Molecular diagnosis: commercial or home-made tests?

Molecular diagnosis should be done using CE - marked commercial kits.

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IVDs: Today and the Future: technological advances

Today

- the variety and complexity of diagnostic tests and instruments available has evolved dramatically, IVDs have been an area of constant innovation
- Automation for NA extraction, amplification and detection platforms
- Fast cyclers allowing to identify a disease earlier
- User friendly devices can be used by medical staff (POCT) or by patients (self-testing) on smaller and less traumatic samples

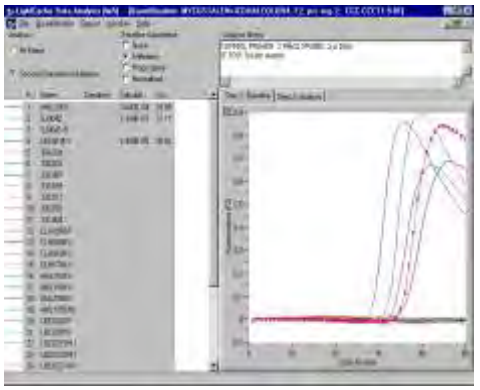
The future

- New innovative detection systems
- Multiplex capabilities expanding
- Full integration of MDx steps
- Molecular “Point of Care” testing

Choice of platforms and assays: Commercial versus in-house tests?

"commercial" tests: FDA approved or CE labeled IVD

- More or less extensive validation and standardization
  - More extensive validation for FDA approval required
- positive controls included
- Quantification standards often included
- internal controls often included



"In house tests": developed & used for diagnostic purposes within a single health institution

- degree of validation and standardization is often not transparent or even lacking; large variability of results within and between laboratories

Accreditation to the requirements of ISO 15189 *(Medical laboratories-Particular requirements for quality and competence)*

Molecular diagnosis should be done using CE-marked commercial kits

- Extensive validation and standardization
  - Validation versus Verification
- Regulatory requirements
- Performance of CE-marked versus in-house tests
- Technological advances: moving to molecular POCT
  - Rapid screening
    - Influenza-RSV
    - GBS
    - *Clostridium difficile*
  - Multiplex syndromic approach

Verification and validation requirements of molecular tests

- The complexity and the extent of verification or validation depends on whether an IVD-CE labeled test or an “in-house” test is concerned

Verification	Validation
Accuracy	Accuracy
	Sensitivity
	Specificity
Precision (intra- and inter-assay)	Precision (intra- and inter-assay)
Linearity (if quantitative)	Linearity (if quantitative)

- *evaluation of an assay’s performance in relation to its known or reported performance, e.g. as specified by manufacturer*

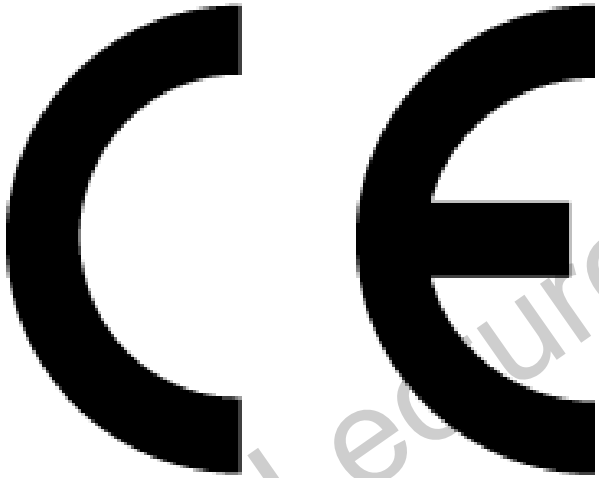
- *evaluation of an assay’s performance in order to determine its fitness for use*

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
	Negative	3	3	3	3
Sensitivity	Positive	-	-	10	10
	Low positive*	-	-	10	10
Specificity	Negative	-	-	20	20
Intra-assay precision	Positive	1	3	1	6
	Low positive*	1	3	1	3
Inter-assay precision	Positive	1	1	1	2
	Low positive*	1	1	1	1
Linearity	Positive	-	1	0	2

* Up to 1 log 10 over the LOD

Rabenau et al, 2007



Conformité **E**uropéene

indicates **compliance** with the **relevant legislation** and **conformity with the directive.**

a **“passport”** allowing a manufacturer to **freely circulate products** within the European market

To ensure that only safe and functional products are sold on the European market

EU In vitro directive 98/79/EC: some key objectives

ensure that only safe and functional products are sold in the EU market

- ”to provide patients with a high level of health protection”
  - Safety and performance of the IVD
  - Quality of the test results
- ”to provide users and third parties with a high level of health protection”
  - Safety and safe use of the IVD

provide ‘clear’ regulations on manufacturing, import, marketing of IVD’s

- Notification of IVD by the manufacturer to the “Competent Authority”
- Verification of conformity by assigned “Notified Body”

Technical harmonisation: rules on safety, quality and performance of IVDs

- Essential requirements, Common technical specification
- Communication between “Competent Authorities”

Conformity assessment of IVDs

- Variety of conformity assessment procedures related to various classes of risks: “CTS” which detail required performance evaluation criteria, reevaluation ….
- High risk IVDs may require lab based performance evaluations in accordance with CTS: HIV, HCV,...
- Notified Body involved in assessment of ‘higher risk’ products
  - Independant organisation
  - May involve external experts, reference labs
- Self certification for IVDs considered “low risk”

EUROPEAN COMMISSION

A new proposal to replace DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT

Brussels, 26.9.2012
COM(2012) 541 final

2012/0267 (COD)

Proposal for a

REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

on *in vitro* diagnostic medical devices

(Text with EEA relevance)

