Workflow automation in molecular diagnostics

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Department of Medical Microbiology

- Bacteriology (incl. mycology & parasitology)
- Virology
- Infection Control & Hospital Hygiene
- Research

- Total department: ~250 employees (including research)
- Diagnostics: ~200.000 Samples/year
  - 80% own hospital, 20% external (mainly from GP-labs)

Our molecular diagnostics (MDx) portfolio

- ~ 70.000 MDx tests per year (mainly multiplex realtime-PCR)
- >90 different MDx tests (including many multiplex PCRs)
- Rapidly expanding MDx portfolio

~30% commercial kits (e.g. HIV, HCV, CT/GO, MTB, MRSA)
- Predominantly high volume tests OR rapid molecular prescreens
- CE/IVD

~70% In house assays (mainly realtime-PCR based)
- All remaining pathogens (low and high volume!)
- Continuous expansion of portfolio
- Flexibility to act and implement quickly (Flu, VRE, CPE, ...)
- Extensive validation mandatory (ISO 15189)

Assay Setup

In house Realtime PCR assays

Need to validate the complete proces prior to introduction of PCR (ISO 15189)

Clinical sample

↓

Nucleic Acid extraction

↓

Realtime Amplification

↓

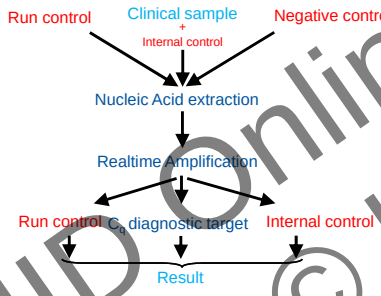
C_q diagnostic target

↓

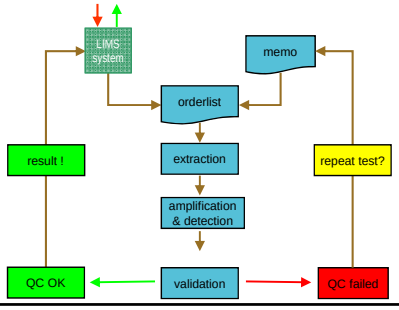
Result

Assay Setup

In house Realtime PCR assays

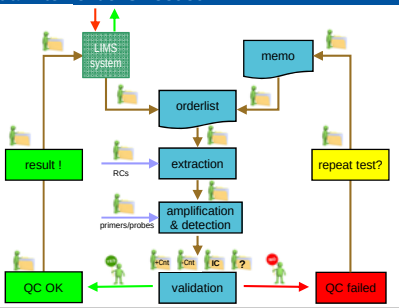


MDx process



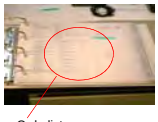
MDx process

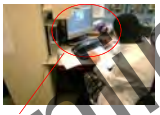
Many manual interventions needed

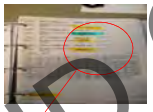


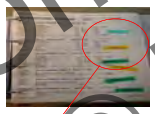
LDT MDx

Manual datamanagement


Orderlist

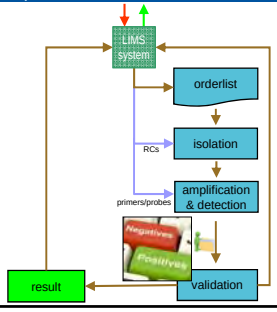

Data analysis


Reagent lots


Run control lots

MDx process

Ideal optimized setup



Route towards an optimized MDx setup

MDx optimization will only be successful if the laboratory process (logistics) is completely redesigned

In other words: lab-automation is not just about replacing a technician by a robot/liquid-handling system, but…

One needs to connect all equipment to LIMS
***and** achieve full process control*

Advantage of integrated MDx system

- Advantages of connecting equipment to LIMS
  - Improves lab efficiency
  - Reduces data entry errors
  - Automated technical validation and interpretation
  - Online logging reagent lots (stock management)
  - Automated realtime QC (e.g. Shewart plots from internal and external controls)
  - Data mining possible for all assay parameters & reagents in case of problems
- Advantages of full process control
  - Barcoding of samples and reagents
  - Tracking and tracing of all samples, reagents and results
  - Integration of individual equipment into a controlled workflow environment
- Net result: improved efficiency and more reliable MDx
- **More reliable tests = improved safety for the patient**

