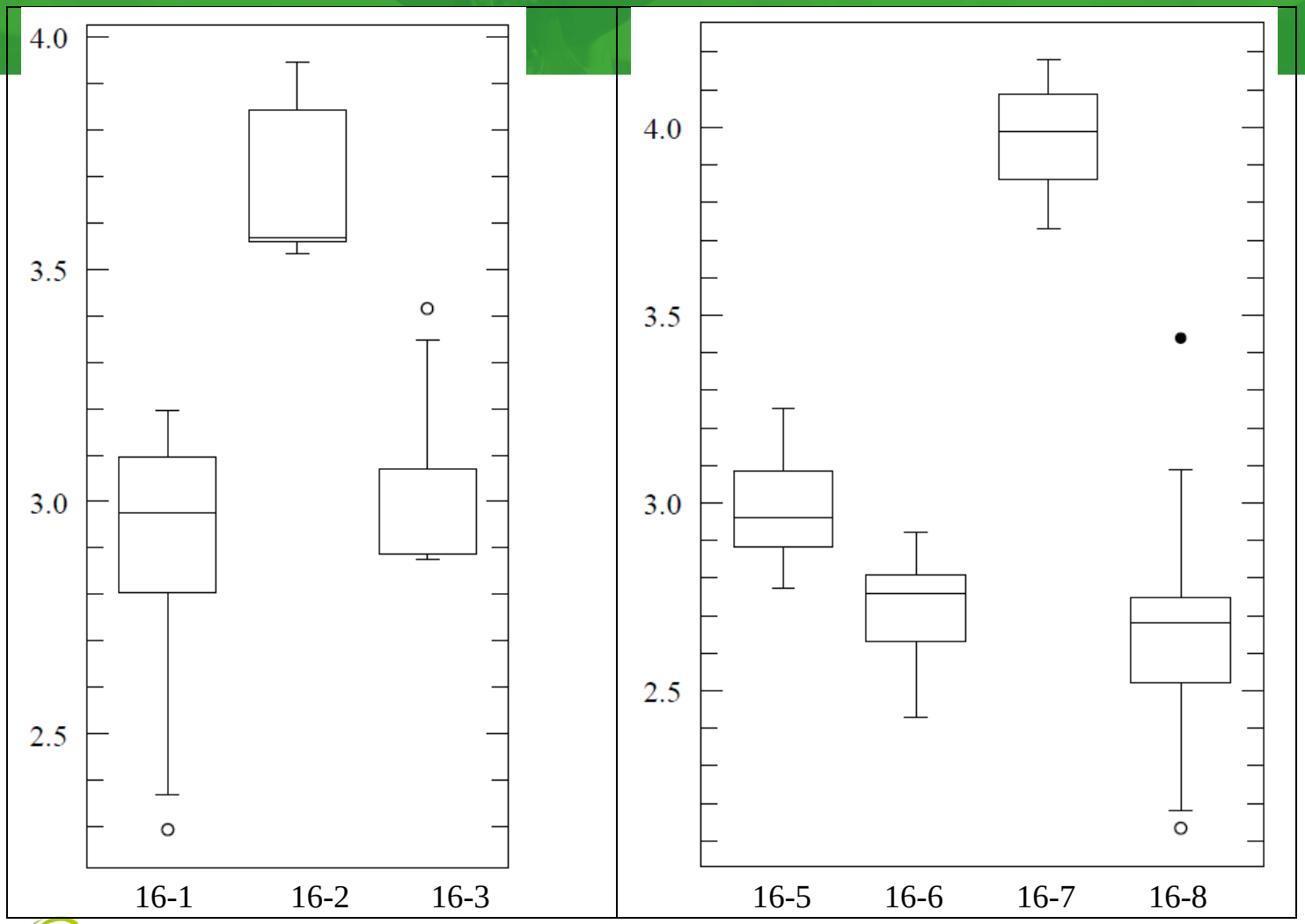
Box-plots

LogIU/mL



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Quantitative results

Target value (M): median of the participants
Z score= (X-M)/SD
X: participant value
SD= standard deviation= (P75-P25)/1.349
|Z| <3

Sample	Z <1	1<Z<2	2<Z<3	Z>3	Comment
HBV16C1-01	16	5	0	1	1 cited laboratory
HBV16C1-02	14	6	2	0	OK
HBV16C1-03	19	3	0	0	OK
HBV16C3-01	13	6	2	0	OK
HBV16C3-02	14	6	1	0	OK
HBV16C3-03	13	8	0	0	OK
HBV16C3-04	15	4	0	2	2 cited laboratories

