National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

QCMD
Quality Control for Molecular Diagnostics

18 years of performance evaluation of molecular detection of enteroviruses by QCMD

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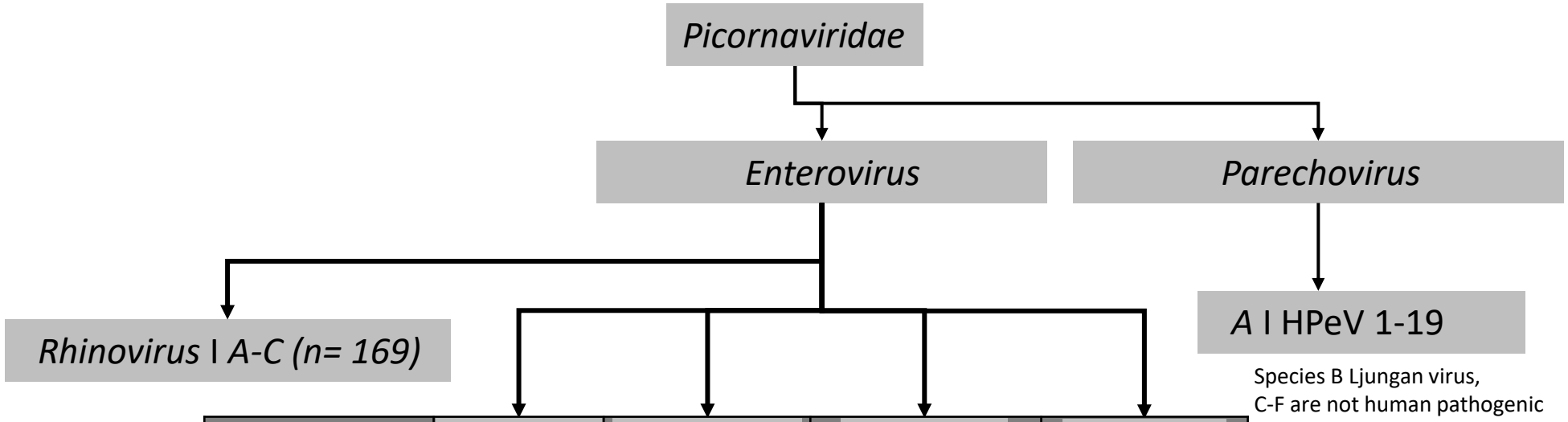
EQALM Symposium 2024
Vienna, Austria, 16-18 October

Outline

- Enteroviruses – Need for early, rapid detection & surveillance
- QCMD Enterovirus RNA EQA Programme, 2005-2022
- Performance data by laboratory type, method/assay, and pathogen type
- Discussion of results

Human enteroviruses

https://ictv.global/report/chapter/picornaviridae/picornaviridae/enterovirus



	A (n= 20)	B (n= 59)	C (n= 23)	D (n= 4)
polioviruses			1-3	
coxsackie A viruses (CVA)	2-8, 10, 12, 14, 16	9	1, 11, 13, 17, 19-22, 24	
coxsackie B viruses (CVB)		1-6		
echoviruses (E)		1-7, 9, 11-21, 24-27, 29-33		
enteroviruses (EV-) (not serotyped)	71, 76, 89-91, 114, 119-121	69, 73-75, 77-88, 93, 97, 98, 100, 101, 106, 107, 111	95, 96, 99, 102, 104, 105, 109, 113, 116-118	68, 70, 94, 111

