

Qiagen 7th Diagnostic Days, Dusseldorf, 27th-29th November 2013

Rationalization and optimization of *CMV* monitoring

CRISTINA COSTA

SC Microbiologia e Virologia U

Azienda Ospedaliera Città della Salute e della Scienza di Torino

**QIAGEN would like to thank
our speaker, Dr. Cristina Costa,
for her presentation.**

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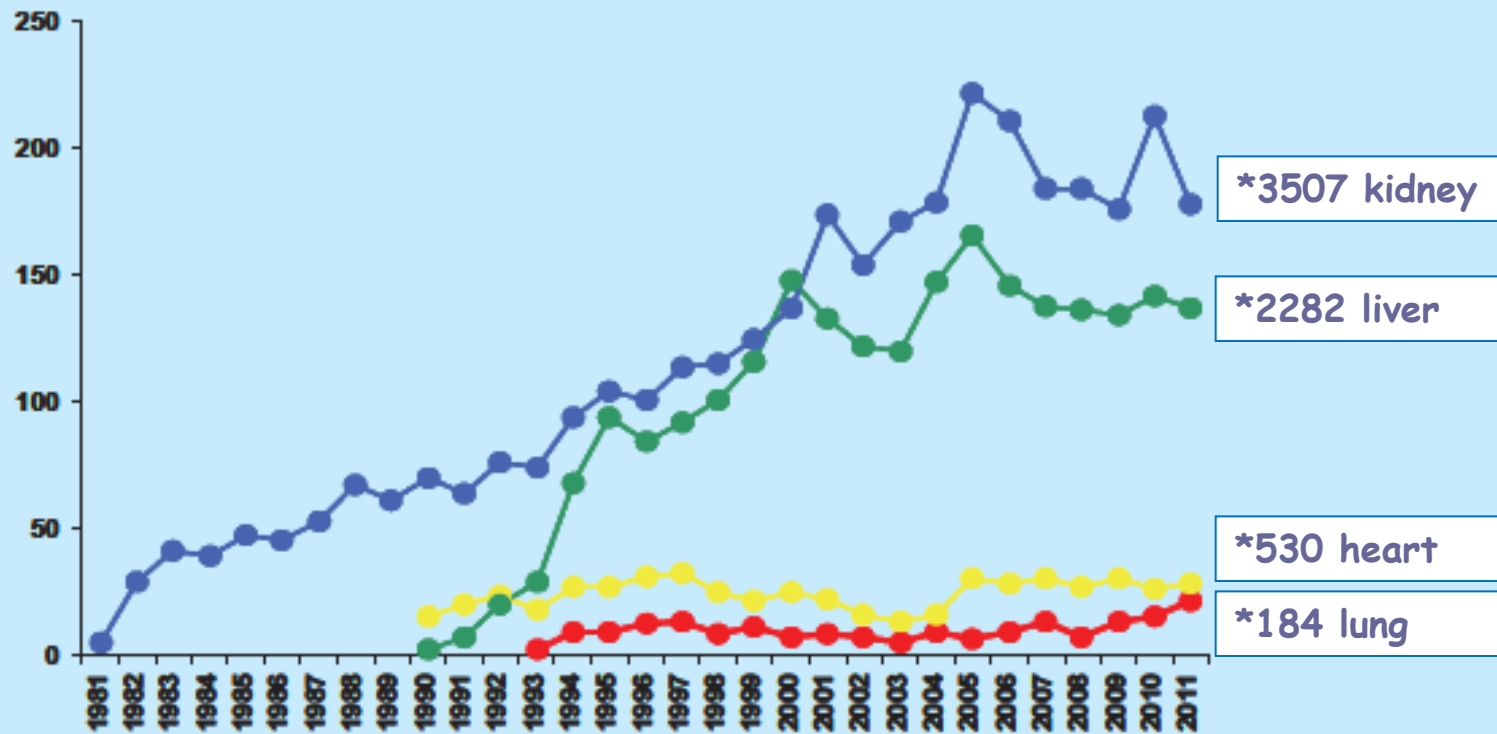
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Virology at Turin: 2004-2013

- Volume of activity
- Diagnostic methods
- Human resources
- Quality management system and certification



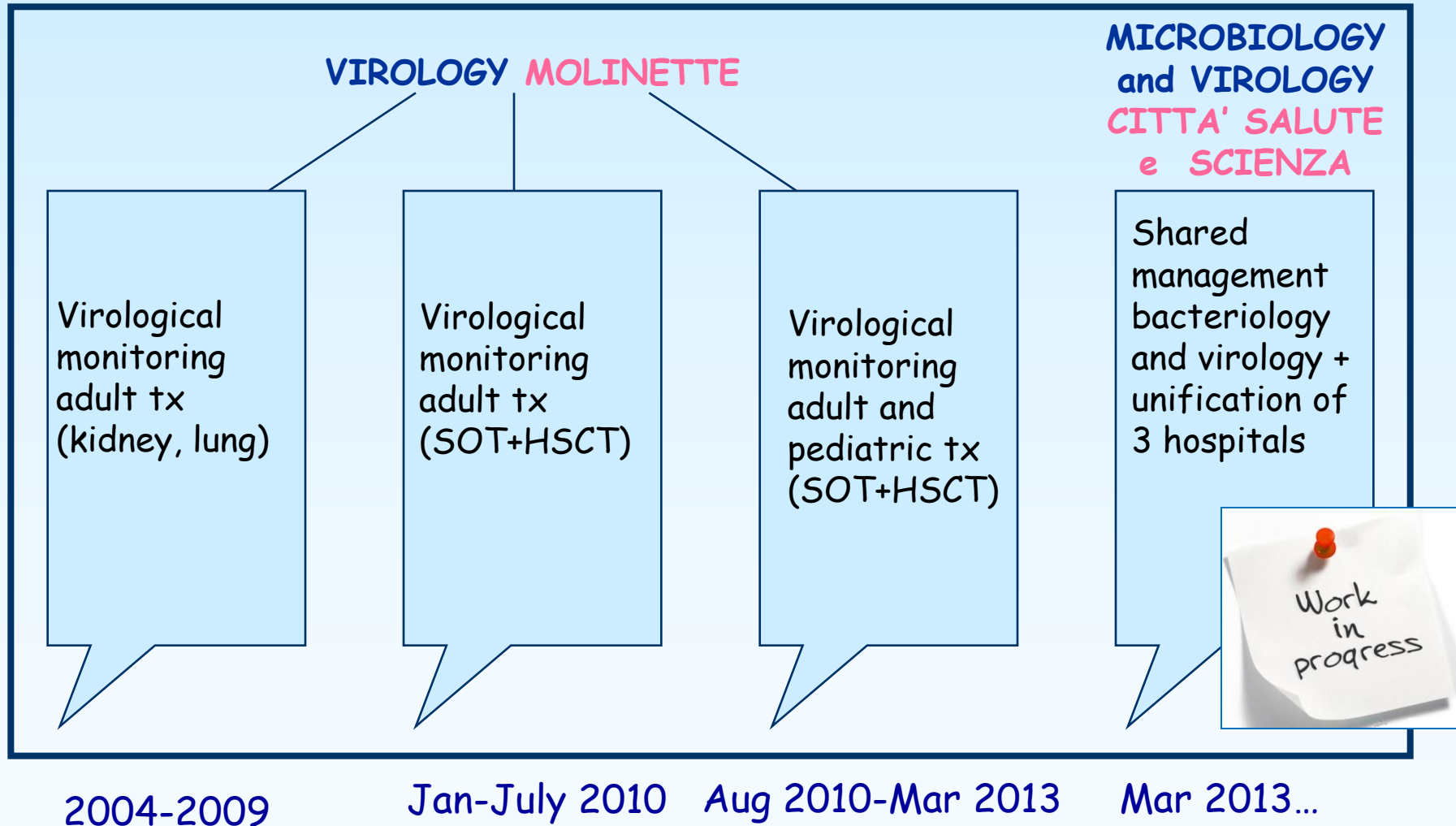
Piemonte and Valle d'Aosta Regional Transplant Center