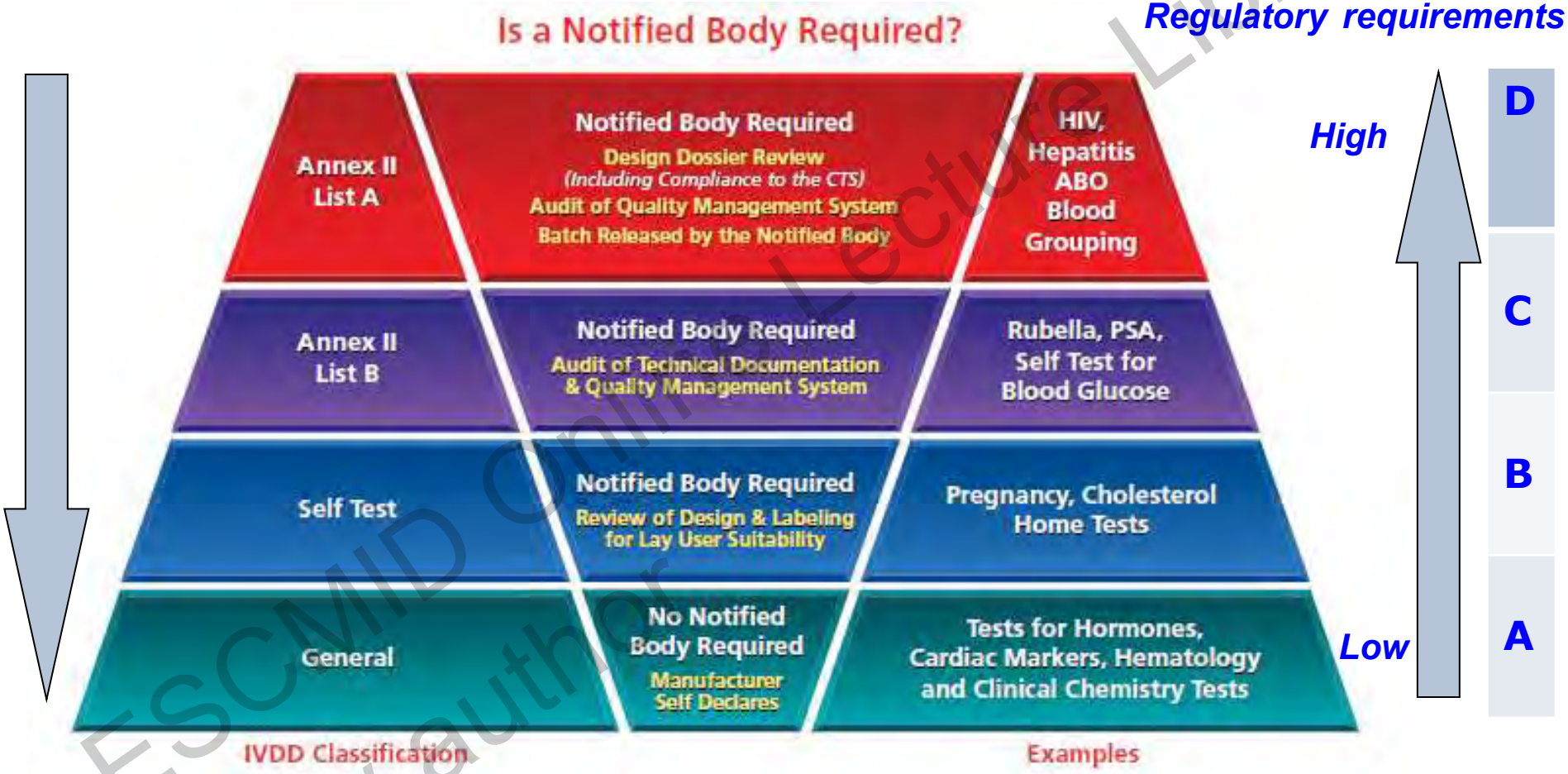
{SWD(2012) 273}
{SWD(2012) 274}

Main elements of new Proposal for IVD Regulation

- **Stronger supervision of independant assessment bodies by national authorities**
  - with permanent “in-house” competent expertise
    - in technical fields linked with the assessment of performance
    - and in the medical field.
- More **power** and **obligations** for **assessment bodies** to ensure thorough testing and **regular checks on manufacturers**
- Clearer rights and responsibilities for manufacturers
- Stricter requirements for ***clinical evidence***, ***vigilance and market*** surveillance to ensure patient safety

⇨ **To increase quality guarantees on commercial tests**

Rules based risk classification of IVDs by new proposal and the directive 98/79/EC



Descending order of risk → *In line with GHTF*

Proposal for a new regulation on In Vitro Diagnostics (IVDs)

Who will benefit?

- Patients and consumers
  - Since all devices will have to undergo thorough assessment of safety and performance
  - Control processes are radically reinforced
- Healthcare professionals
  - Better information on benefits, residual risks,…
- Manufacturers
  - Clearer rules

A new proposal for a Regulation on in vitro diagnostic medical devices

Amendment 70: Article 4 – paragraph 5 – subparagraph 1 and 2

Text proposed by the Commission Amendment

"Member States …..… and may make the manufacture and use of the devices by health institutions concerned ***subject to further safety Requirements***".

"Devices classified as class D in accordance with the rules set out in Annex VII, ***even if*** manufactured and used within ***a single health institution***, shall ***comply with the*** requirements of this Regulation***."***

Even for "in house" developed tests:

for critical tests: regulation will become similar to the one for manufacturer's of commercial kits!

For any test: notification may be required!

