One-assay approach and principles of CE-IVD (in vitro diagnostics).

M. Ieven
Maastricht
07.03.2014

Vaccine & Infectious Disease Institute

Universiteit Antwerpen

IVDs: Today and the Future: technological advances

Today

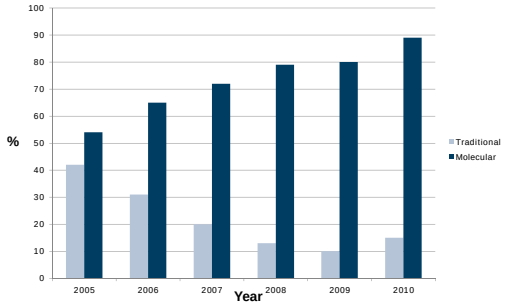
- the variety and complexity of diagnostic tests and instruments available has evolved dramatically, IVDs have been an area of constant innovation
- Automation for NA extraction, amplification and detection platforms
- Fast cyclers allowing to identify a disease earlier
- User friendly devices can be 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
- Next gen sequencing

1

Increase in laboratory's reported use of Molecular Diagnostics output.

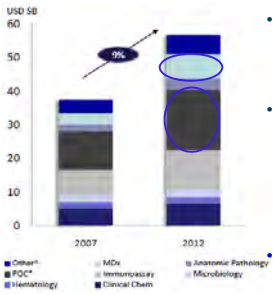


'Traditional includes' : culture, serology, etc

Kindly provided by QCMD

2

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 time required for results from hours to minutes and permit improve outcome for patients and revolutionize the field of infectious diseases
- ***However this is only true if the result delivered is accurate and reliable.***

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

Source: Sientia analysis, Kalorama, Frost & Sullivan.

3

One assay approach? issues and regulation we have to think about

- Organization of the laboratory
  - Certification or accreditation
- Choice of platforms and assays available
  - Extraction and amplification platforms?
  - Qualitative versus quantitative?
  - Limited target versus multiplex detection?
- In house versus CE and CE/IVD assays?
- What can we expect from assays we are using?
- What demands for CE marked and in-house developed assays? The clinical use of them

4

One assay approach: issues and regulation we have to think about

- Organization of the laboratory
  - Certification or accreditation?
- Choice of platforms and assays available
  - Extraction and amplification platforms?
  - Qualitative versus quantitative?
  - Limited target versus multiplex detection?
- In house versus CE and CE/IVD assays?
- What can we expect from assays we are using?
- What demands for CE marked and in-house developed assays? The clinical use of them

5

Choice of platforms and assays available

Extraction System

QIAsymphony RGQ, System

Nuclisense EasyMag System

The MagNA Pure 96 System

One assay approach ⇨ consolidation on same platform

Amplification System

Cobas Amplicor System

LightCycler® 480 Instrument

CFX96 Touch™ RT PCR

One assay approach ⇨ consolidation on same platform

6

Consolidation of extraction methods: EQA panel to evaluate the performance

Sample Content	Conc. / Dilution	Centre 2 Own extraction	Centre 2 EasyMAG	Centre 3 Own extraction	Centre 3 EasyMAG
HMPV-I	$1x10^{-5}$	45,1	40,5	37,93	39
PIV2	-34	33,1	30,9	32,7	31,57
INF B	$1x10^{-6}$	39,3	0	38,9	36,43
HMPV-II	$1x10^{-5}$	41,5	0	37,85	36,52
INF A	$1x10^{-7}$	39,5	0	0	37,9
PIV1	-30	34	33,8	30,53	29,87
INF A	$1x10^{-6}$	0	36,7	40,39	35,05
PIV1	-34	36,1	36	33,71	34,08
INF A	$1x10^{-6}$	36,3	37,2	37,75	35,97
INF A	$1x10^{-5}$	34,4	35	35,38	33,75
RSVA	$1x10^{-5}$	40,7	35,3	32,26	30,07
PIV1	-27	31,1	30,9	27,85	27,19
RSVA	$1x10^{-3}$	29,5	29,8	24,24	27,75