* Including combined transplants and transplants from living donor

Source CRT: Activity data

Evolution of Virology at Turin: 2004-2013



Quality management system and certification: from 2011

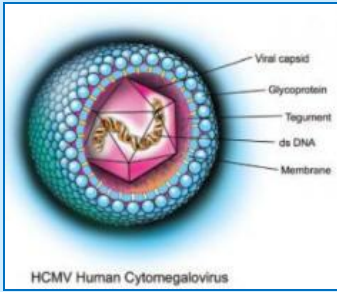
EFFICACY: reliable results, adequate timing

EFFICIENCY: optimization of human, instrument and economic resources

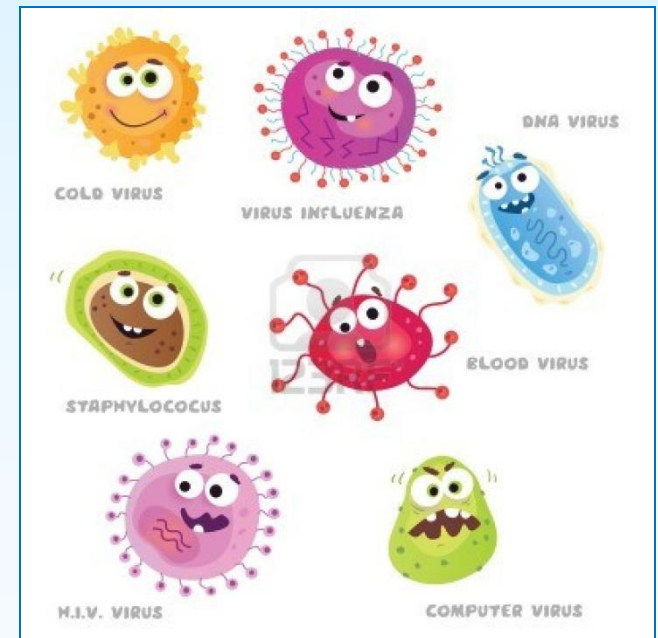
TRACEABILITY: patient, specimen, test, reagents, controls, instrument, operator



**QUALITY =
PATIENT'S
SATISFACTION**

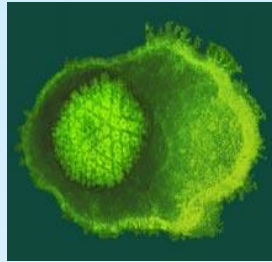
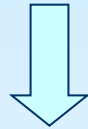


CYTOMEGALOVIRUS



CMV and transplantation

LATENCY REACTIVATION



- * INFECTION: positive virological tests in the absence of symptoms
- * DISEASE: symptomatic infection

RISK FACTORS

SEROLOGICAL MATCHING D/R
(high risk D+/R-)

LEVEL OF
IMMUNESUPPRESSION

Table 1. Incidence of Cytomegalovirus in Solid Organ Transplantation⁴

Organ	Infection	Disease
Kidney	8%–32%	8%
Heart	9%–35%	25%
Liver	22%–29%	29%
Lung or heart/lung	39%–41%	39%
Pancreas or kidney/pancreas	50%	50%

Antiviral strategies and monitoring of CMV at Turin

High risk patients (D+/R-)