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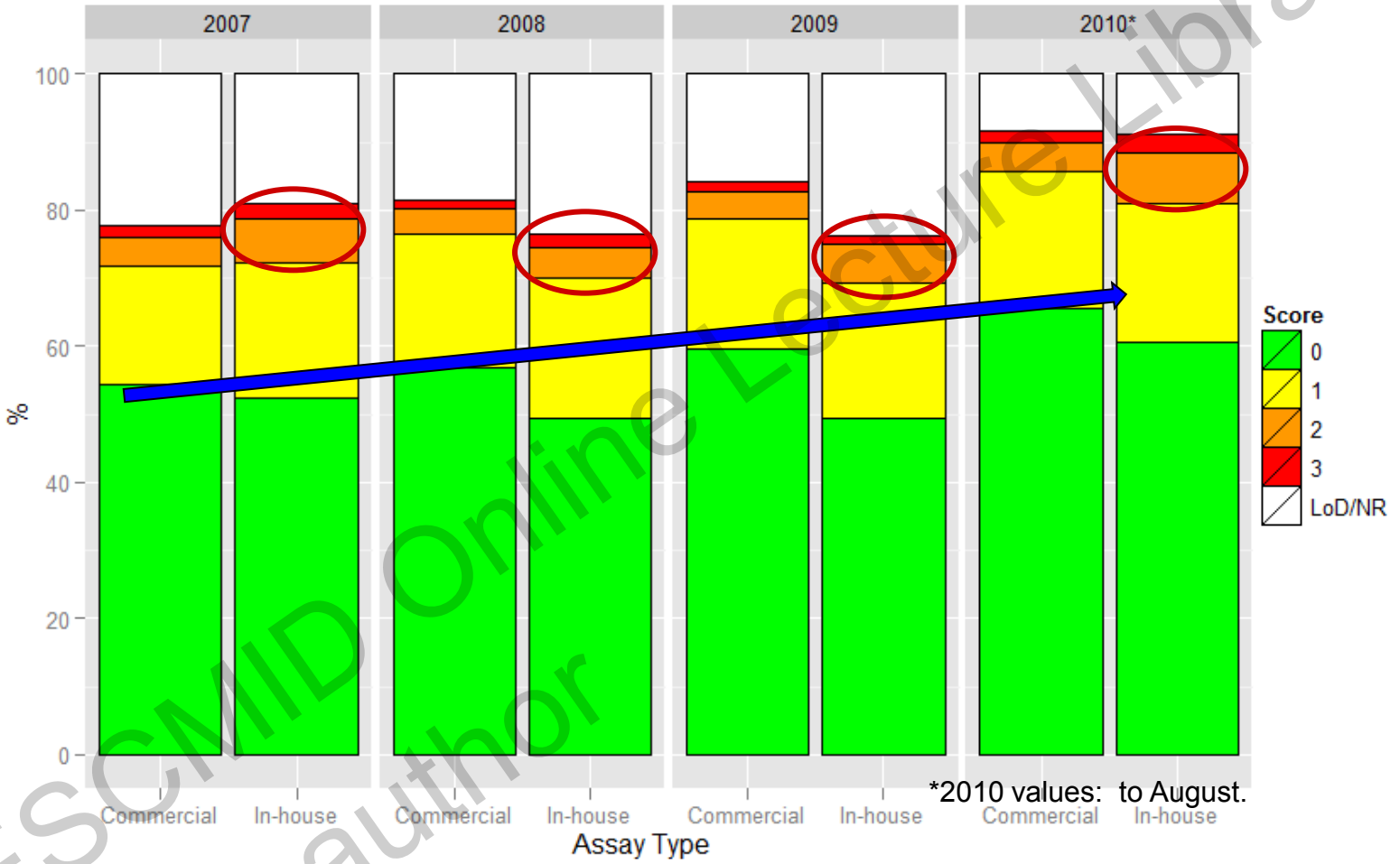
Do CE marked test perform better?

Performance of molecular tests for the detection of blood borne viruses (HIV, HBV, HCV, CMV, …): belonging to Annex II List A or B

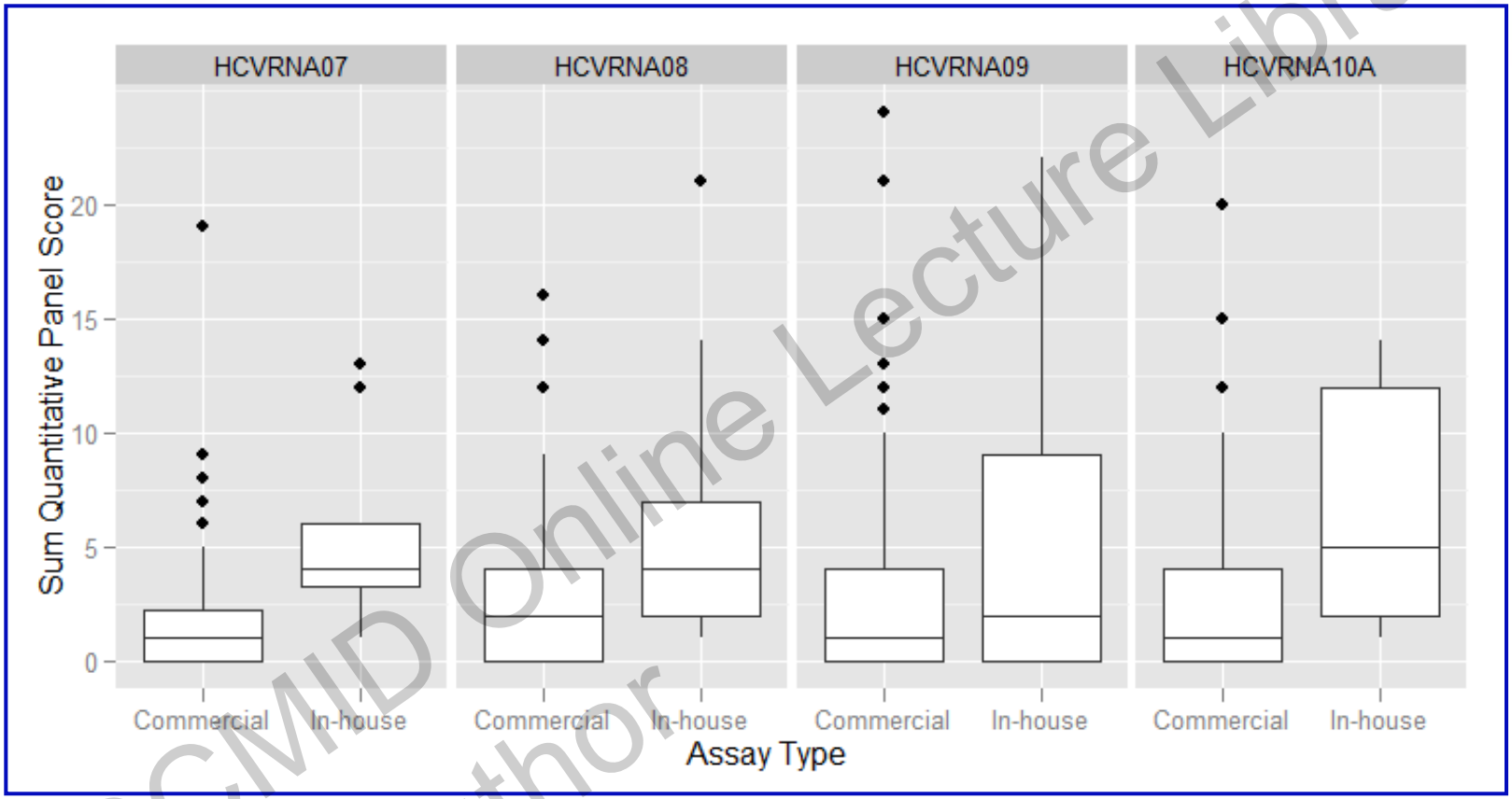
QCMD Quantitative scores for EQA panel samples, 2007-10