One assay approach ⇨ consolidation on same platform

EasyMag was implemented for all extractions

7

Choice of platforms and assays available

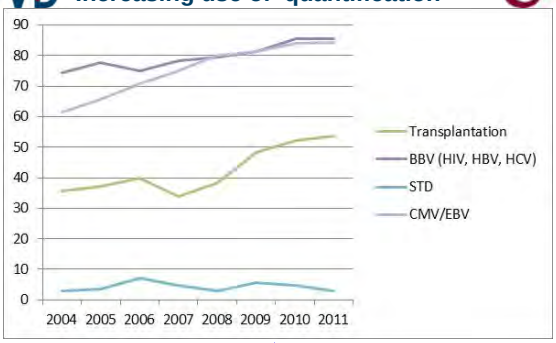
- Conventional versus real-time assays?
- Qualitative versus quantitative?
- Limited target versus multiplex detection?
- Commercially available versus in-house tests?

- Choices influenced by:
  - the application e.g. respiratory vs STD testing
  - The clinical context: e.g. outbreak management vs clinical diagnosis
  - Diagnosis versus monitoring e.g. detection of HCV RNA
  - Availability of test platforms
  - Cost

➡ **What is appropriate for one lab may not be for another**

8

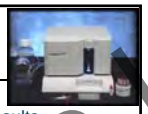
Qualitative vs quantitative testing? Increasing use of 'quantification'



One assay approach ⇨ **RT-PCR approach**

Kindly provided by QCMD

Limited target versus multiplex detection e.g. for respiratory pathogens



Limited target detection

- Usually ⇧analytical sens.
- Mostly lower cost
- Often lower TAT
- In outbreak situations
  - SARS Coronavirus
  - Influenza, H1N1
  - RSV
- As first approach
  - in high prevalence periods
  - if therapeutic implications
- Outside normal working hours

Multiplex detection

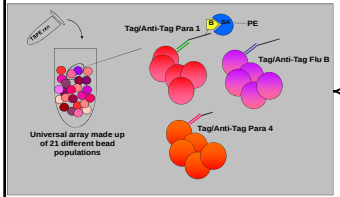
- In >90% similar results
- Expensive
- TAT usually > 4-6hours
- For epidemiological studies
  - Prevalence of respiratory etiologies
  - Role of respiratory viruses
- As add-on diagnostic test
  - In severely ill patients
  - In immunocompromised
- For virus discovery studies

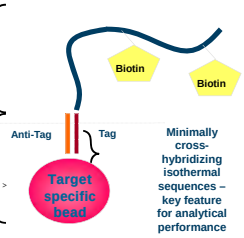
One assay approach ? Evaluate intended use, advantages, limitations

Luminex xTAG Universal Bead Array

Universal Array Sorting

Oligo-coupled beads used with Tag-It products. Use MFI for every target-specific primer to determine presence or absence of each virus



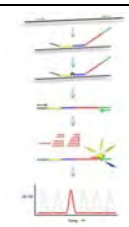


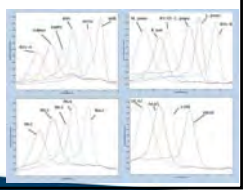
Minimally cross-hybridizing isothermal sequences – key feature for analytical performance

RVP 10-12 hr to RVP Fast 4-5 hr

RespiFinder Mx assay, Pathofinder

- Rapid, conventional & real-time PCR
- Multiplex capabilities expanding till up to 25 targets
- 18 viral + 4 bacterial pathogens in 1 RT assay
- Gene-specific Mx reverse transcription step
- 2 pathogen specific probes hybridized by ligation, amplified and detected by melting curve analysis
- Contains a competitive internal amplification control
- Diagnosis within 6 hours
- Validated on QCMD panels
  - CE-IVD labelled





Limited target versus Mx detection: Influenced by intended use eg in LRTI

- Public health impact on outbreak management
  - Rapid detection of influenza
  - Rapid detection of RSV
- Medical impact on epidemiological information
  - Distribution of respiratory pathogens in LRTI
- Individual patient care
  - impact on patient management: Cost-efficiency aspects?
    - Stop inappropriate AB use: reduces overall costs, resistance
    - Start quickly specific antiviral treatment
    - Decrease unnecessary diagnostic studies