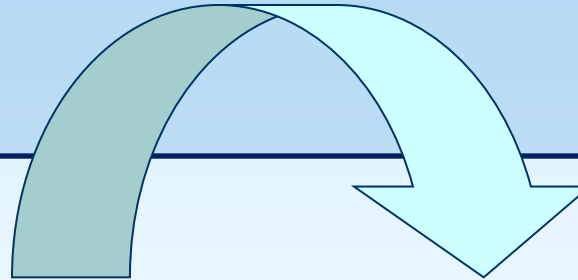
Infection

Disease

ANTIVIRAL PROPHYLAXIS

PRE-EMPTIVE THERAPY

ANTIVIRAL THERAPY



VIROLOGICAL MONITORING

DNAemia on whole blood

weekly up to 3 months

monthly up to 12 months

in case of positivity: twice weekly
until negativization

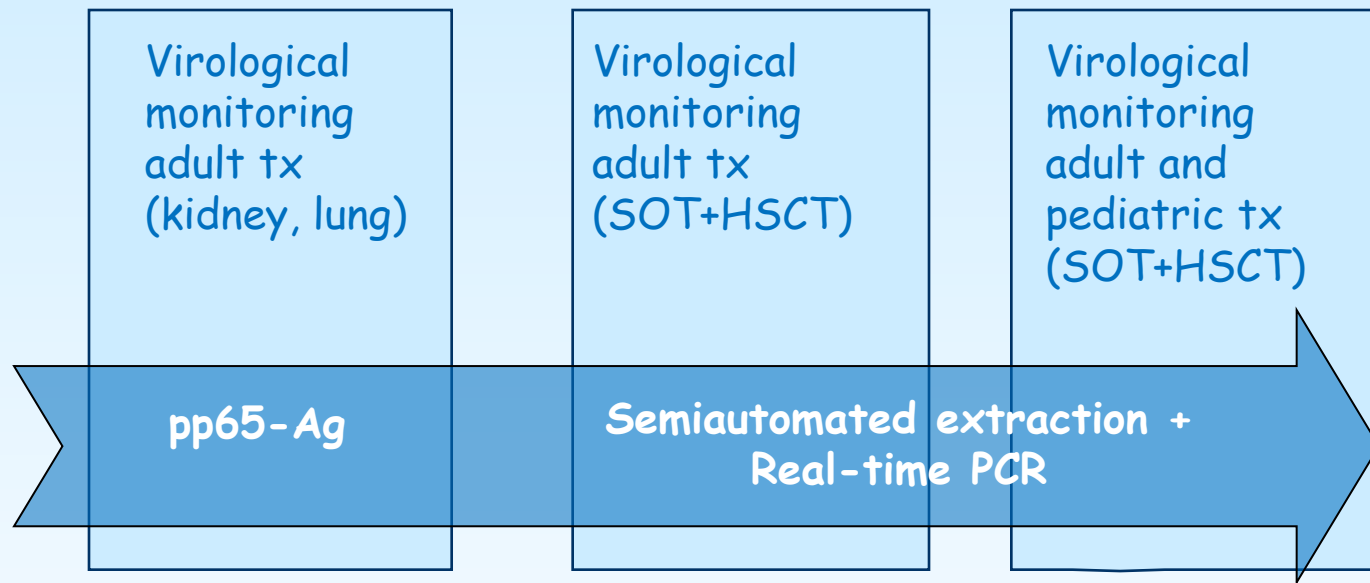
IMMUNOLOGICAL MONITORING

CMV-specific T-cell response

by Elispot assay

at 1, 3, 6, 12 months

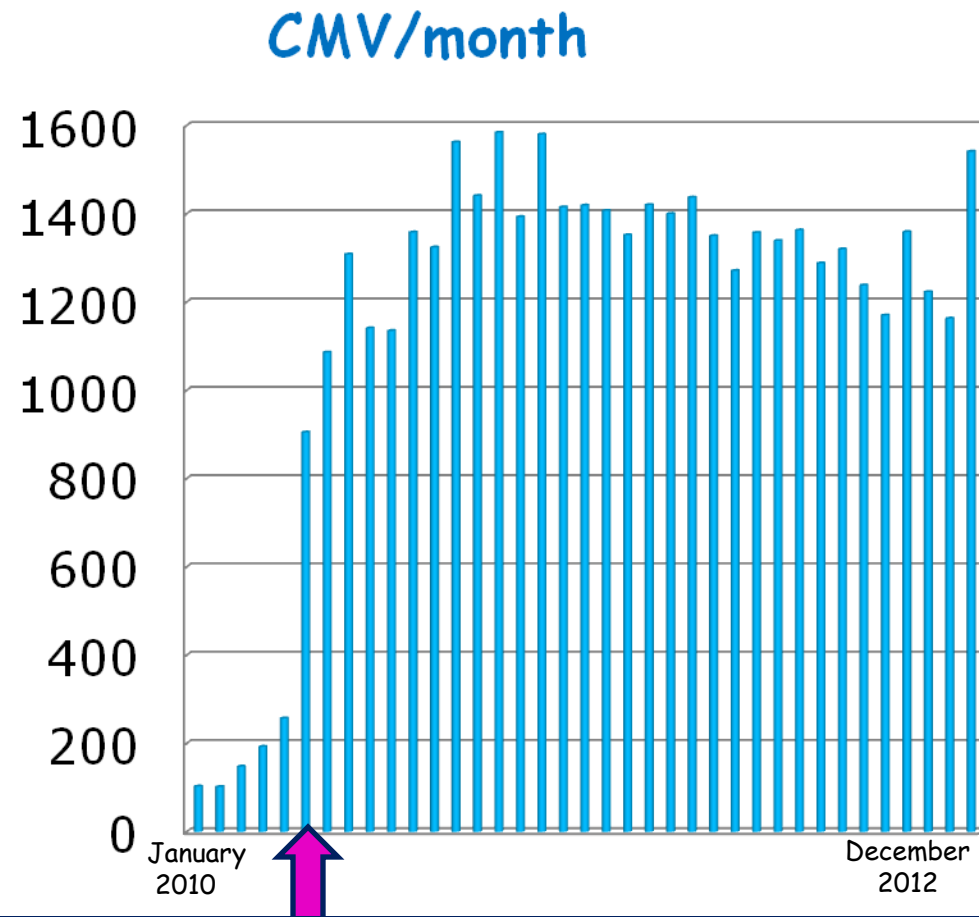
Diagnostics of CMV at Torino: 2004-2010



2004-2009

Jan-July 2010 Aug 2010-Mar 2012

Volume of activity CMV 2010-2012

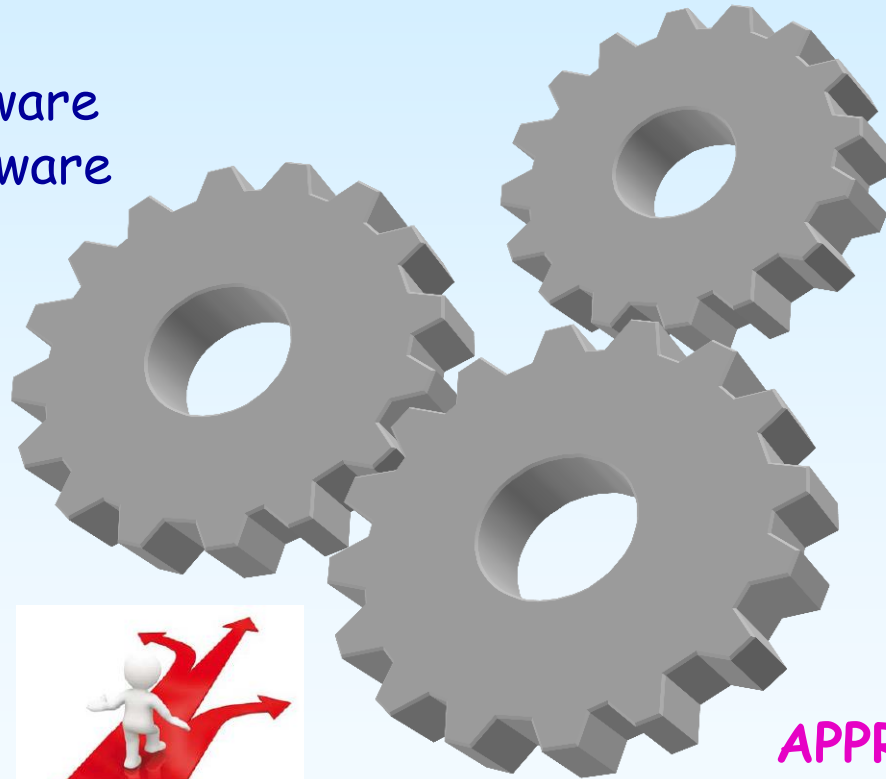


June 2010: virological tests on all adult tx;
August 2010: virological tests on all pediatric tx

Optimization and rationalization

AUTOMATION + INFORMATIZATION

Hospital LIS
Instrument software
Management software



APPROPRIATENESS

"Patient tailored"
diagnostic pathways

Technical implementation of diagnostic activity March 2012

Semiautomated
extraction +
Real-time PCR



Automated
extraction +
Real-time PCR



Result analysis
Transfer to LIS



AMPLIFICATION

Reception of extraction and
assay setup files
Assay setup transfer to ABI



Reception of
work list



SAMPLE PREPARATION

ASSAY
SET-UP

Automated transfer
of eluates
Automated setup based
on work list

LABORATORY
Request for test check-in



Daily management of CMV



Weekly schedule of Molecular Biology laboratory

	PLATE 1	PLATE 2	PLATE 3
MONDAY	CMV	EBV (WB Friday+other specimens)	BK
TUESDAY	CMV	VZV, HHV8, HSV-1 e 2	HHV6, Parvo B19, Adeno
WEDNESDAY	CMV (WB + other specimens)	EBV (WB monday + tuesday)	HHV7
THURSDAY	CMV	JCV	RT+Entero
FRIDAY	CMV	EBV (WB wednesday + thursday)	Quantitation on tissutal specimens

Quantitative result: CMV, EBV, BKV, Parvo B19, Adeno, HSV1, HHV6, Entero

Preliminary qualitative result: VZV, HSV2, JCV, HHV7, HHV8

Respiratory viruses panel by multiplex PCR

EXTRACTION FROM
WHOLE BLOOD FOR CMV -
EBV - DAILY



AMPLIFICATION CMV
DAILY

AMPLIFICATION EBV
3/WEEK

**TODAY WITH
ONLY ONE
QIASYMPHONY**

EXTRACTION FROM
WHOLE BLOOD FOR
OTHER VIRUSES
WEEKLY



AMPLIFICATION
WEEKLY

EXTRACTION FROM
OTHER SPECIMENS
3/WEEK



MANUAL TRANSFER OF ELUATES
INTO A QIASYMPHONY PLATE
FOR ASSAY SETUP



AMPLIFICATION
ACCORDING TO
WEEKLY SCHEDULE

EXTRACTION FROM
WHOLE BLOOD FOR CMV -
EBV DAILY



AMPLIFICATION CMV
DAILY

AMPLIFICATION EBV
3/WEEK

EXTRACTION FROM
WHOLE BLOOD FOR
OTHER VIRUSES
WEEKLY



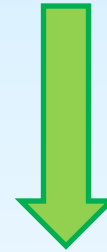
AMPLIFICATION
WEEKLY

EXTRACTION FROM
OTHER SPECIMENS ON
DEMAND WITH
AUTOMATED ASSAY
SETUP



AMPLIFICATION
ACCORDING TO
WEEKLY SCHEDULE

COMING SOON
WITH 2
QIASYMPHONYS



... AUTOMATION,
TRACEABILITY, TIME,
RESOURCES ...