Overall commercial quantitative assays score a little better; sometimes improved after feed-back from clinical labs (e.g. HCV viral load molecular assay)

Data kindly provided by QCMD

HCV: commercial vs in-house?

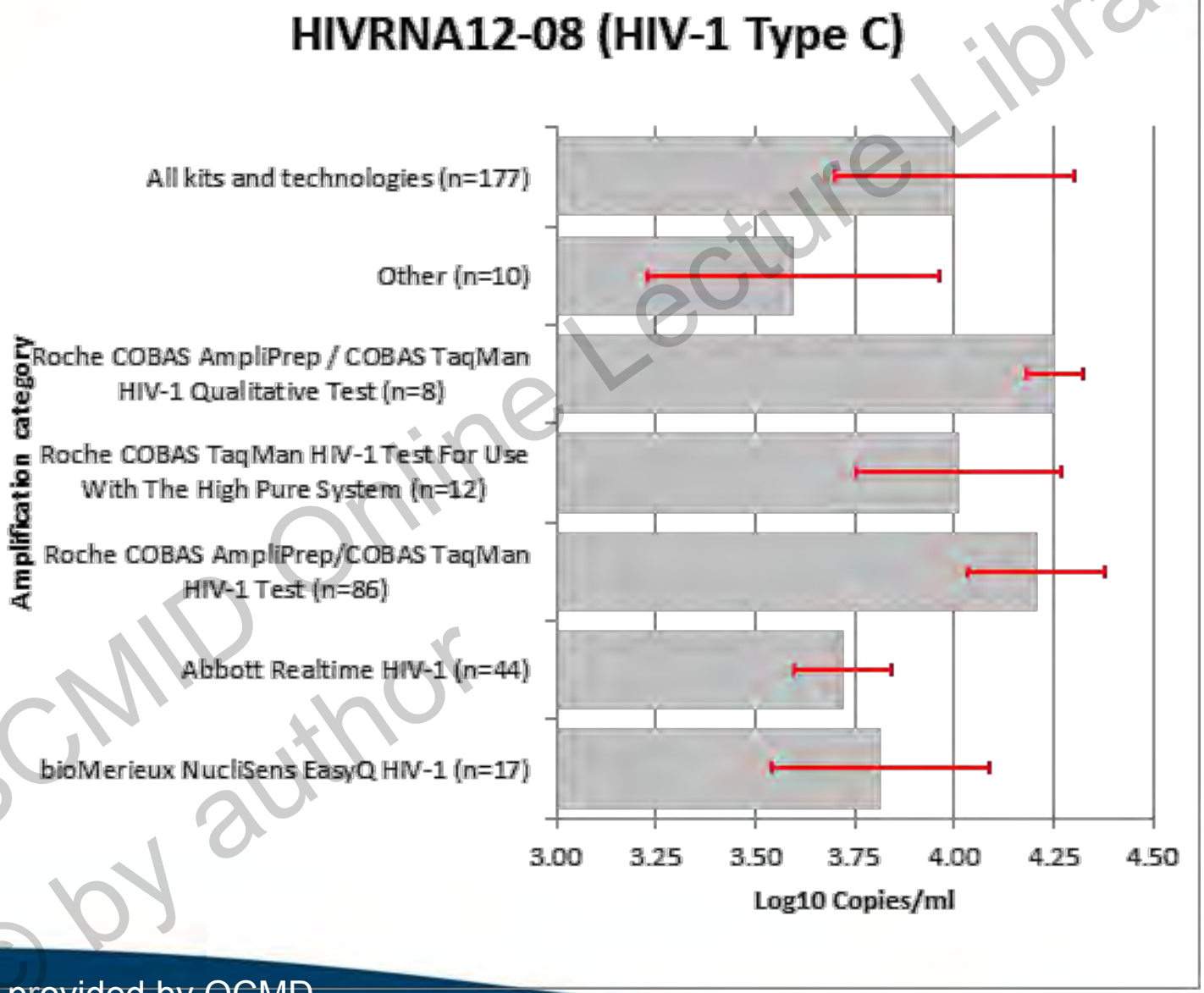


	Number of datasets scored			
	HCVRNA07	HCVRNA08	HCVRNA09	HCVRNA10A
Commercial	68	103	139	170
In-house	10	17	13	13