13

Limited target versus Mx detection: flu H1N1 outbreak management

- Semiautomated and fully automated assays for detection of FluA (including H1N1) and FluB:
  Flu A sensitivities varying between 93% - 98%,
  specificities varying between 99 -100%

Sails AD et al. J Virol Methods 2009, 162: 88-90
Ginocchio CC et al J Clin Virol 2009; 45: 191-195
Casalegno JS et al Clin Microbiol Infect 2009; 16: 326-329
Beck ET, et al J Mol Diagnostics 2010; 12: 74-81

⇨ Automated **high throughput molecular system** allows clinicians and public health officials to **react quickly during outbreaks**

14

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

15

Impact of molecular methods on the diagnosis of respiratory tract infections

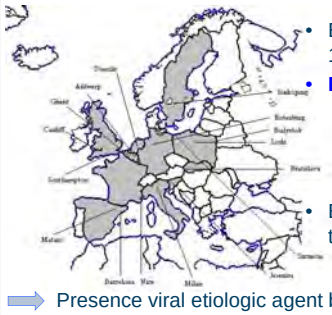
- Public health impact on outbreak management
  - Rapid detection of influenza
  - Rapid detection of RSV

- Medical impact on epidemiological information
  - Distribution of respiratory pathogens in LRTI

- Individual patient care
  - impact on patient management: Cost-efficiency aspects ?
    - Stop inappropriate A/B use: reduces overall costs, resistance
    - Start quicker specific antiviral treatment
    - Decrease unnecessary diagnostic studies

16

GRACE: Etiologic diagnosis of LRTI in primary care: impact of RT-PCR



- EU FP6 Network of excellence 16 PCN in 11 countries
- **From 10/2007 – 12/04/2010**
  - 3102 adult patients with LRTI
  - 2984 controls
  - 16 PCN in 12 countries
- Blood & respiratory samples taken, transported to Antwerp

⇒ Presence viral etiologic agent by RT-PCR: **53.7%**
⇒ In total: etiology found in **> 70%** in LRTI in community

leven M et al on behalf of GRACE ECCMID 2010

17

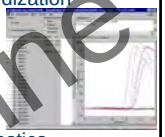
Significance of etiologic agents?

Target	Patient with LRTI prevalence n/total (%) first visit (n=3058)	follow up visit (n=2549)	*P*-value
Rhinovirus	590 (19.3)	111 (4.4)	< 0.00001
Influenza A/B	315 (10.3)	11 (0.4)	< 0.00001
Coronaviruses	233 (7.6)	71 (2.8)	< 0.00001
RSV	144 (4.7)	13 (0.5)	< 0.00001
Human MPV	142 (4.6)	7 (0.3)	<0.00001
Parainfluenza 1-4	83 (2.7)	13 (0.5)	< 0.00001
Human AV	47 (1.5)	44 (1.7)	0.26
Polyomavirus WU	44 (1.4)	54 (2.1)	0.06
Polyomavirus KI	27 (0.9)	29 (1.1)	0.51
Bocavirus	18 (0.6)	11 (0.4)	0.96
Total viruses	**1643 (53.7)**	364 (14.2)	

18

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
- internal controls sometimes included



"In house tests"; developed & used for diagnostics purposes on the premise of the developer / user
- 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)*

19

Verification and validation requirements of molecular tests

- The complexity and the extent of verification or validation procedure depends on the fact whether an IVD-CE labeled test or a self-developed ("home-brewed") 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)

Rabenau HF et al., J Clin Virology 2007; 40: 93-98

20

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

21

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"

22

Conformity assessment of IVDs

- Variety of conformity assessment procedures related to various classes of risks (for Annex II List A "CTS" which detail required performance evaluation criteria, reevaluation ….)
- High risk IVDs may require lab based performance evaluations in accordance with CTS: HIV, HCV,...only?
- Notified Body involved in assessment of 'higher risk' products
- More than one possible assessment route for 'higher risk' products
- Self certification for IVDs considered "low risk"
- Limited review of tests for home use