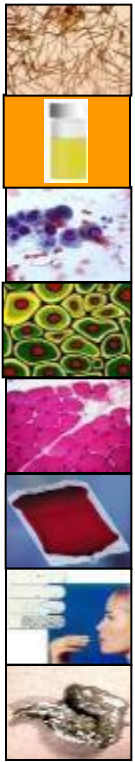
... EZ1 for extraction
of few specimens
QIAgility, liquid
handling

The Qiagen system and its components

Different
sample types

Sample purification & Assay setup

Molecular testing



QIASymphony
Sample Preparation



QIASymphony
Assay Setup

Rotor-Gene Q



Kit artus MDx



Home made assays

Qiasymphony

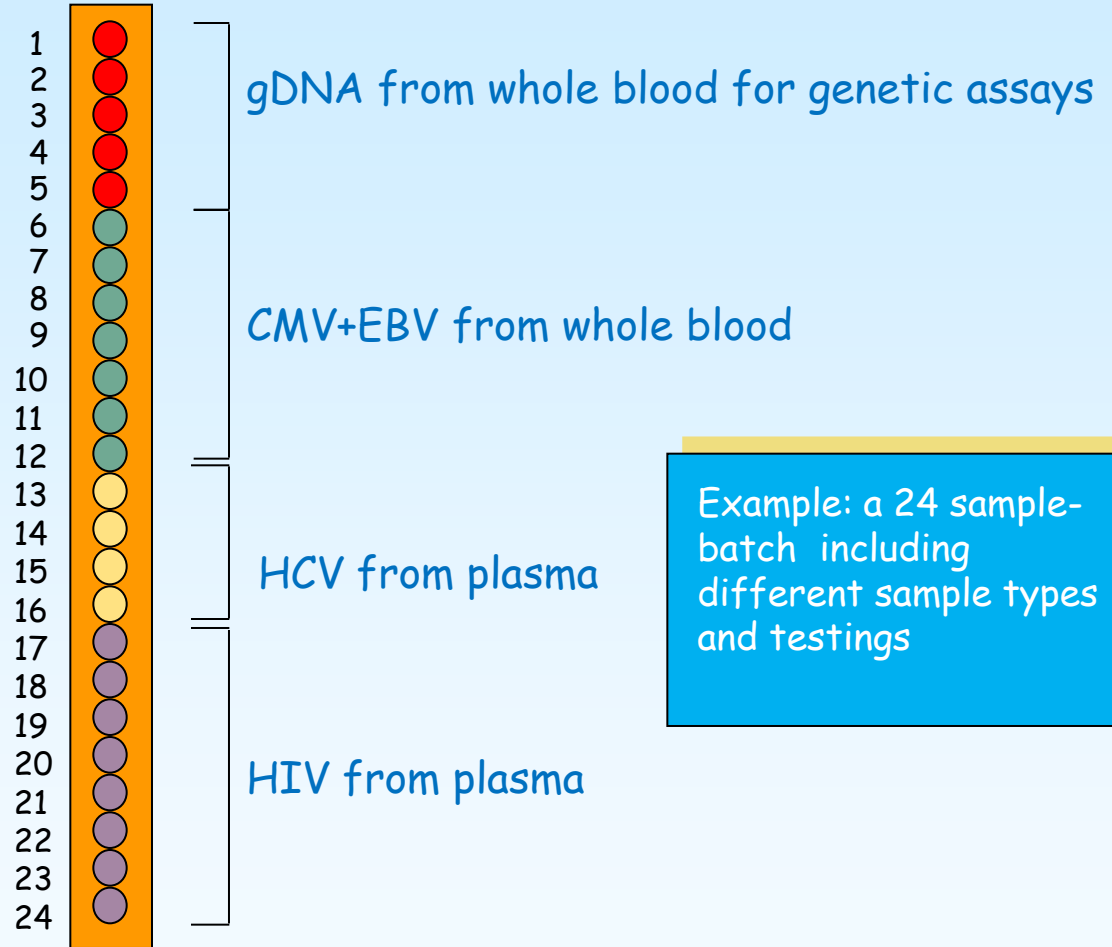
**Rapidity, flexibility and
walkaway automation**

continuous sample loading and walkaway
automatic barcode reading during sample loading
LIS interface

4 batches of up to 24 samples = 1-96 samples
original tubes (adapters $\varnothing$ from 10-13 mm to 15-17 mm)
sample volume up to 1 ml and different elution volumes



Combination of different protocols and testings



Example: a 24 sample-batch including different sample types and testings

Rapid instrument setup



Reagents and consumables drawer

96 samples per run

tip racks (200 μ l and 1500 μ l)

unit boxes (sample cartridge and 8-Rod Cover)