- Lower mean score for commercial panels
- Smaller range

Data kindly provided by QCMD

Performance based on amplification assays



Data kindly provided by QCMD

Performance per HIV core panel member

HIVRNA12-04 (HIV-1 Type B)

								Participant A		Participant B	
			Reported range					Assay	All	Assay	All
Assay / Technologies	N=	Mean log copies	Low	high	1sd	2sd	3sd	Your Result	Your Result	Your Result	Your Result
bioMerieux NucliSens EasyQ HIV-1	17	3.60	3.39	3.82	0.22	0.43	0.65	0.21			
Abbott Realtime HIV-1	44	3.41	3.21	3.61	0.20	0.40	0.61			0.41	
Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test	86	3.59	3.45	3.72	0.13	0.26	0.40				
Roche COBAS TaqMan HIV-1 Test / With The High Pure System	12	3.55	3.32	3.78	0.23	0.46	0.69				
Roche COBAS AmpliPrep / COBAS TaqMan HIV-1 Qualitative Test	8	3.58	3.53	3.64	0.05	0.11	0.16				
Other	10	3.49	3.12	3.87	0.37	0.75	1.12				
All kits and technologies	177	3.54	3.34	3.73	0.20	0.40	0.59		0.21		0.41

This monitoring and evaluation of performance:

⇒ **only possible with commercial tests**

Data kindly provided by QCMD

Many different 'molecular testing workflows' within the Molecular microbiology laboratory

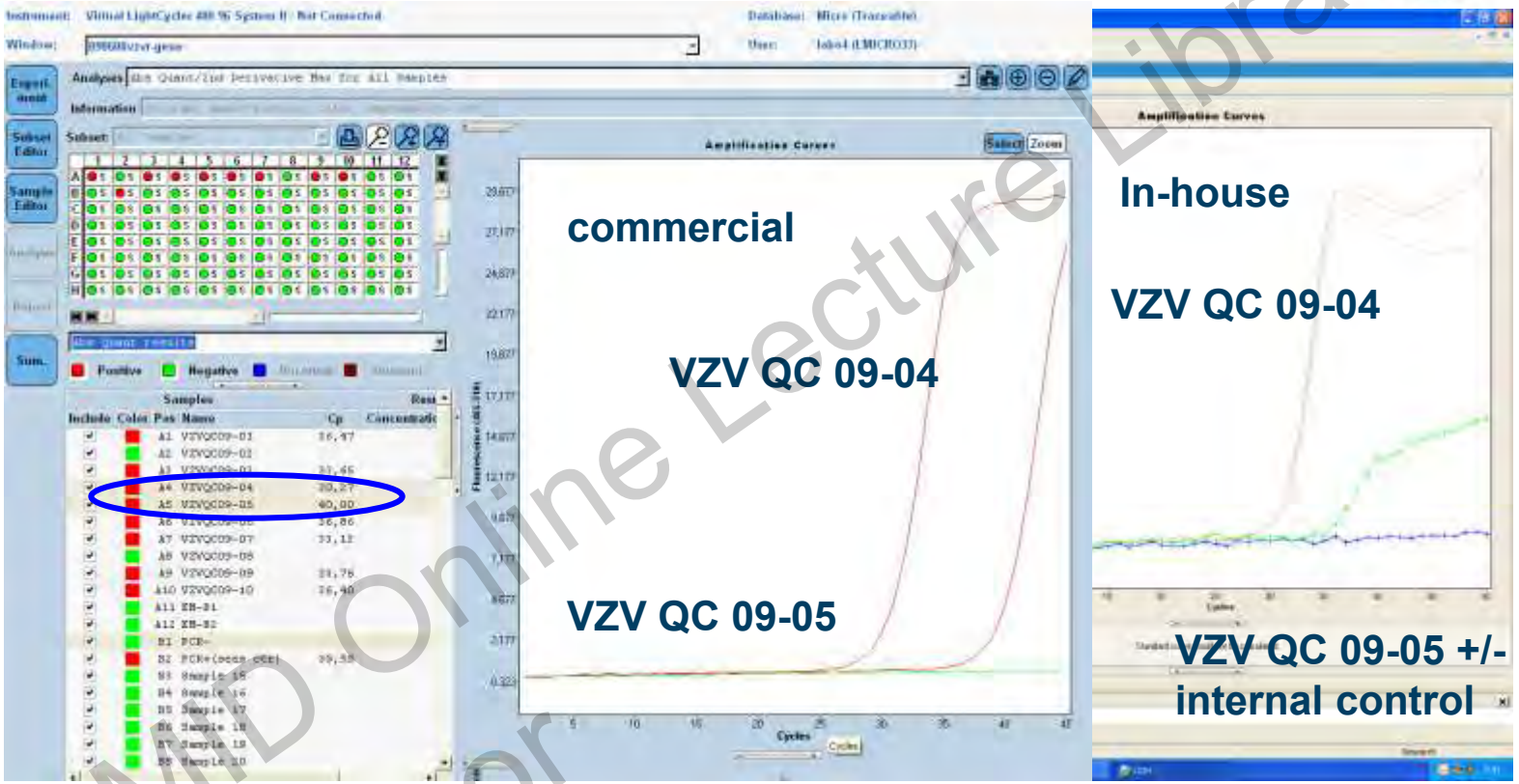
TEST TARGET Mycobacterium tuberculosis (MTB)	Sample Pre-treatment method	Extraction kit / assay	Extraction Platform	Nucleic Acid Amplification Platform	NA Detection Kit
Laboratory A	'In house' MTB method from sputum	Nordiag Sputum NA extraction kit	Nordiag ARROW platform	ABI 5700 (PCR platform)	Nanogen MTB kit
Laboratory B	'In house' MTB method from sputum	'In house' NA extraction method	**Nordiag ARROW platform**	ABI 5700 (PCR platform)	**Nanogen MTB kit**
Laboratory C	ROCHE AMPLICOR® Respiratory Specimen Preparation Kit	ROCHE MagNA pure DNA isolation kit	ROCHE MagNA pure isolation Platform	ROCHE 'COBAS® TaqMan® 48 Analyzer	ROCHE COBAS® **TaqMan®** MTB Test

Different combinations... significant impact on performance / variability.