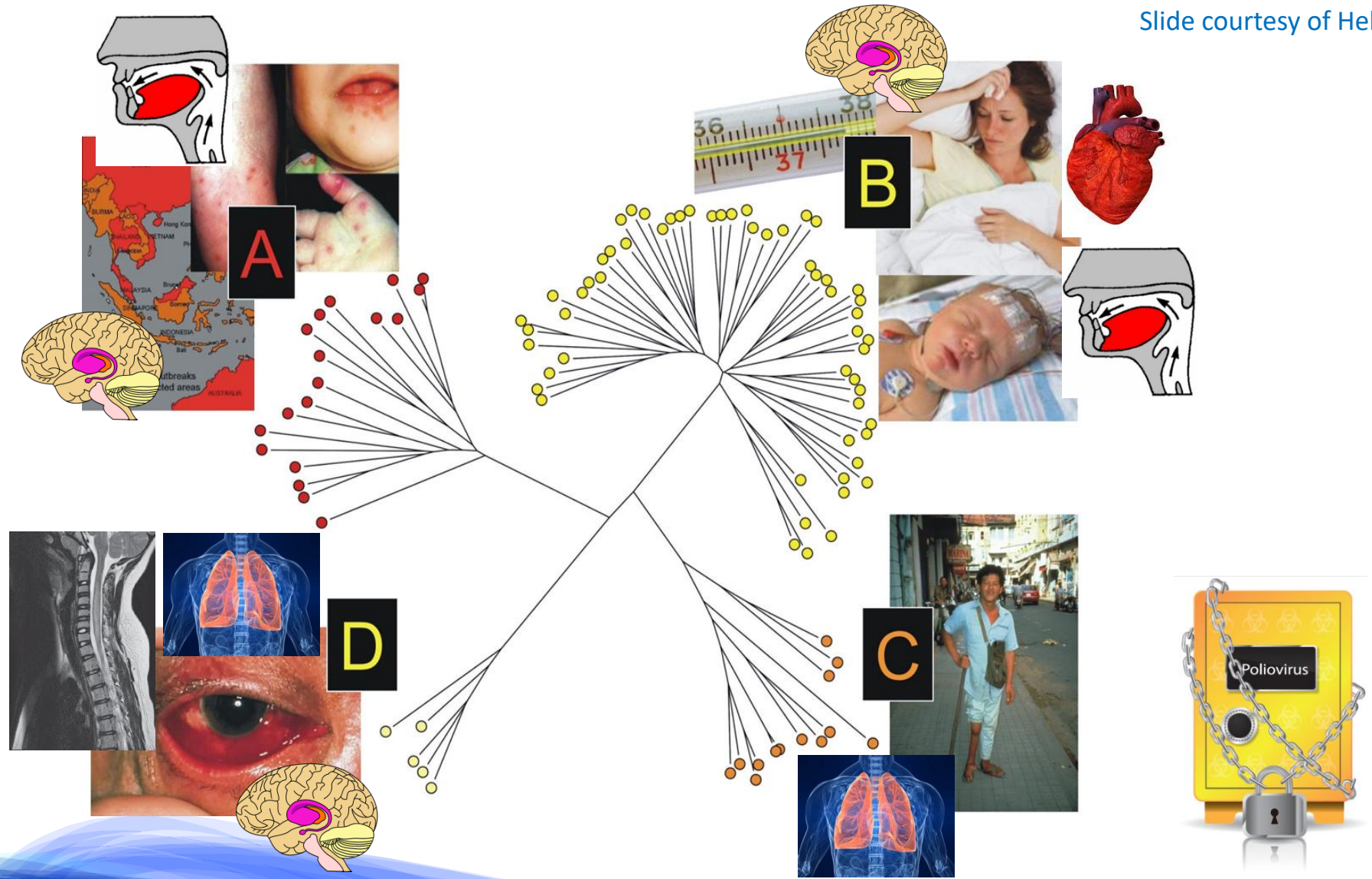
Species E-L are not human pathogenic

Polioviruses

Non-polio enteroviruses (NPEVs)

Enteroviruses and their clinical manifestations

Slide courtesy of Heli Harvala (modified)



Validated and quality-assured EV diagnostic

Crucial for:

- **Prompt diagnosis** (↓antibiotic usage/complications; limit unnecessary investigations)
- **Prognosis and supportive care** in case of severe diseases
- **Immediate infection and outbreak control** measures; monitoring of (new/recombinant) EVs
- **Assessing disease burden** of severe conditions
- **Identifying alternative treatment options** (novel antivirals, immunotherapies, vaccines)
- **Excluding circulation of wild or vaccine-derived poliovirus**

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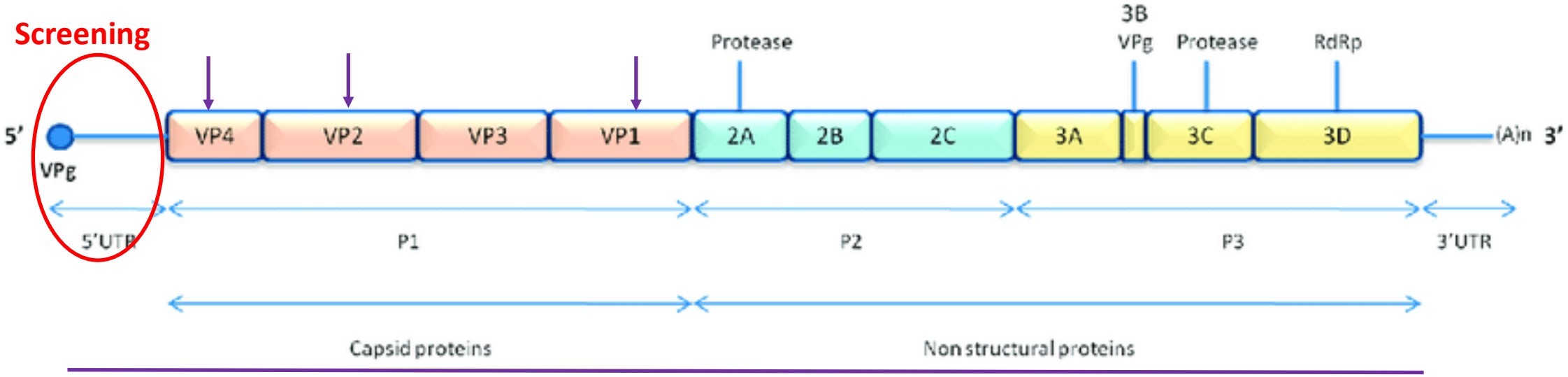
RT-PCR targeting 5'UTR for screening



- Recommended as primary assay for EV detection/screening.
  (fast turn-around time and high sensitivity over virus isolation)

- Typically detects all EV types/species with equal sensitivity (but also potentially RVs);
  IMPORTANT: assays need to be frequently updated to ensure that all types will be detected.

Harvala H, Broberg E, Benschop K, Berginc N, Ladhani S, Susi P, et al. Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. J Clin Virol. 2018 Apr;101:11-17.

Outline

- Enteroviruses – Need for early, rapid detection & surveillance
- **QCMD Enterovirus RNA EQA Programme, 2005-2022**
- Performance data by laboratory type, method/assay, and pathogen type
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QCMD Enterovirus RNA EQA Programme

- Introduced in 1998
- Accredited since 2011

Aim: To assess the ability of laboratories molecular assays to detect different types and concentrations of enterovirus (EV).

- **Material source:** Cultured and/or Clinical material
- **Sample matrix:** Transport medium (TM)
- **Covering clinical range** (diluted samples are from same stock/batch within a panel)
- **Sample formats:** Lyophilised (prior to 2014), liquid frozen (from 2014 onwards)

Year	Panels			
	Challenge	No. of samples	Core samples	Educational samples
2005	S	12	12	0
2006	S	12	12	0
2007	S	12	12	0
2008	S	12	12	0
2009	S	12	12	0
2010	S	12	12	0
2011	S	12	12	0
2012	S	12	12	0
2013	S	12	9	3
2014	S	12	9	3
2015	C1	5	5	0
	C2	5	4	1
2016	C1	5	4	1
	C2	5	4	1
2017	S	10	9	1
	C1	5	4	1
	C2	5	5	0
2018	S	10	9	1
	C1	5	4	1
	C2	5	5	0
2019	S	10	9	1
	C1	5	4	1
	C2	5	5	0
2020	S	10	9	1
	C1	5	5	0
	C2	5	5	0
2021	S	10	10	0
	C1	5	5	0
	C2	5	5	0
2022	S	10	10	0
	C1	5	5	0
	C2	5	5	0

QCMD EV Performance Study, 2005-2022

Panel distribution & participation

- **32 panels** conducted
- Either single annual (S) or biannual (C1,C2)
- Since 2015, flexible formats to meet regulatory needs