Reagent cartridge

prealiquoted, sealed, barcoded

all the reagents

automatically recognized and opened

utilization based on the number of extractions

seals for reuse up to 14 days at room temperature

complete traceability



Process safety on Qiasymphony

Inventory Scan, Prevention of contamination



Inventory scan

drawers, reagents levels

barcode reading of reagents

NO errors in loading

Prevention of contamination

mobile UV lamp and drop catchers

closed system

separate and safe waste
compartment

TRACEABILITY

Report file; data export and print via USB port and network

Dedicated extraction kit

For all biological matrices

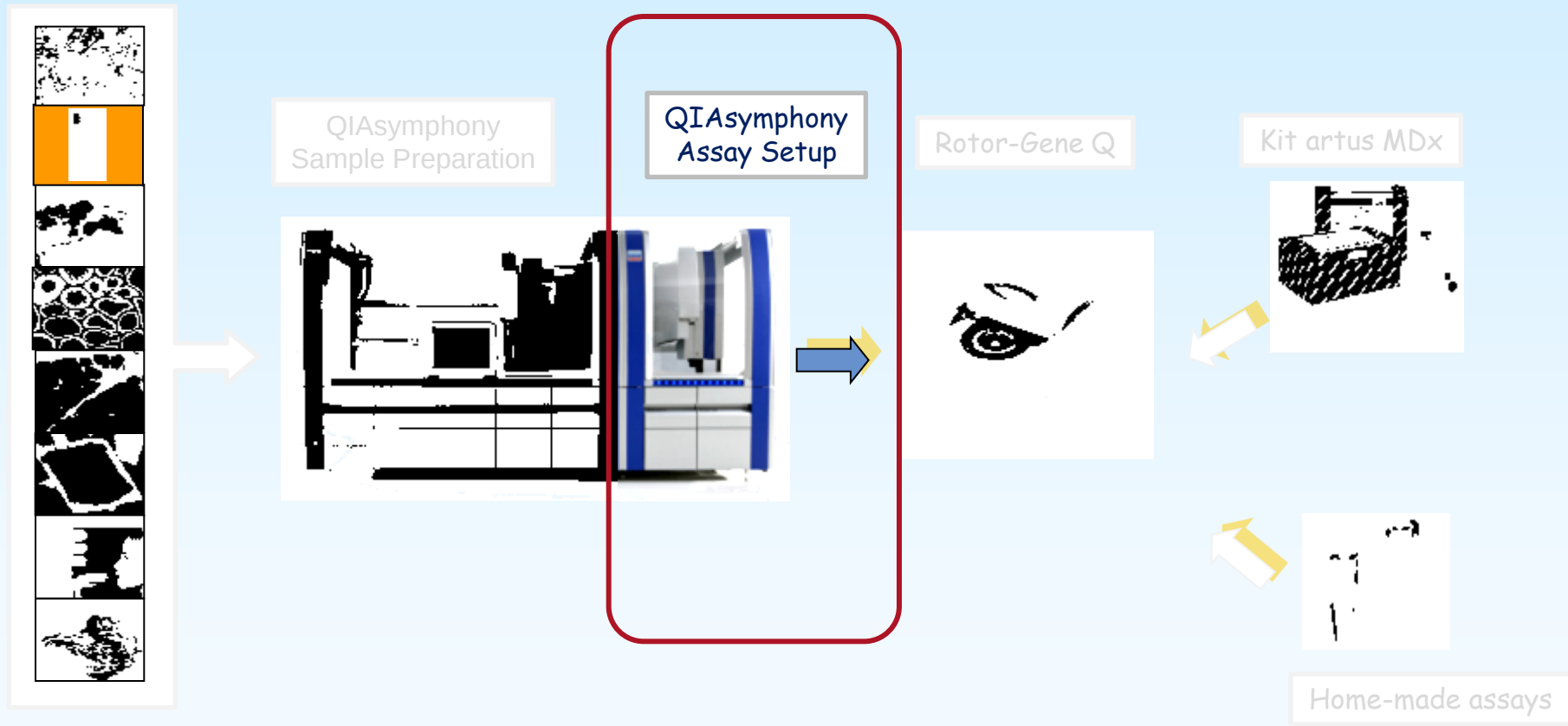
<u>QIAasymphony DNA Midi Kit</u> 96 preps 1000 μ l each or 144 preps 400 μ l each	Biological fluids with high cellularity (whole blood) Fresh, frozen and FFPE tissues
<u>QIAasymphony DNA Mini Kit</u> 192 preps 200 μ l each	
<u>QIAasymphony Virus/Pathogen Midi kit</u> 96 preps 800-1000 μ l each	Biological fluid with low cellularity (serum, plasma, CSF, urine, fecal supernatant)
<u>QIAasymphony Virus/Pathogen Mini Kit</u> 192 preps 200 μ l each	
<u>QIAasymphony PAXgene Blood RNA Kit</u> 96 preps	Total cellular RNA from whole blood