Do CE marked test perform better?

What about the performance of other molecular tests and emerging rapid tests not belonging to Annex II List A or B of the Directive 98/79/EC?

VZV: commercial vs in-house?



- VZV09-04: Ellen: 21184 copies/ml
- VZV09-05: 9/84 : 148 copies/ml

→ **Commercial test more sensitive than in-house test**

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IVDs: the Future: All Inclusive Systems

"We're a little slow in the lab today, so I'd like to run some tests."

Shifting diagnostics from:

University lab ⟹ to the community hospital

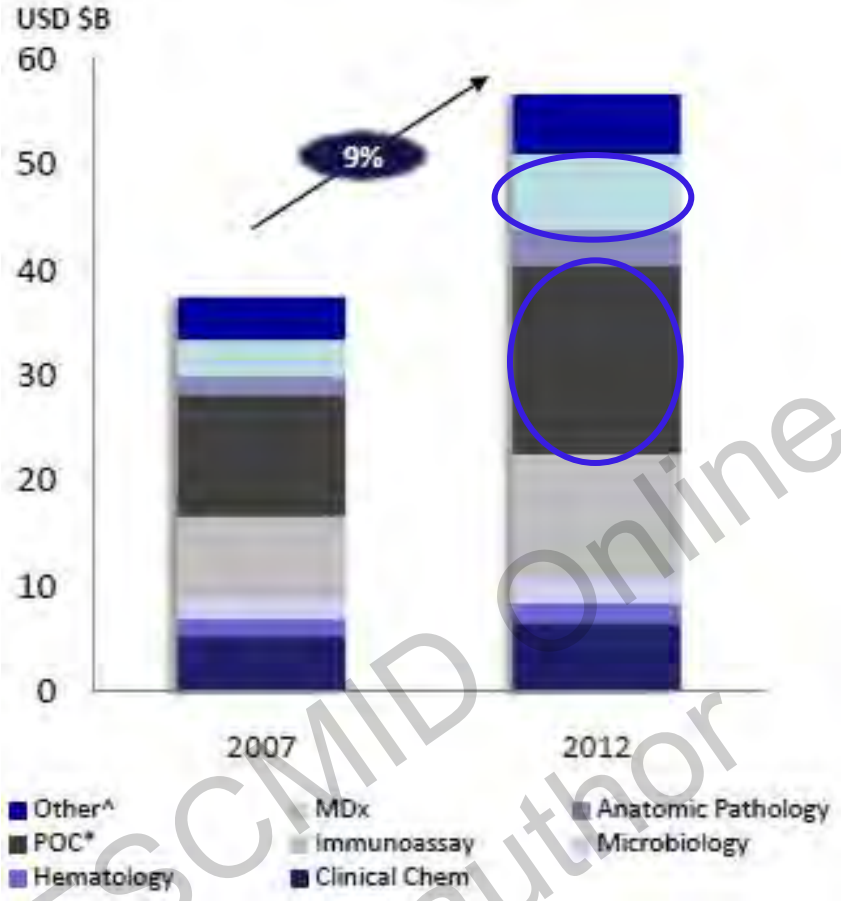
The laboratory ⟹ to the bedside:

IVDs: the Future: All Inclusive Systems

Shifting diagnostics from:

Sample in…..⟹ result out

IVDs: the Future: Global Growth in molecular Diagnostics in IVD Market



- MDx is the fastest growing segment. Rapid MDx for infectious disease, is likely to see explosive growth
- The emerging technologies have the ability to decrease the **TAT or time required for results from hours to minutes** and permit improve outcome for patients.

*Includes blood glucose & pregnancy testing
^Includes coagulation, flow cytometry, drug of abuse, therapeutic drug monitoring, nephelometry, and others

Source: Sientia analysis, Kalorama, Frost & Sullivan.

All Inclusive IVDs: Molecular Point of Care Testing

Advantages

- Well validated according to FDA or EU directives
- Many new interesting technologies:
  - “Biochips”, “Lab-on-a-chip”, “nanotechnology”, POCT
- Simple and small instrumentation: sample in result out
- Often easy to perform, not requiring extensive expertise
- Applicable to various targets, even multiplex formats
- Diagnostics becoming available to every lab
- **Added clinical benefit through rapid diagnosis**

IVDs: the Future: All Inclusive Systems

sample in → **qualitative result out**

Different single use cartridge based tests are available
RVP panel: from 2 to up to 6 targets
TAT from 30 min. to 1.5 - 2hr

Impact on patient management: Need for speed! TAT is crucial!

- Evaluation of 2 real-time RT-PCR assays (Cepheid, Sunnyvale, USA) on SmartCycler **(TAT < 1hour)**
  - RSV Analyte Specific Reagent (ASR) bead
  - Influenza A/B ASR bead
- Comparison with “in-house” multiplex real-time PCR for flu and RSV

- Results:
  - RSV: sens: 98.2%, spec: 100%
  - Influenza A/B: sens: 96.5%, spec: 100%

Compared to “in house” multiplex: significant ⇩ in TAT

facilitates urgent testing outside batched runs or normal working day

Sails AD et al. J Virol Methods 2009, 162: 88-90

Rapid etiologic diagnosis by RT-PCR can reduce antibiotic prescription

- randomised prospective study
- Flu A 48%, Rhino 22%, HCoV 15%
- Antibiotic prescribed in 11% of initial visits
  - Rapid PCR results group: 6.8%
  - Delayed results group: 15.1%