2011 – The options

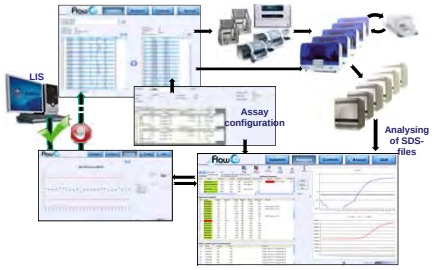
Qiagen Symphony

bodéqio

Roche FLOW

FlowG

(open to multiple MDx components)



The pros and cons (2011)

- **Open Access solutions (local solutions)**
  - Pro: Flexibility (adapted to local situation)
  - Con: Multiple hardware and software partners involved
    - *Software needs to be developed /adapted*
    - *Stability of operation & support*
    - *System updates (file formats) may affect performance*
    - *Responsibility*
- **Dedicated solutions (e.g.Roche FLOW)**
  - Pro: One stop shop
    - Multiple users (experience groups)
    - Integrated equipment and software validation
  - Con: Reduced freedom in operational setup (local solutions)
    - Slow implementation of new middleware and hardware developments

From separate modules…

Roche MP96

Flow Middleware

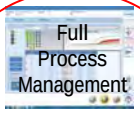
Hamilton Liquid Handling

LC480 II qPCR System

…to integrated MDx modules

Roche MP96

Full Process Management



FLOW Middleware

Hamilton Liquid Handling

LC480 II qPCR System

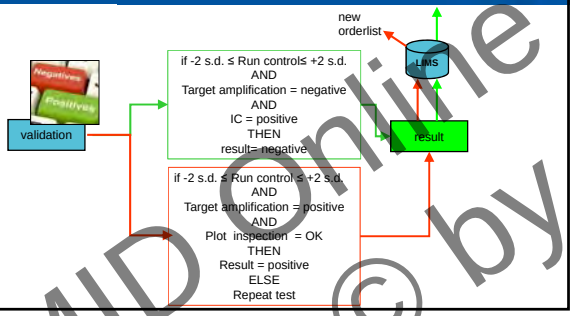
The FLOW Components

PSH | MagNA Pure 96 | PSU | LC480 II | FLOW software

Middleware

- Bi-directional communication with LIMS
- Bi-directional communication between various pieces of equipment
- Realtime presentation of assay progress on 'Dashboard'
- Automated logging of assay performance parameters
- Automated technical validation of assay results
- Stock monitoring *(pending)*
- Trend analysis *(pending)*

Automated QC and result calling



Assuring daily operation

UMCU Microbiology

Virology

Bacteriology

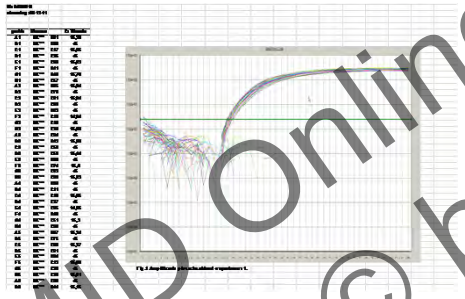
- Double FLOW lines with identical set up
- Swapping between lines in case of component failures prior to the start of a run
- Automated and continuous mirroring of data
- Manual procedures available to safeguard clinical samples in case of component failures **during** a run
- Service and maintenance contracts with Roche

A few examples of FLOW performance

- Checkerboard experiment (to monitor sample cross-over contamination)
- EQA results
- Various specimen types

Example of FLOW performance (1)

Checkerboard experiment



Example of FLOW performance (2)

QCMD HSV EQA panel

Panel code	Test panel code	*Sample description*	EQA Consensus	Core Proficiency	Ct FLOW		Ct MHC	
HSVDNA13-07	HSVDNA13R-05	Herpes Simplex virus (HSV-1, Macintyre)	9940	Core	32.79	HSV-1	31.27	HSV-1
HSVDNA13-04	HSVDNA13R-08	Herpes Simplex virus (HSV-1, Macintyre)	189	Educational	37.78	HSV-1	37.88	HSV-1
HSVDNA13-01	HSVDNA13R-07	Herpes Simplex virus (HSV-1, 95/1909)	9755	Core	29.05	HSV-1	31.13	HSV-1
HSVDNA13-08	HSVDNA13R-03	Herpes Simplex virus (HSV-1, 95/1506)	480	Core	29.58	HSV-1	37.31	HSV-1
HSVDNA13-02	HSVDNA13R-10	Herpes Simplex virus (HSV-2, MS)	6353	Core	27.66	HSV-2	28.53	HSV-2
HSVDNA13-05	HSVDNA13R-01	Herpes Simplex virus (HSV-2, MS)	249	Educational	34.87	HSV-1	35.74	HSV-1
HSVDNA13-03	HSVDNA13R-04	Herpes Simplex virus (HSV-2, 09-015981)	4335	Core	26.74	HSV-2	30.29	HSV-2
HSVDNA13-09	HSVDNA13R-09	Herpes Simplex virus (HSV-2, 09-015981)	91	Educational	35.62	HSV-2	36.34	HSV-2
HSVDNA13-10	HSVDNA13R-02	Varicella Zoster Virus		Core		Neg		Neg
HSVDNA13-06	HSVDNA13R-06	Negative		Core		Neg		Neg