The Qiagen system and its components

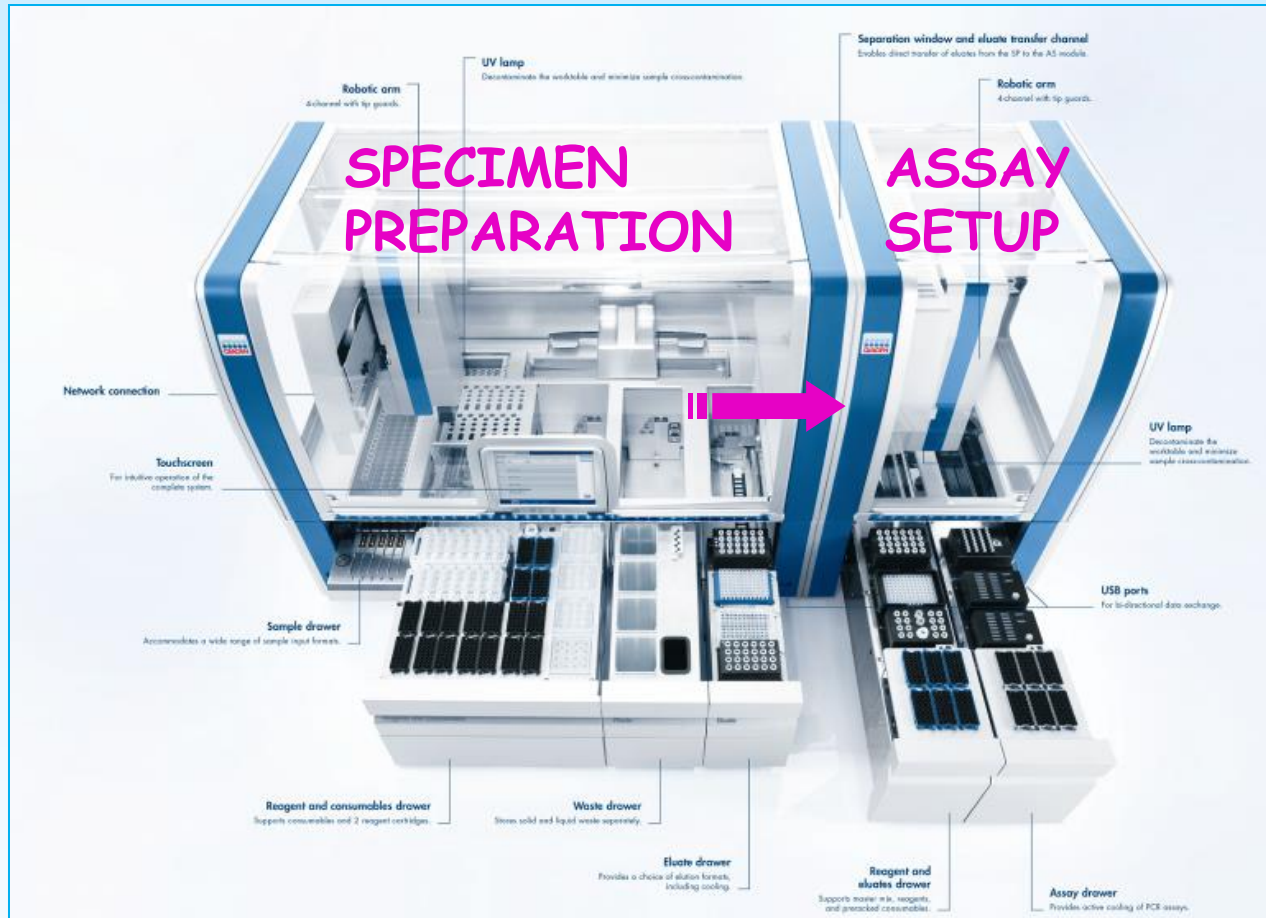
Different sample types

Sample purification & Assay setup

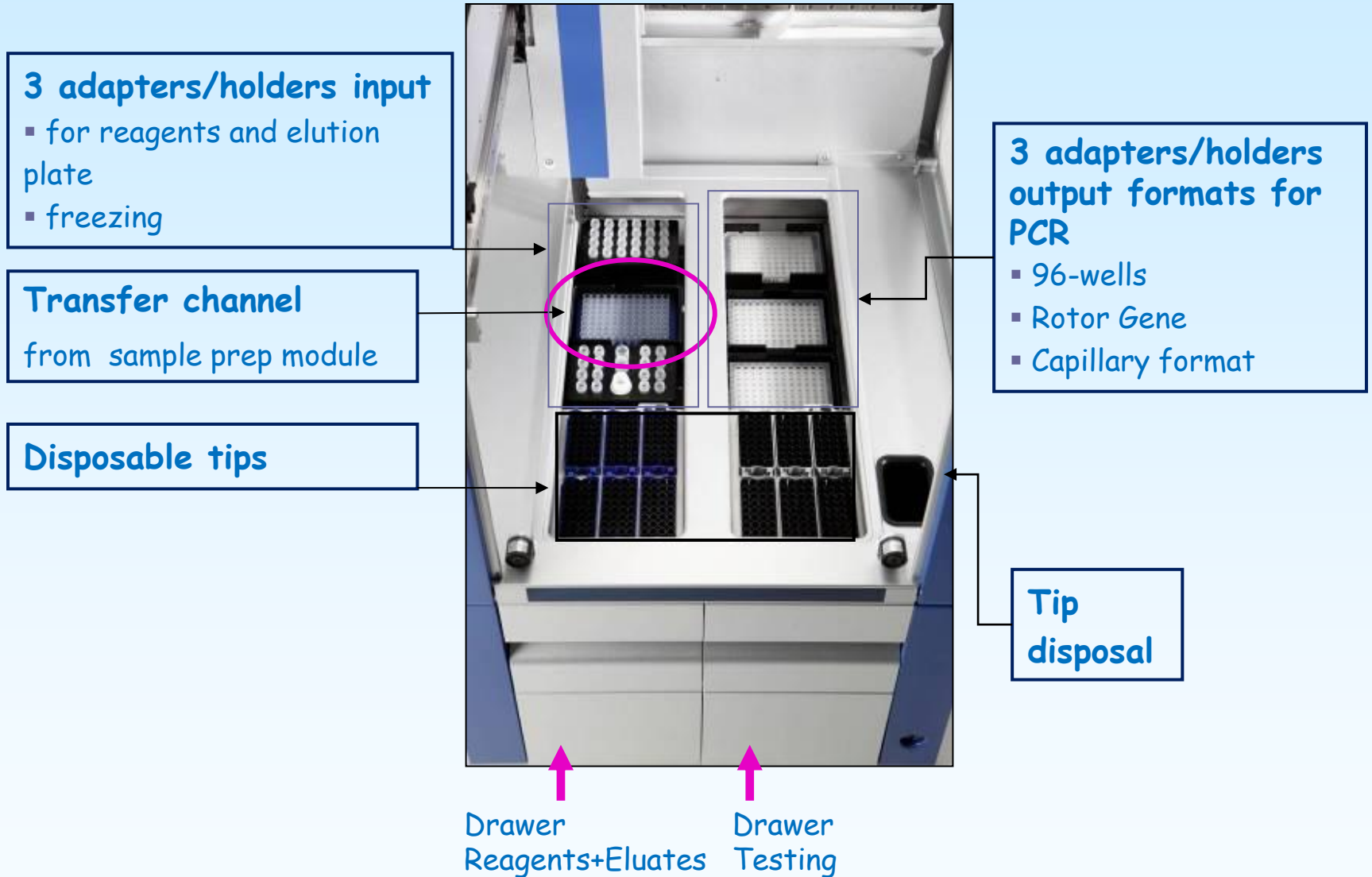
Molecular testing



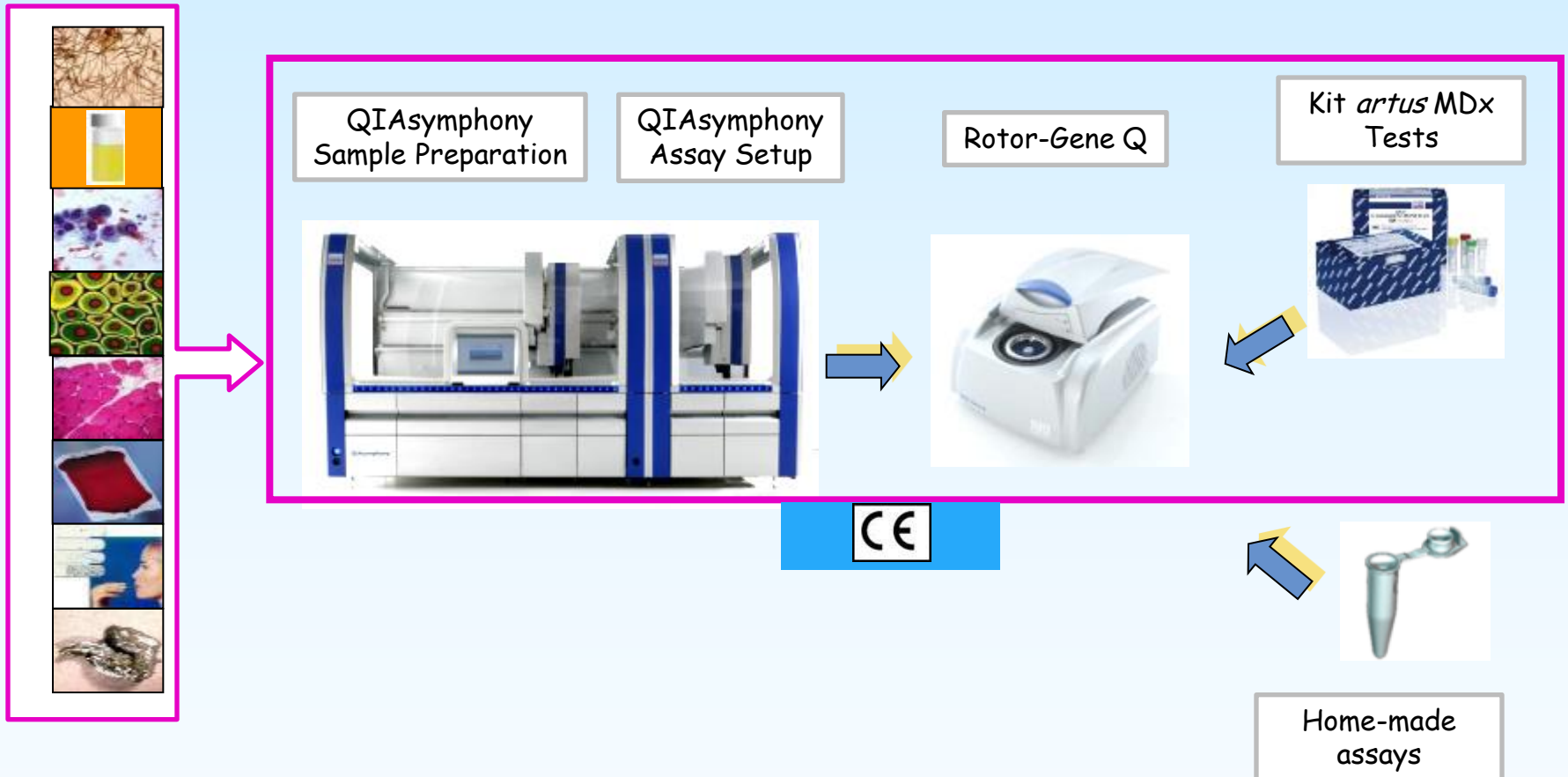
Automated transfer of elution plate for assay setup