- **3,675 datasets with results** evaluated
- Returned by **699* participants** worldwide (via ITEMS)
  - 621* Diagnostic laboratories
  - 78* Public Health (PH) laboratories

- Each panel: **'Core'** and/or **'core' and 'educational'** samples

*counted only once independent of participation frequency

Panel compositions, 2005-2022

Number of samples included in the panels per virus type (core and educational)

Positives: EV types/species A B C D

[sensitivity: to detect true pos. samples correctly]

Negatives

[true neg. (false positivity)/ non-EV types (specificity)]

Year	Challenge	CVA16	EV-A71	CVA9	CVB3	E6	E 9	E11	E16	E18	E25	E30	CVA21	CVA24	PV3	EV-D68 P	EV-D68 B3	Negative	HPeV-1	HPeV-3	RV-A16
2005	S	-	2	-	3	1	2	-	-	-	-	-	-	1	-	-	-	1	1	-	1
2006	S	1	-	-	3	-	-	-	1	-	-	2	-	-	1	1	-	3	-	-	-
2007	S	-	1	-	3	-	-	2	-	-	-	2	-	-	1	-	-	1	-	2	-
2008	S	1	2	-	3	-	-	-	1	-	-	-	-	1	1	-	-	1	-	2	-
2009	S	1	2	-	3	-	-	1	1	-	-	-	-	-	1	-	-	1	-	2	-
2010	S	-	2	2	1	-	-	2	-	-	-	2	-	-	-	-	-	1	-	2	-
2011	S	1	2	-	-	-	-	2	-	-	-	1	1	1	-	2	-	1	-	-	1
2012	S	1	1	1	2	-	-	2	-	-	-	1	-	1	-	2	-	1	-	-	-
2013	S	1	1	1	2	-	-	2	-	-	-	1	-	1	-	2	-	1	-	-	-
2014	S	1	2	1	2	-	-	2	-	-	-	-	-	1	-	1	-	2	-	-	-
2015	C1	-	1	-	2	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-
	C2	-	-	1	3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
2016	C1	-	-	1	1	-	-	1	-	-	-	1	-	1	-	-	-	-	-	-	-
	C2	-	2	-	1	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-
2017	C1	-	-	1	1	-	-	1	-	-	-	1	-	1	-	-	-	-	-	-	-
	C2	-	2	-	1	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-
	S	-	2	1	2	-	-	1	-	-	-	1	-	1	-	1	-	1	-	-	-
2018	C1	-	1	1	-	-	-	-	-	-	-	1	-	1	-	1	-	-	-	-	-
	C2	-	1	-	2	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-
	S	-	2	1	2	-	-	1	-	-	-	1	-	1	-	1	-	1	-	-	-
2019	C1	-	-	1	-	-	-	-	-	-	1	-	-	1	-	1	-	1	-	-	-
	C2	-	1	-	-	1	-	-	-	1	-	1	-	-	-	1	-	-	-	-	-
	S	-	1	1	-	1	-	-	-	1	1	1	-	1	-	2	-	1	-	-	-
2020	C1	-	-	1	-	-	-	-	-	-	1	-	-	1	-	1	-	1	-	-	-
	C2	-	1	-	-	1	-	-	-	1	-	1	-	-	-	1	-	-	-	-	-
	S	-	1	1	-	1	-	-	-	1	1	1	-	1	-	1	1	1	-	-	-
2021	C1	-	-	1	-	-	-	-	-	-	1	-	-	1	-	-	1	1	-	-	-
	C2	-	1	-	-	1	-	-	-	1	-	1	-	-	-	1	-	-	-	-	-
	S	-	1	1	-	1	-	-	-	1	1	1	-	1	-	1	1	1	-	-	-
2022	C1	-	-	1	-	1	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-
	C2	-	1	-	-	-	-	-	-	1	-	1	-	-	-	1	1	-	-	-	-
	S	-	1	1	-	1	-	-	-	1	1	1	-	1	-	1	1	1	-	-	-

Number of samples tested, 2005-2022

Spilt into core samples only and all samples tested by laboratory type and assay type

	Core samples n=41087				All samples n=44434			
	Diagnostic laboratories n=36383		Public health laboratories n=4704		Diagnostic laboratories n=39366		Public health laboratories n=5068	
Year	In-house n=19588	Commercial n=16795	In-house n=3537	Commercial n=1167	In-house n=20959	Commercial n=18407	In-house n=3794	Commercial n=1274
2005	876	132	180	24	876	132	180	24
2006	1224	240	180	12	1224	240	180	12
2007	1284	360	180	24	1284	360	180	24
2008	1428	480	180	24	1428	480	180	24
2009	1332	720	228	84	1332	720	228	84
2010	1308	696	228	132	1308	696	228	132
2011	1368	888	264	168	1368	888	264	168
2012	1428	1080	252	132	1428	1080	252	132
2013	1053	954	144	99	1404	1272	192	132
2014	999	1098	225	99	1332	1464	300	132
2015	953	936	211	58	1060	1045	235	65
2016	860	1004	180	60	1075	1255	225	75
2017	985	1490	171	58	1095	1655	190	65
2018	927	1592	180	45	1030	1765	200	50
2019	993	1511	180	45	1105	1680	200	50
2020	845	1169	159	38	885	1230	165	40
2021	885	1250	200	30	885	1250	200	30
2022	840	1195	195	35	840	1195	195	35

- **Total of 44,434 samples**
- Performance analysis for:
  - **Core (n= 41,087)**
  - All samples (incl. educational, n= 3,347)