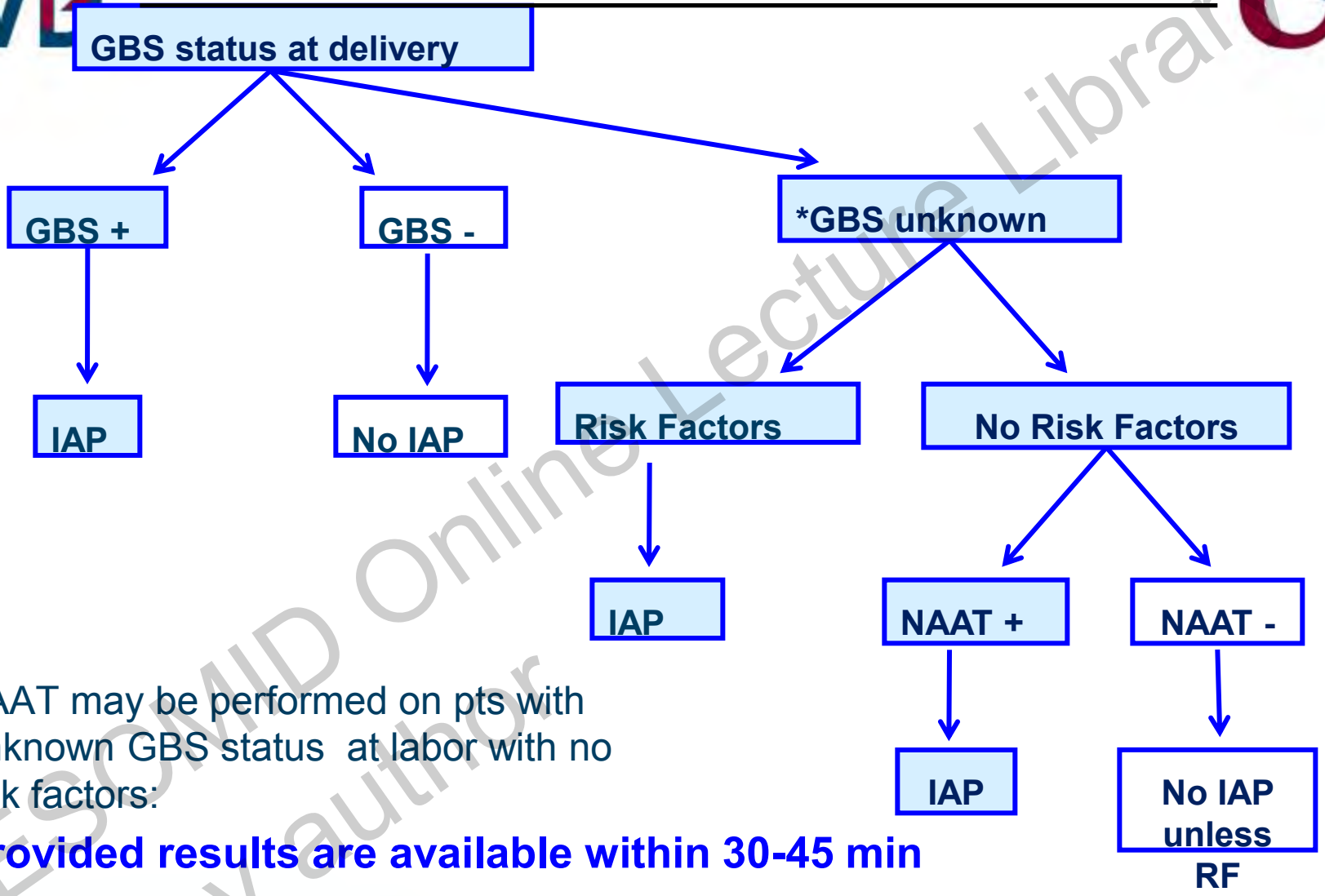
P= 0.01

Rapid etiologic diagnosis influenced physicians to prescribe less antibiotics

AB prescription reduced by half in group randomised for rapid results

Brittain-Long R et al. ECCMID 2010, P1332

Intrapartum Testing by NAAT Tests



NAAT may be performed on pts with unknown GBS status at labor with no risk factors:

provided results are available within 30-45 min

Provided the availability of a POCT : NOT POSSIBLE WITH IN HOUSE

Cost & effectiveness of Intrapartum GBS screening : expected advantages

- Intrapartum GBS PCR, (968 Pregnant women) Vs intrapartum culture
  - **Sensitivity 98.5%**
  - **Specificity 99.6%**
  - **PPV 97.8%**
  - **NPV 99.7%**
  - GBS POCT PCR (35-45 min)

Najoua El Helali, et al. *Clin Infect Diseases* 2009;49:417–23

- Intrapartum PCR in 2010 vs antenatal GBS screening in 2009:

	2010	*2009*
GBS colonization rate:	**16.7%**	**11.7%**
Probabilities of GBS disease:	**0.9%**	**0.5%**
Total cost/delivery in $	**1,754 +/- 842**	**1,759 +/-1,209 (*P*=0.9)**

- Intrapartum PCR screening: **only possible with commercial POCT**

⇒ **Increased accuracy of GBS status at time of labor & delivery**

⇒ **Improvement of prevention**

Najoua El Helali, et al. *Obstet Gynecol* 2012;119:822–9

Towards « European Consensus » for GBS screening and treatment

Decision taken by a European working party

(Neonatologists, obstetricians, microbiologists)

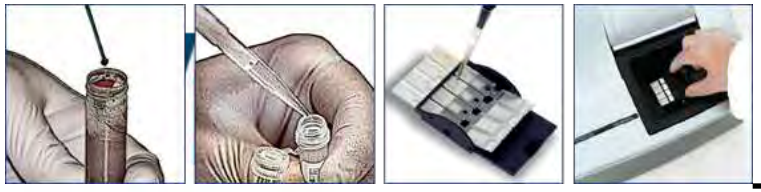
including countries with screening-based IAP, with risk-based IAP strategies or nothing (June 2013, Florence, Italy)