Assay setup



Workflow Qiasymphony & Rotor-Gene

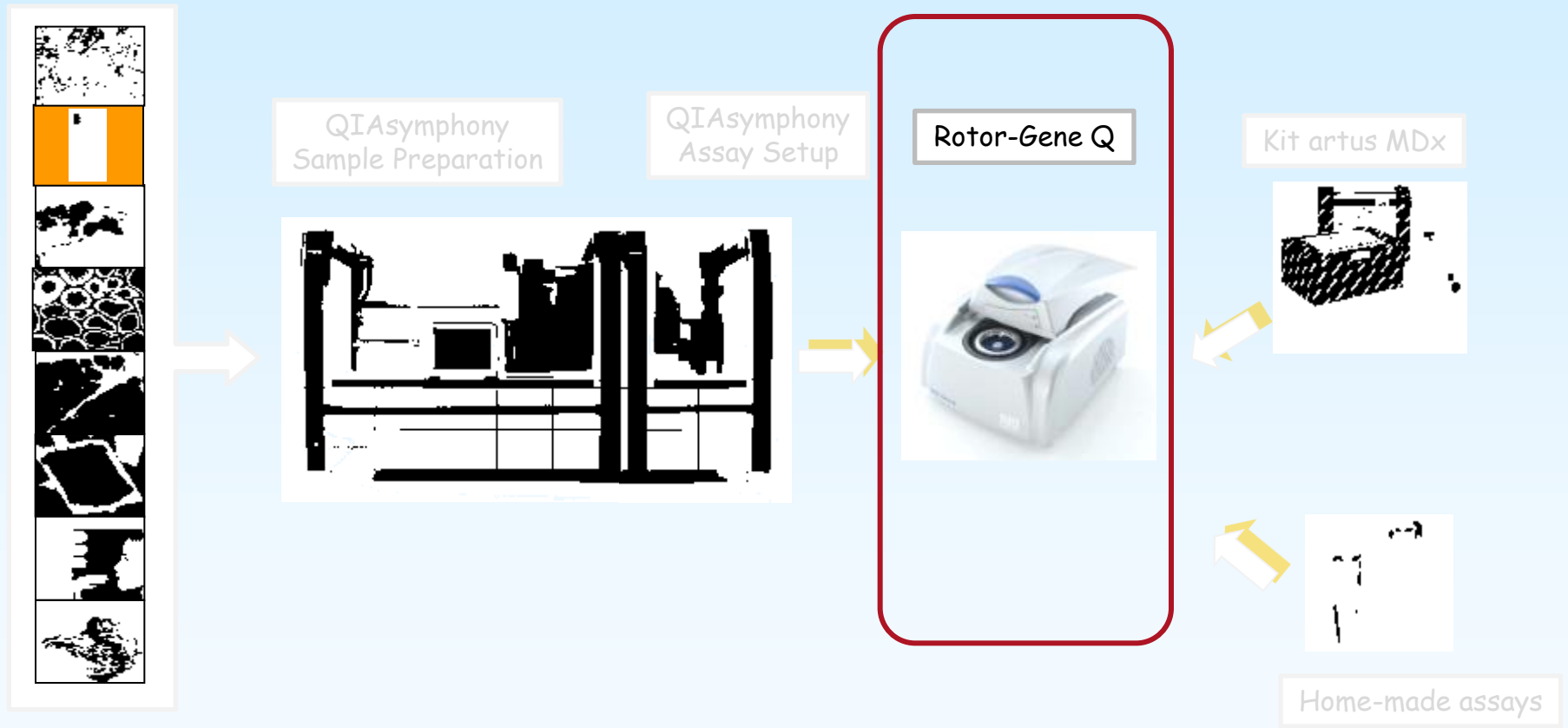


Amplification

Different
sample types

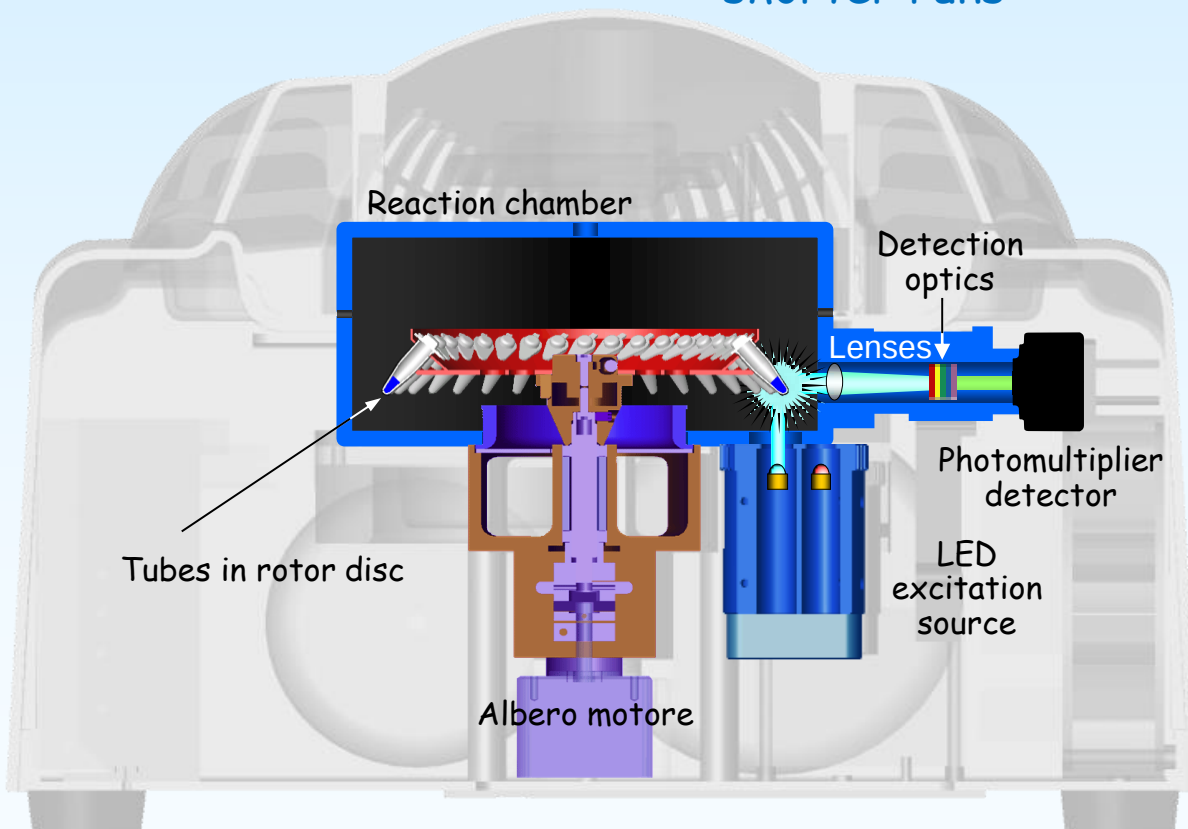
Sample purification & Assay setup

Molecular testing & Diagnostic result



Rotor-Gene Q - Pure Detection

Rotary format, forced air cycler
superior temperature uniformity
rapid temperature ramping
higher precision
shorter runs