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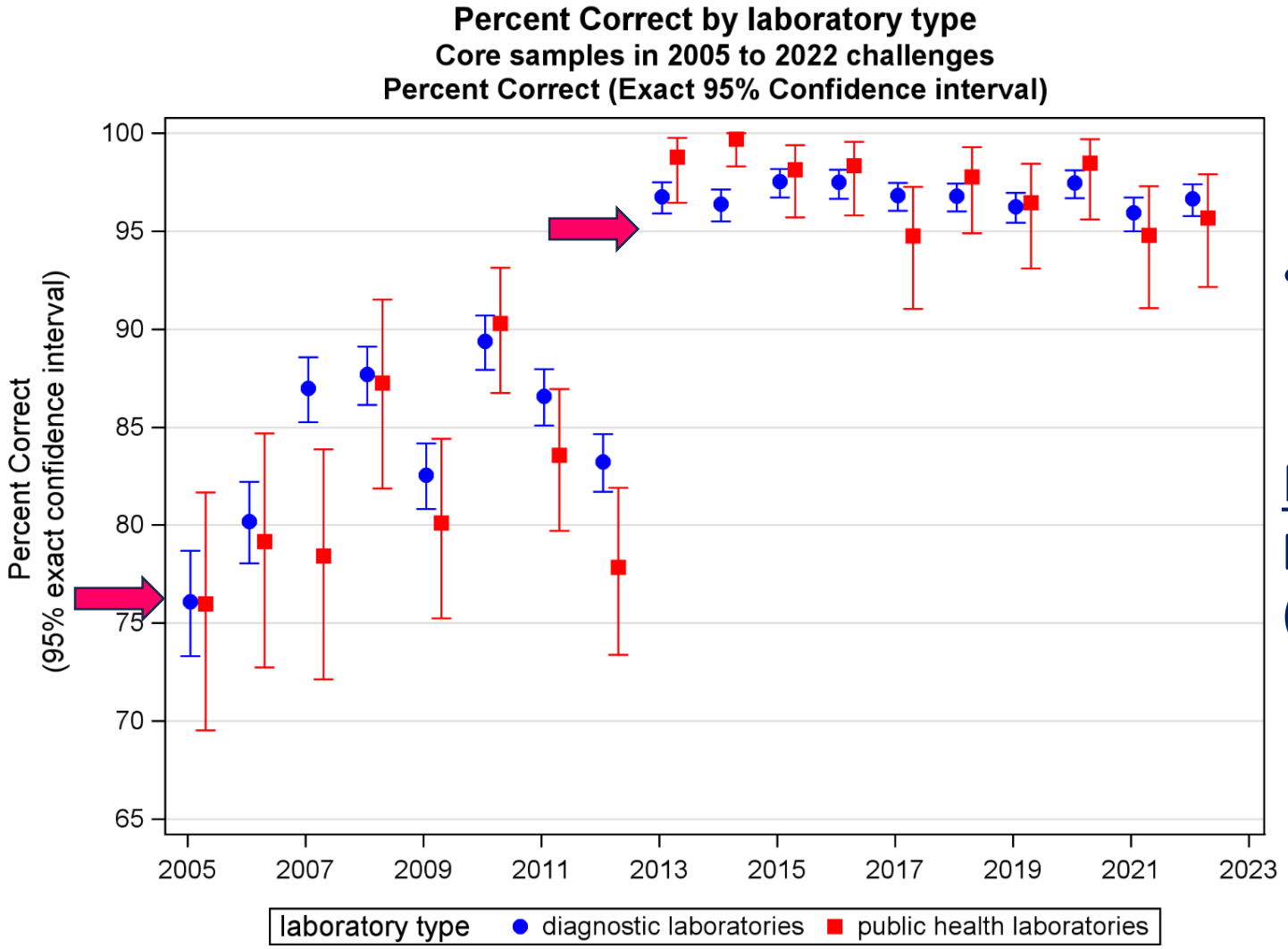
In-house vs commercial assays

Year	Diagnostic laboratories			Public health laboratories		
	In-house n (% of total)	Commercial n (% of total)	Total n	In-house n (% of total)	Commercial n (% of total)	Total n
2005	69 (88.5%)	9 (11.5%)	78	14 (87.5%)	2 (12.5%)	16
2006	89 (83.2%)	18 (16.8%)	107	12 (92.3%)	1 (7.7%)	13
2007	99 (82.5%)	21 (17.5%)	120	15 (88.3%)	2 (11.7%)	17
2008	111 (75.5%)	36 (24.5%)	147	15 (88.3%)	2 (11.7%)	17
2009	105 (67.3%)	51 (32.7%)	156	17 (73.9%)	6 (26.1%)	23
2010	106 (66.3%)	54 (33.7%)	160	15 (57.7%)	11 (42.3%)	26
2011	109 (61.2%)	69 (38.8%)	178	18 (58.1%)	13 (41.9%)	31
2012	106 (56.7%)	81 (43.3%)	187	17 (60.7%)	11 (39.3%)	28
2013	108 (54.3%)	91 (45.7%)	199	14 (58.3%)	10 (41.7%)	24
2014	105 (50.0%)	105 (50.0%)	210	18 (62.1%)	11 (37.9%)	29
2015	102 (51.3%)	97 (48.7%)	199	18 (75.0%)	6 (25.0%)	24
2016	102 (47.0%)	115 (53.0%)	217	18 (72.0%)	7 (28.0%)	25
2017	103 (41.9%)	143 (58.1%)	246	14 (66.7%)	7 (33.3%)	21
2018	97 (39.1%)	151 (60.9%)	248	16 (76.2%)	5 (23.8%)	21
2019	98 (40.3%)	145 (59.7%)	243	16 (76.2%)	5 (23.8%)	21
2020	85 (42.5%)	115 (57.5%)	200	15 (79.0%)	4 (21.0%)	19
2021	83 (42.6%)	112 (57.4%)	195	16 (84.2%)	3 (15.8%)	19
2022	79 (41.6%)	111 (58.4%)	190	17 (81.0%)	4 (19.0%)	21
Total	1756 (53.5%)	1524 (46.5%)	3280	285 (72.2%)	110 (27.8%)	395

- **Diagnostic laboratories showed transition from in-house to commercial assays.**

Number of panels tested by laboratory type and assay type, 2005-2022 (**3,675 datasets**)

Overall performance over time (laboratories)