Main recommendations

- Universal screening at time of delivery
  - POCT with high PPV and NPV
    - Real time PCR or other methods
  - TAT < 1 hour
- IAP for all GBS positive pregnant women
  - documented by intrapartum testing (or late pregnancy test if performed)

Kindly provided by Pierette Melin

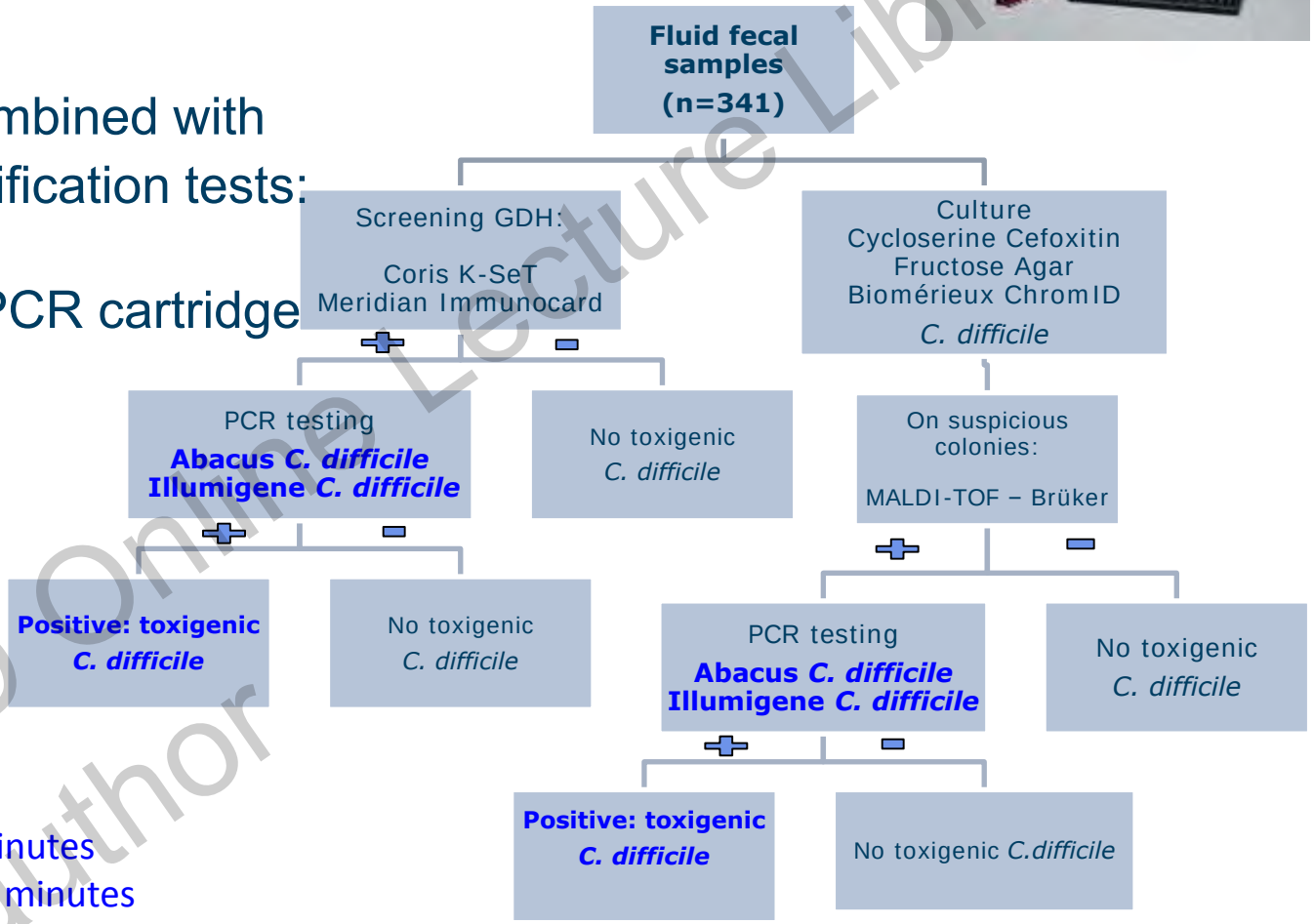
⇒ Can only be realized with the use of a commercial test



Towards POCT for *C. difficile*

Algorithm with:

- GDH based test combined with
- 2 CE marked amplification tests: LAMP, PCR
- Reagent strip and PCR cartridge



Hands on time: 3-5 minutes
Time to final result: 50-60 minutes

Rapid results available provided the availability of a commercial test

Ho E et al. ECCMID 2014, eP1332

All Inclusive Systems for Multiplex syndromic approach

- FDA cleared Mx panel for the detection of respiratory pathogens
- Closed system integrates sample prep, amplification, detection and analysis
- Performance characteristics for the detection of Influenza:
  - Sensitivity: ranging from 78% - 95%

	Prodesse ProFLU+	Prodesse ProFAST+	FilmArray RP	Verigene RV+
Pathogens	Flu A, B, RSV	Flu A, B, RSV	17 viruses, 3 bact	Flu A, B, RSV
Sample prep	Not included	Not included	Included	Included
TAT in hrs	**3**	**3**	**1**	**2.5**
Hands-on time	1.5h	1.5h	2 min	5 min
throughput	14 samples/run	14 samples/run	1 sample/instrument	1 sample/processor

High-risk patients could greatly benefit from a broad and rapid screening of different respiratory pathogens.

Van Wesenbeeck L et al *J Clin Microbiol.* 2013; 51: 2977–2985

Molecular diagnosis: commercial or home-made tests? Conclusions

Commercial tests

Standardization
Validation
Test performance
TAT: very short
Ease of use
Multiple targets
Multiplex

In-house tests

??
??
Test performance
TAT: longer
User friendliness?
Multiple targets
Multiplex