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Methods

Method	N	HBV16C1-01 Target value (Log10 UI) 2,976 SD (Log10 UI) 0,193 Z-score	HBV16C1-02 3,619 0,146 Z-score	HBV16C1-03 2,98 0,199 Z-score
Abbott Realtime	10	-1,02	-1,04	-1,42
		-0,59	-0,44	0,035
		-1,22	-0,56	-0,78
		-0,89	-1,50	-0,65
		-0,68	-1,24	-1,12
		-0,10	0,50	0,18
		0,06	-0,02	-0,30
		-1,12	-0,62	-0,47
		0,69	1,13	0,04
		0,31	0,85	0,62
Altona Diagnostics RealStar	1	-3,61	-0,10	-0,99
Qiagen Artus Real Time	3	0,15	-0,60	0,59
		0,89	-0,27	-0,57
		0,61	0,51	0,65
Real-time In-House PCR	2	0,32	0,02	0,61
		-1,01	-1,34	-0,92
Roche Cobas Taqman	6	0,11	0,22	0,42
		0,62	2,03	0,83
		1,14	1,60	0,71
		-0,13	0,90	-0,04
		0,72	2,14	1,11
		-0,23	0,40	-0,79

Error type 1: lack of sensitivity

In sample CTB08-05 (50 copies/tube): 50% false negative answers

CT: Swedish variants (concerned several methods)

In 2010, for the detection of TG in Amnios (5 copies/ml): 36/60 good answers (65%).

In sample BMM 2015/11 (8 copies TG/mL): 71.4% false negative results

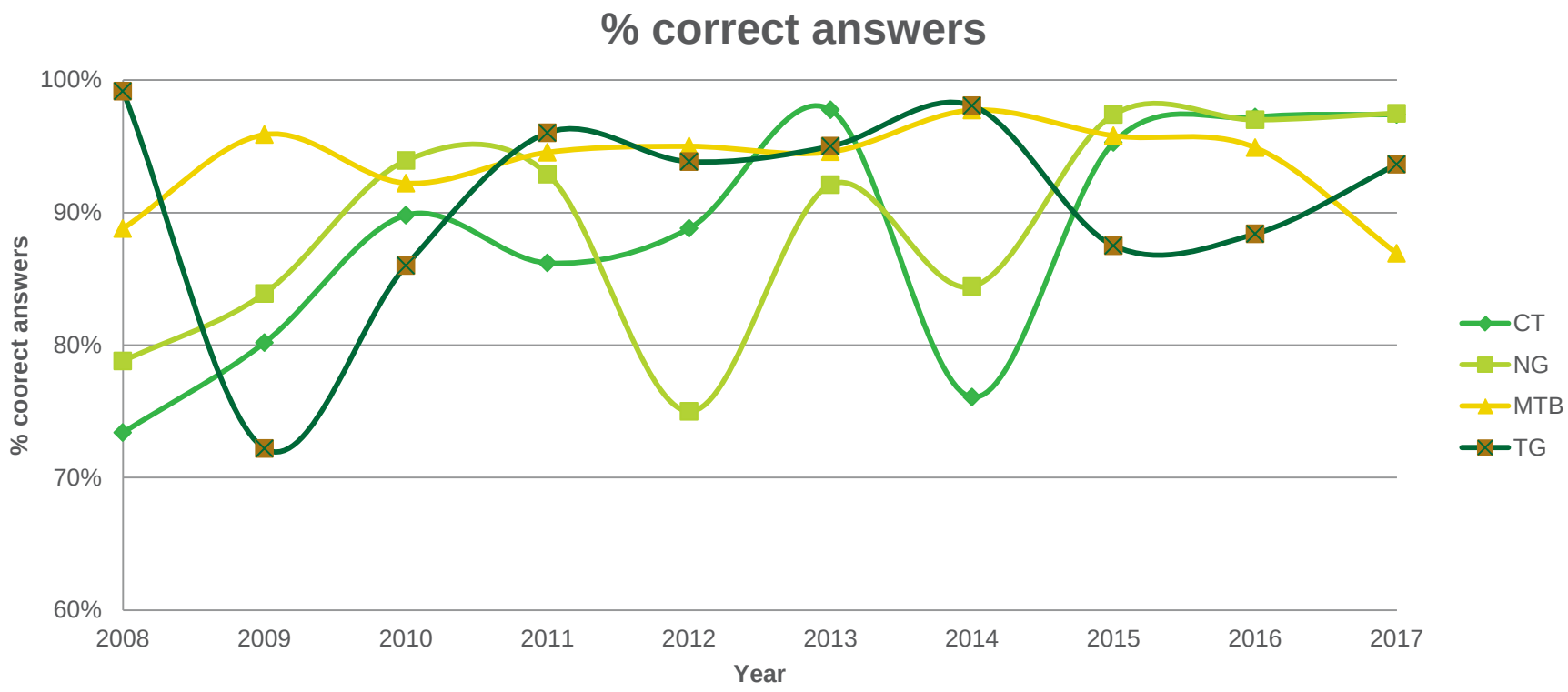
Error type 2: Lack of specificity

Example: *Neisseria gonorrhoeae*

In 2009, *N. lactamica* in sample NG 09-08 was detected as NG positive by 5 out of 29 participants (17.2%).