- **Performance improved, however PH laboratories showed large variation.**

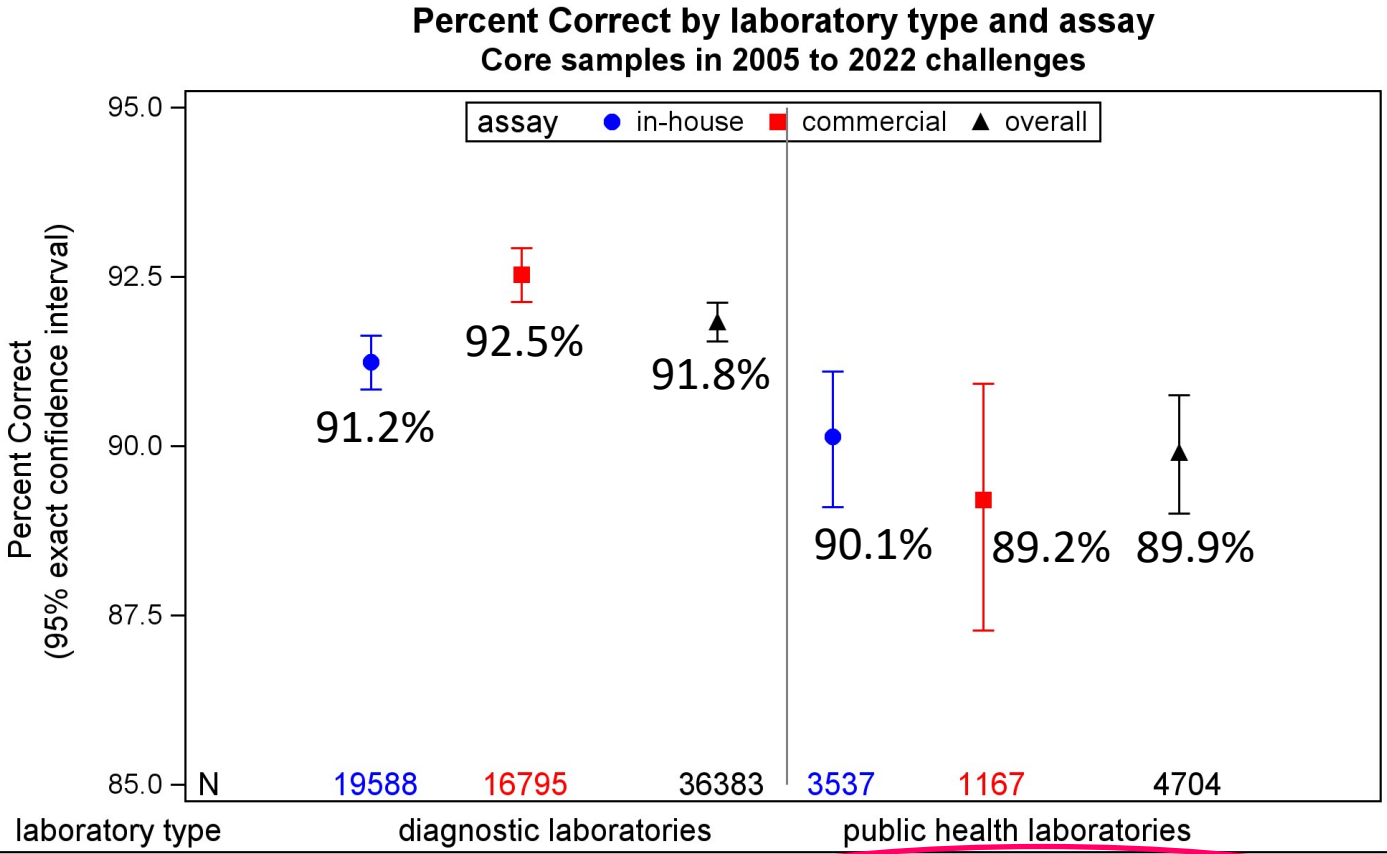
Pooled data over time:

Diagnostic laboratories > PH laboratories

(odds ratio= 1.26 (95% CI: 1.14 - 1.40); p<0.0001)