23

Classification of IVDs by the in vitro directive 98/79/EC

- **Annex II List A :-**
  Blood grouping, ABO, Rhesus (C, c, D, E, e) anti-Kell, Blood virals, **HIV 1&2, HTLV I&II, Hepatitis B, C, D**
- **Annex II List B :-**
  Irregular anti-erythrocyte antibodies, **Congenital infections: Rubella, Toxoplasma**, Hereditary disease: phenylketonuria, Blood grouping: anti-Duffy, anti-Kidd,Human infections: **CMV, Chlamydia**, HLA tissue groups: DR, A, B Tumoral marker: PSA, Trisomy 21 (including software),Self test: blood sugar
- **IVDs for Self Testing**
- **Most IVDs**

Descending order of risk

24

Classification of IVDs by the in vitro directive 98/79/EC

25

EUROPEAN COMMISSION

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}

A proposal for a Regulation on in vitro diagnostic medical devices

- Current legislation

DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 27 October 1998 on *in vitro diagnostic medical devices*

P7_TA-PROV(2013)0427

In vitro diagnostic medical devices ***I

Amendments adopted by the European Parliament on 22 October 2013 on the proposal for a regulation of the European Parliament and of the Council on in vitro diagnostic medical devices (COM(2012)0541 – C7-0317/2012 – 2012/0267(COD))[1]

(Ordinary legislative procedure: first reading)

- 532 amendments ⇒ 27 compromise IVD amendments
- target for adoption 2014 ⇒ effective from 2015 to 2019.

27

Proposal for a regulation on In Vitro Diagnostics (IVDs)

Main elements of the proposal include:

- Wider and clearer scope of the EU legislation
- **Stronger supervision of independant assessment bodies by national authorities**
- More **power** and **obligations** for **assessment bodies** to ensure thorough testing and **regular checks on manufacturers**
- Clearer rights and responsibilities for manufacturers, applying also for diagnostic services and internet sales
- Stricter requirements for clinical evidence, to ensure patient safety
- Adaptation of the rules to technological and scientific progress

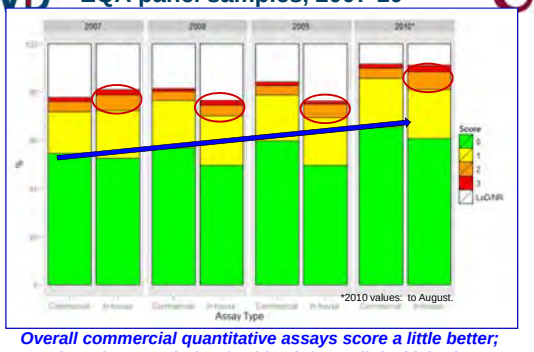
28

Do verification and validation procedures guarantee high quality tests?

Performance of molecular tests for the detection of blood borne viruses (HIV, HBV, HCV, CMV, …: belonging to Annex II List A or B of the Directive 98/79/EC

29

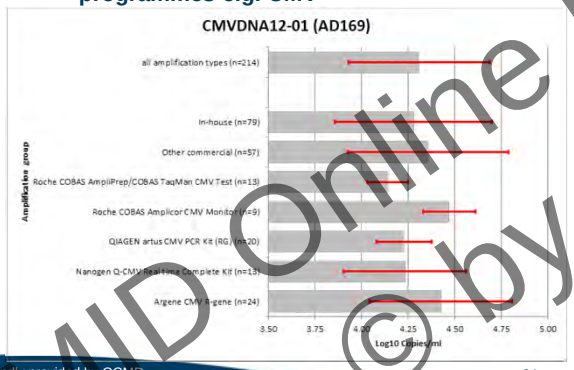
QCMD Quantitative scores for EQA panel samples, 2007-10



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

30

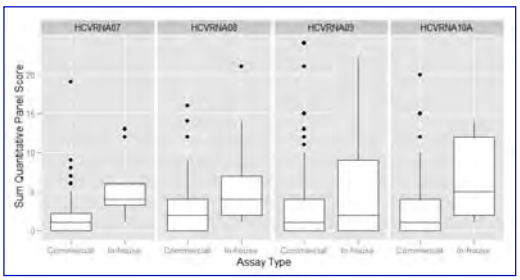
QCMD results of quantitative EQA programmes e.g. CMV



Kindly provided by QCMD

31

HCV: commercial vs in-house?