Example of FLOW performance (3)
CSF

MPLC (Ct) FLOW (Ct)

Glims nummer	Materiaal	Bekend pos voor	Bekende ct waarde diagnostiek	ct waarde FLOW	PhHV	PhHv 1:10 ver	Herhaling op MP32 en FLOW
10-008088	Liquor	HSV-2	30,69-30,95	29,99-29,86	28,46		
10-008089	Liquor	HSV-1	35,26-34,05	35,19-34,77	28,43		
10-008092	Liquor	HSV-2	35,26-33,83	33,64-33,21	28,20		
10-008093	Liquor	HSV-1	37,14-37,81	38,54-37,28	28,15		
10-008094	Liquor	HSV-1	31,88-31,35	31,22-31,44	28,22		
10-008096	Liquor	HSV-2	35,44-36,71	37,72-35,58	27,89		
10-011419	Liquor	JC	30,18-29,64	30,61	28,10		
10-016765	Liquor	JC	24,63-24,91	25,14	27,56		
10-026872	Liquor	HSV-1	29,09-29,08	28,59-28,80	27,70		
10-027138	Liquor	HSV-2	28,20-28,31	29,01-28,60	27,18		
10-047924	Liquor	JC	33,21-36,00	34,45	27,98		
10-049520	Liquor	JC	37,14-36,30	41,00	27,70		
11-005575	Liquor	HSV-1	32,37-32,65	32,7-32,88	27,06		
11-009240	Liquor	Toxoplasma	25,88-25,53	25,21-25,19	28,05		
11-011526	Liquor	JC	24,63-25,04	23,23	27,38		
11-012815	Liquor	Toxoplasma	32,85-33,95	32,21-32,64	28,01		
11-020271	Liquor	HSV-1	27,88-28,04	29,95-29,66	29,70		
11-036575	Liquor	HSV-2	30,11-30,49	29,73-29,21	27,70		
11-040264	Liquor	Toxoplasma	33,88-33,80	32,98-33,51	27,23		
11-043012	Liquor	Toxoplasma	34,12-36,01	37,53-36,40	26,38		
11-046197	Liquor	JC	33,40-33,06	35,01	28,37		
11-046921	Liquor	HSV-1	31,49-31,43	29,89-29,48	28,72		
11-051695	Liquor	JC	26,39-27,83	25,90	28,13		

Example of FLOW performance (4)
Occulair Fluids and Serum

MPLC (Ct) FLOW (Ct)

Glims nummer	Materiaal	Bekend pos voor	Bekende ct waarde diagnostiek	ct waarde FLOW	PhHV	PhHv 1:10 ver	Herhaling op MP32 en FLOW
05-000676	VOK	Toxoplasma	31.68 - 31.76	31,01/30,62	31,43		
05-001586	VOK	HSV-1	33.70 - 33.09	33,18/33,08	31,57		
05-003030	VOK	HSV-1	39.59 - 35.12	35,50/35,37	30,95		
05-003031	VOK	HSV-1	32.07 - 32.39	32,82/32,43	31,27		
05-015682	VOK	VZV	lage ct. Door stroomstoring run opnieuw	26,03/26,26	32,02		
05-017879	VOK	VZV	27.34 - 27.44	24,92/24,78	NIET GETEST		
05-019733	VOK	Toxoplasma	36.20 - 37.04	45/45	31,34		
05-019883	VOK	VZV	28.82 - 29.13	26,77/26,61	31,14		
05-020811	VOK	VZV	30.54 - 31.95	29,29/29,57	28,16		
05-040249	VOK	VZV	29.08 - 29.19	25,86/25,64	31,04		
06-001522	VOK	Toxoplasma	35.20 - 39.49	37,60/34,35	31,63		
06-010203	VOK	Toxoplasma	38.04 - 37.19	37,21/37,56	31,07		
06-010616	VOK	VZV	24.29 - 24.27	22,34/22,80	37,56		
06-014257	VOK	Toxoplasma	42.01 - 35.71	37,23/36,13	32,79		
06-018226	VOK	Toxoplasma	33.23 - 32.56	31,45/33,32	31,51		
06-023346	VOK	VZV	35.94 - 36.42	34,03/34,21	28,93		
06-037150	VOK	VZV	33.52 - 33.19	32,10/31,98	29,4		
06-039154	VOK	HSV-1	33.53 - 33.76	36,05/36,24	32,69		