Performance on core samples tested **by laboratory type**, 2005-2022

Binary logistic regression model on pooled data



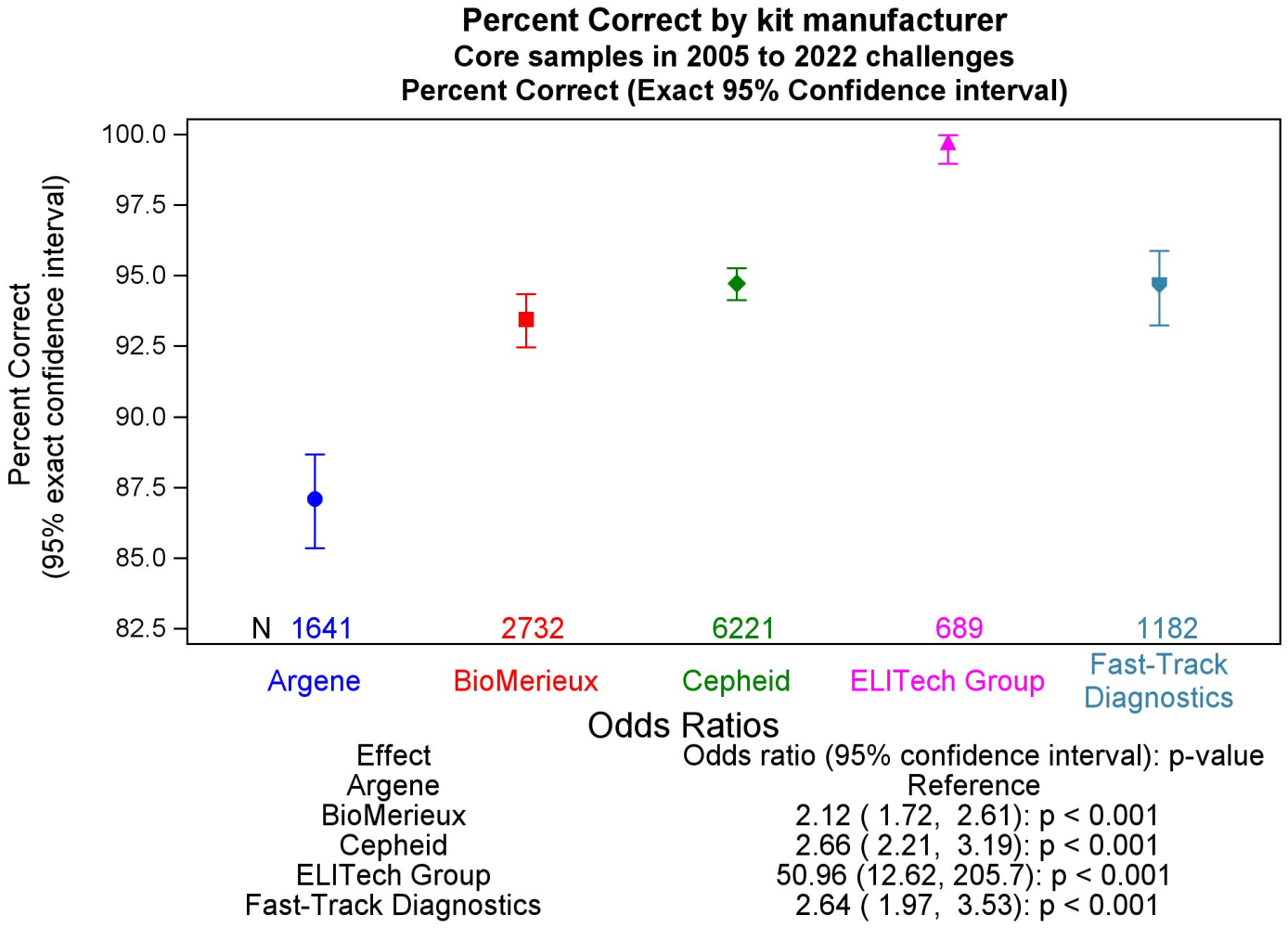
Odds Ratios

	Effect	Odds ratio (95% CI): p-value
laboratory type (Ref: public health laboratories)	commercial	1.16 (1.08, 1.24): p < 0.001
assay (Ref: in-house)	diagnostic laboratories	1.23 (1.11, 1.36): p < 0.001

- **Diagnostic > PH laboratories (varied)**
- **Commercial > in-house assays (linked with Diagnostic laboratories)**

Performance on core samples tested **by laboratory and assay type (pooled)**, 2005-2022

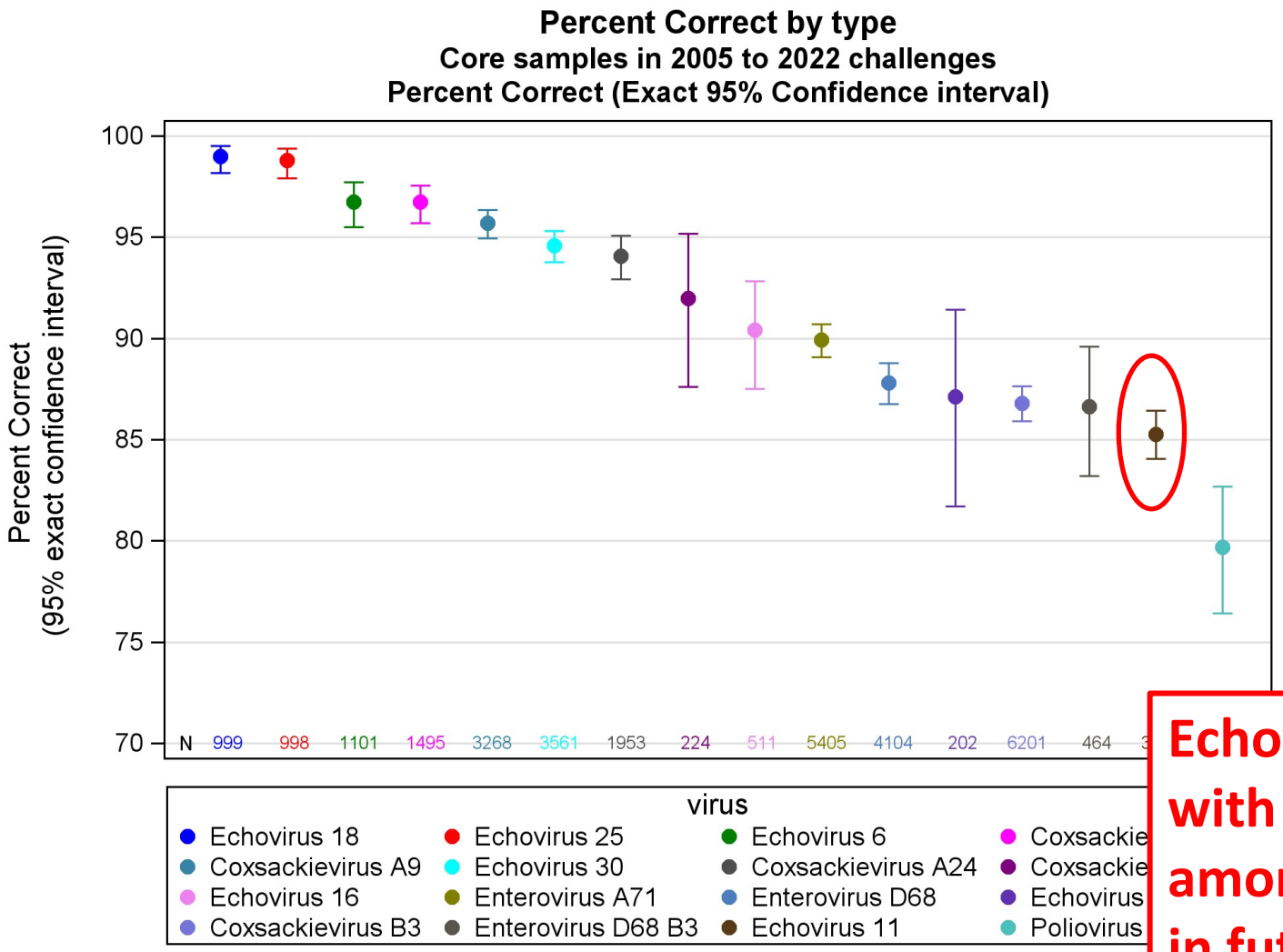
Performance of most frequent used 5 assays



- **Up to 25 different commercial assays were used over time with overall performance of 92.7% (64.4 to 100%).**
- **TOP 5 ranged between 87.1% (Argene) to 99.7% (ELItech)**

Assay-related performance with odds ratios of **top 5 commercial assays most used**, 2005-2022