	Number of datasets scored			
	HCVRNA07	HCVRNA08	HCVRNA09	HCVRNA10A
Commercial	60	163	139	170
In-house	16	17	15	12

- Lower mean score for commercial panels
- Smaller range

32

Why do diagnostic tests differ in performance? Do CE marked test always 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?

33

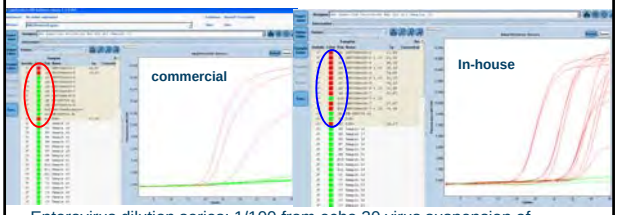
Sensitivity and specificity of two different commercial assays

- Evaluation of 2 CE labeled multiplex assays: RespiFinder (Pathofinder) and xTAG RVP (Luminex) on
- Compared with in-house PCRs used in GRACE

	RespiFinder		xTAG RVP	
Virus	Sensitivity	Specificity	Sensitivity	Specificity
INF	84.8%	98.4	72.3	96.8
HCoV	89.1	99.0	32.6	98.7
hMPV	100	98.5	96.6	99.4
HRV	95.2	98.3	88.7	99.6
RSV	88.5	100	71.4	99.1

- Both commercial assays are less sensitive than in-house PCRs used in GRACE
- RespiFinder (Pathofinder) is more sensitive than Luminex assay

Entero: commercial vs in-house?



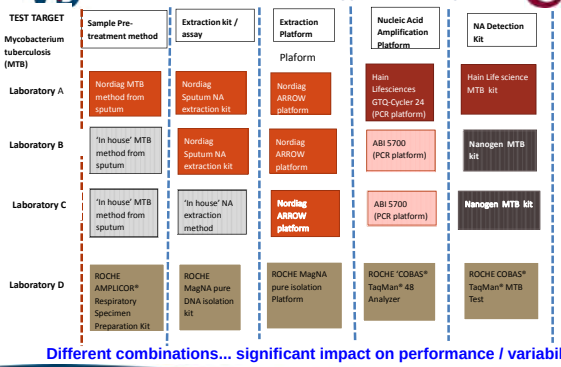
Enterovirus dilution series: 1/100 from echo 20 virus suspension of $5x10^{6.5}$ TCD50/ml: 1/100 dilutions in pool cerebrospinal fluid:

In-house PCR = 2 steps PCR with cDNA step, commercial PCR = 1 step
If commercial PCR with cDNA ⟹ sensitivity can be improved

➡ **In-house test more sensitive than commercial test**

35

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



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

Kindly provided by QCMD

36

Possible reasons of poor performance of some CE marked IVDs

- **Variables in performance depending on test itself?**
  - Clinical sensitivity
    - Clearance after comparison with suboptimal **"gold standard"** reference test
    - Influenced by multiplexing
  - Clinical specificity:
    - Primer/probe design
    - Changing/emerging pathogens: Revalidate specific target or complete assay?
- **Variables in end-user that affect assay performance?**
  - Sample integrity, sampling technique, sample preparation
    - Use of other sample preparation than what is recommended by manufacturer
  - Operator experience and training
  - Inappropriate interpretation of results, absence of appropriate QC

37

One assay approach: issues and regulation we have to think about: CONCLUSIONS

- Important factors in choice of tests and organisation in lab
  - Clinical relevance and utility in own setting
  - Level of experience and training and familiarity of the user with test procedures is essential to the validity of the test result
  - Infrastructure and equipment available
  - Cost efficiency items should be considered
- CE/IVD's should be reviewed, monitored on ongoing basis
  - participation in EQA schemes by IVD providers is recommended .
- EQA help the clinical lab's for accreditation requirements
  - Certification of primers and probes
  - Validation of in house versus commercial methods

➟ **Support choices of reagents and methods**

38