In 2010, *N. cinerea* in sample NG10-2 was detected as NG positive by 6 out 31 participants (19%).

Summary of the results (I)



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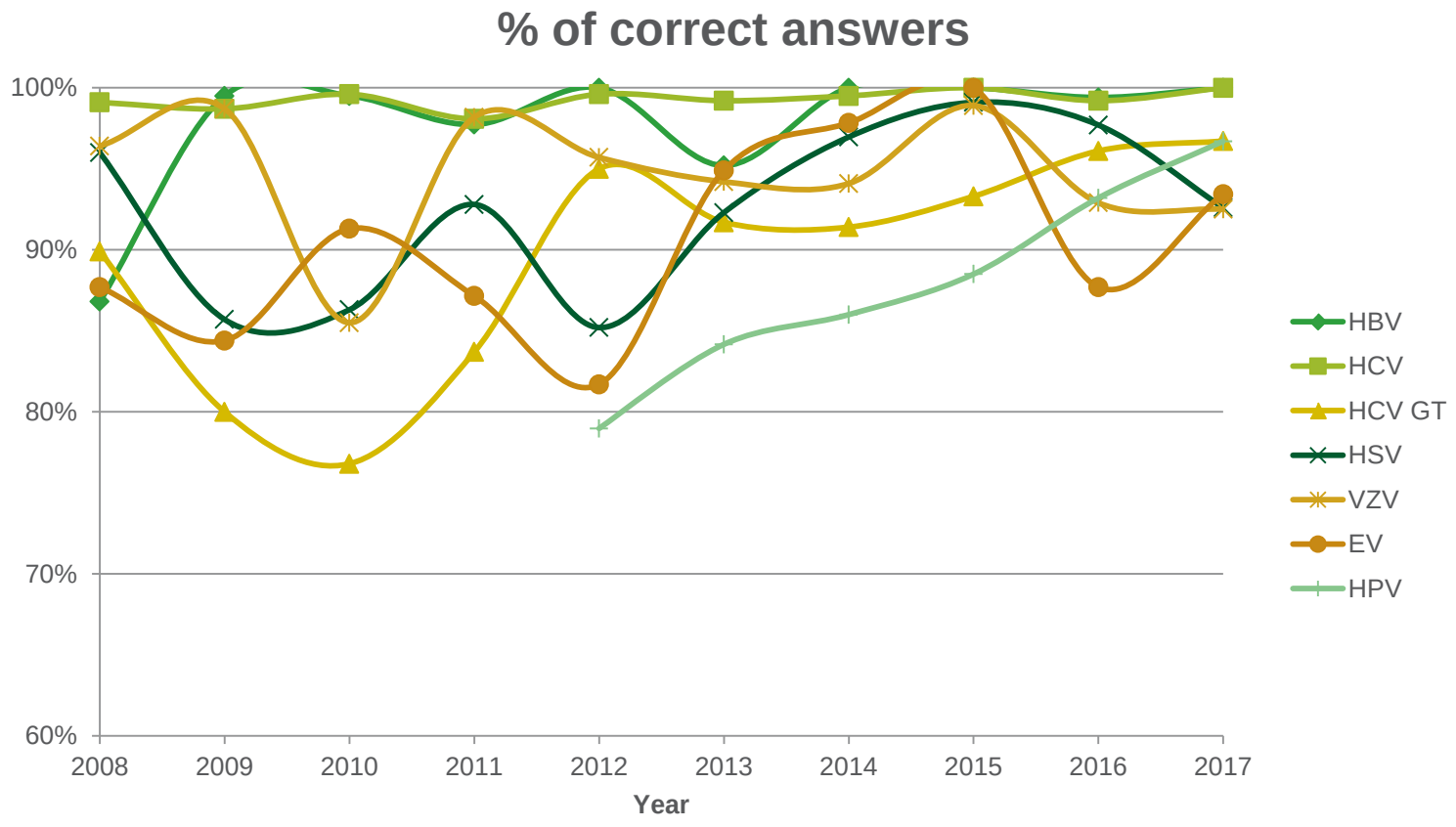
Summary of the results (II)



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Conclusions

EQA allows to detect systematic biais

All the users of a method or a kit will be impacted

EQA allows to improve the quality of the assay

Fail→ what are the reasons for? → correction and prevention → Next EQA round → Improvement?

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