Detection of different EV types pooled over time



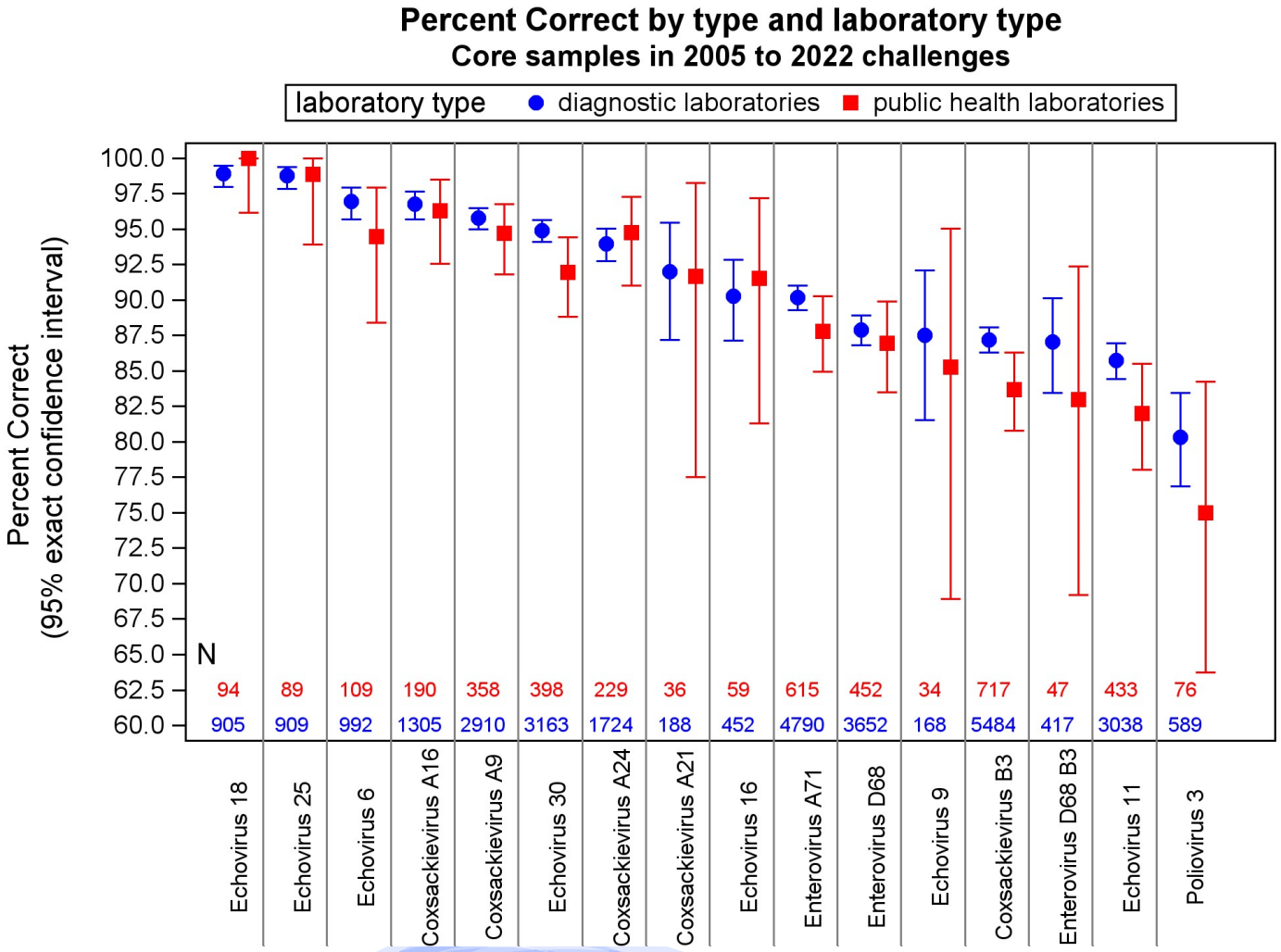
- **Overall sensitivity improved: 71.0% (2005) to 96.4% (2022)**

- **Per EV type, varying sensitivity among participants: 79.7% (PV3) to 99.0% (E18)**

- **Lowest % correct rate among NPEVs: 85.2% (for E11 positive samples)**

Echovirus 11 has recently been associated with severe hepatitis and mortality among neonates and should be (re-)included in future EQA panels.

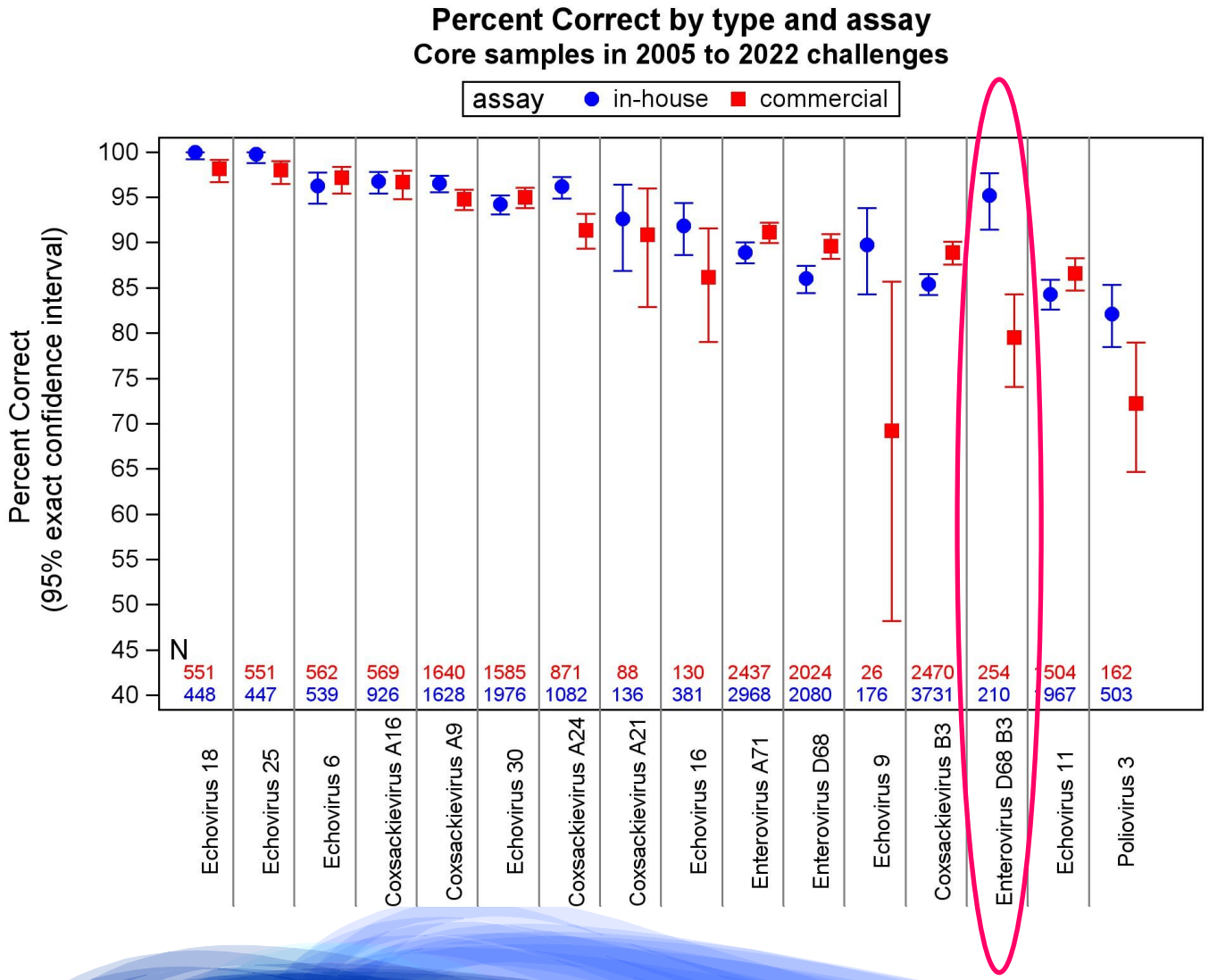
Detection of EV types by laboratory type