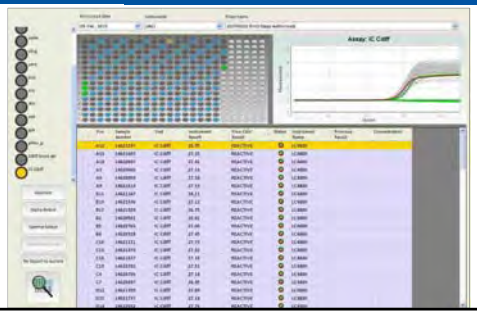
Glims nummer	Materiaal	Bekend pos voor	Bekende ct waarde diagnostiek	ct waarde FLOW	PhHV	PhHv 1:10 ver	Herhaling op MP32 en FLOW
12-028797	Serum	CMV	30,87 - 30,75	32,86	33,35		
12-030641	Serum	CMV	37,33 - 39,34	38,25	33,51		

Example of FLOW performance (5)
Biopsy samples

MPLC (Ct) FLOW (Ct)

Glims nummer	Materiaal	Bekend pos voor	Bekende ct waarde diagnostiek	ct waarde FLOW	PhHV	hHv 1:10	Herhaling op MP32 en FLC
12-013154	Biopten	EBV	33,25 - 34,38	33,03	34,64	32,68	
12-013155	Biopten	EBV	34,84 - 33,85	35,65	45	33,79	
12-017669	Biopten	EBV	32,37 - 32,37	31,52	33,71		
12-017671	Biopten	EBV	35,62 - 34,55	32,43	34,72	33,27	
12-017674	Biopten	EBV	39,81 - 35,34	33,12	33,6		
12-021348	Biopten	HHV-6	26,67 - 26,69	25,97	32,61		
12-021349	Biopten	HHV-6	25,66 - 25,64	25,11	33,21		
12-022945	Biopten	EBV	33,86 - 33,05	29,65	32,51		
12-024297	Biopten	PARVO	35,32 - 35,91	36,1	32,89		

Example of FLOW performance (6)
Control PCR of many targets



Workflow solution for UMCU

Integrated (modular) workflow solution running for >3 years now
- 12 DNA targets in Bacteriology
- 25 DNA targets in Virology
- 2 RNA targets in Virology (more pending)

Reliable performance despite 'early adapter challenges'
- Operational back-up scenario
- Easy switching between hardware components of both Flow-lines

Allows efficient and highly automated workflow

Development of new high sample volume MDx tests now viable option

Consequences of MDx automation

- **Complete change in MDx laboratory setup:**
  - From pathogen / syndrome driven run setup to 'full-portfolio' MDx runs
  - Reduced TTR (twice/thrice daily reporting possible)
  - Reagent supplies (ready to use assay components, eg. Primer/Probe mixes)
  - Reduced human error and sample cross-contamination rates
- **Fewer laboratory staff needed for routine MDx**
  - Only 1–2 technicians per FLOW line (vs 3-4 techs for manual procedures)
  - More resources available for test development/implementation, QC, QMS-tasks
- **Education level laboratory staff**
  - Less highly educated technicians needed for daily MDx
  - More knowledge and expertise needed on liquid handling and ICT processes
- **Structural changes**
  - Result validation now by senior technicians (instead of Molecular Microbiologists)
  - Backup system essential (not enough personnel left to run manually)
- **Financial consequences**
  - Increased hardware/software/IT costs
  - Reduced personnel costs
  - Revalidation on FLOW needed for all running tests (~2-6 weeks per test)

Acknowledgements

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Roche Molecular Solutions International

Sanguin International software solutions

Xiril Robotics

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