- **PH laboratories showed a larger variation similar as for the overall performance.**

True positive rates (sensitivity) on core samples **by laboratory type**, 2005-2022

Detection of EV types by assay type



- **Commercial assays showed lower detection rates for E9, EV-D68 B3, and PV3.**

True positive rates (sensitivity) on core samples **by assay type**, 2005-2022

False positivity & detection of non-EV types

Year	% correctly detected Sensitivity: True positive core EV types	False positivity: True negative	% incorrectly detected Specificity: Parechovirus 1	Parechovirus 3	Rhinovirus 16	all non-EV types	True negative and non-EV types combined
2005	71.0	3.0	7.9		14.9	11.4	8.6
2006	74.8	4.1					4.1
2007	83.1	7.8		3.9		3.9	5.2
2008	84.8	4.5		3.7		3.7	4.0
2009	77.6	6.1		2.8		2.8	3.9
2010	88.1	6.6		6.1		6.1	6.3
2011	84.6	2.7			10.7	10.7	6.7
2012	81.0	0.8					0.8
2013	96.7	0.4					0.4
2014	96.5	2.0					2.0
2015	97.5	1.7					1.7
2016	97.4	1.2					1.2
2017	96.3	0.3					0.3
2018	96.6	1.0					1.0
2019	96.0	1.6					1.6
2020	97.5	2.2					2.2
2021	95.6	2.1					2.1
2022	96.4	1.8					1.8

- **False positivity rate was low (overall 2.5%) and varied over time.**

No significant difference between laboratory types. Commercial assays had a lower FP rate compared to in-house assays (data not shown).

- **Overall rate of incorrectly detected specificity samples was 5.7%.**

Highest for Rhinovirus 16, followed by HPeV-1 and HPeV-3

No significant difference between laboratory types or assay types (data not shown).

Summary

- **Quality control of EV molecular assays is key for maintaining high-quality diagnostic**

Performance analysis from 18 years consecutive proficiency testing results shows:

- **Overall performance improved** for both diagnostic and PH laboratories over time
- In-house assays were mainly used; however, **transition to commercial assays was seen**
- **In-house assays and commercial assays showed similar performance**
- **CAVE:** varying performance, certain types can be missed, not always distinguishing RVs/EVs!

Conclusion

The inclusion of different EV types of clinical and public health relevance remains a crucial part of the EQA, as differentiation between these types should be regularly evaluated considering their varying disease patterns, changing epidemiology and emergence of new/recombinant strains.

Limitation of the current EQA schemes are:

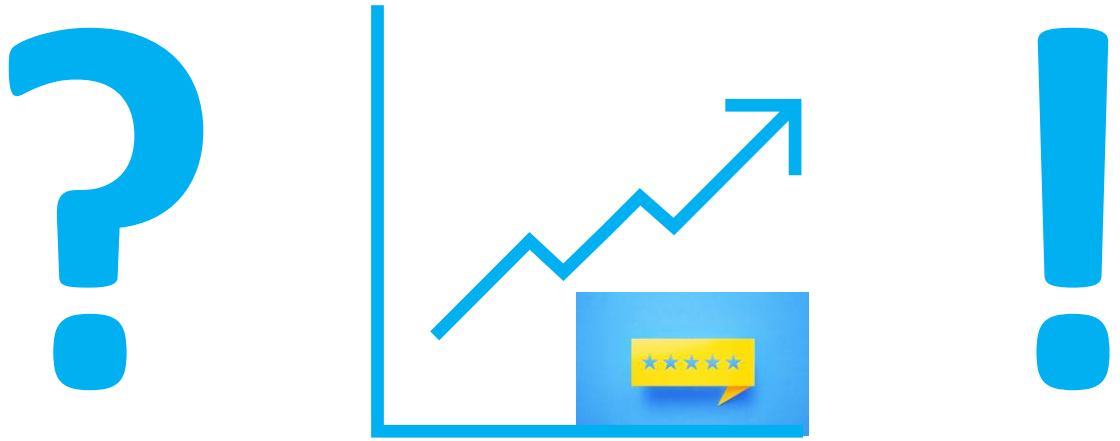
- inclusion of virus strains difficult to culture is not possible with our approach (using cultured materials);

- due to low number of quantitative results, these analyses were not part of this evaluation.

Feedback & Questions

PD Dr. Oliver Donoso Mantke

QCMD Scientific Advisor



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