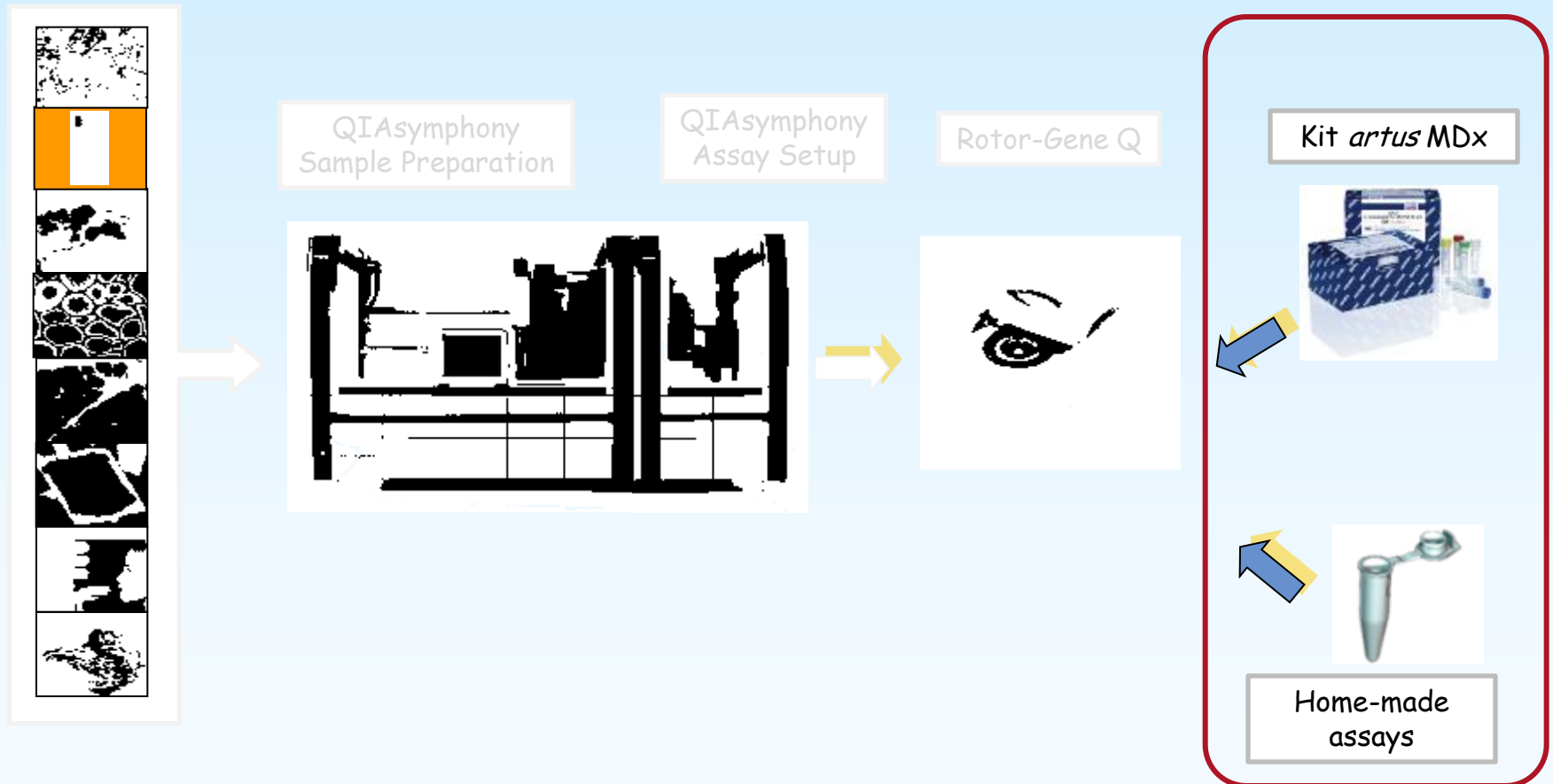


Amplification

Different
sample types

Sample purification & Assay setup

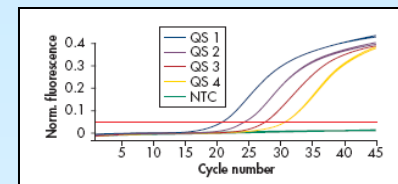
Molecular testing & diagnostic result



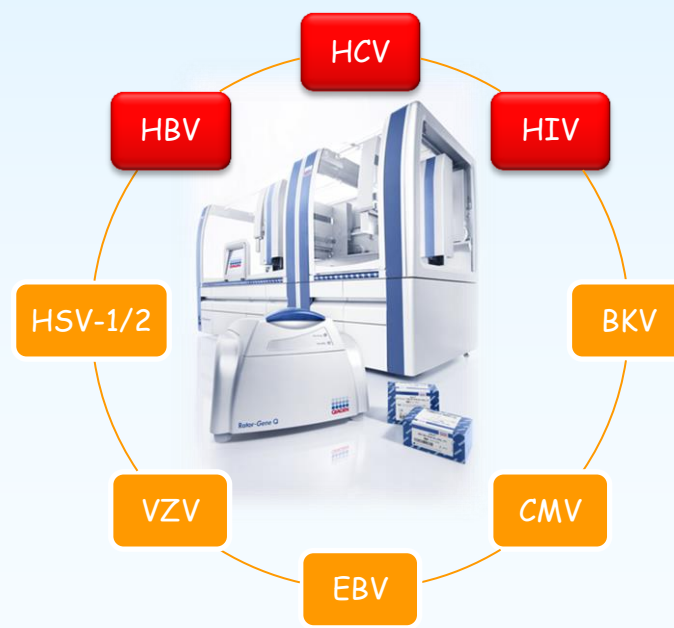
artus QS RGQ Real-Time PCR Kits

Sensitive and reliable pathogens detection

- High sensitivity and specificity
- Reliability (internal control)
- Quantitative results
- Ready-to-use Kits
- Optimized for automated setup
- Unique thermal profile and same reaction volume
- CE-IVD marked

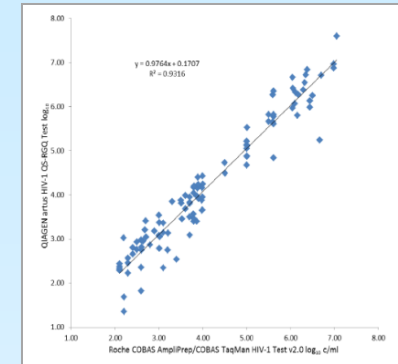


	LOD	Linear range
HIV	76.4 IU/ml (34 copies/ml)	100 to 1×10^8 IU/ml (45 to 45×10^6 copies/ml)
HBV	10 IU/ml	31.6 to 20×10^6 IU/ml
HCV	21 IU/ml	35 to 17.7×10^6 IU/ml
CMV	42.5 copies/ml	79.4 to 100×10^6 copies/ml
EBV	157.3 copies/ml	631 to 10×10^6 copies/ml
VZV	80.7 copies/ml	500 to 100×10^6 copies/ml
HSV 1/2	57.3 copies/ml(HSV-1) 65.7 copies/ml(HSV-2)	
BKV	26.7 copies/ml	50 to 92.6×10^6 copies/ml

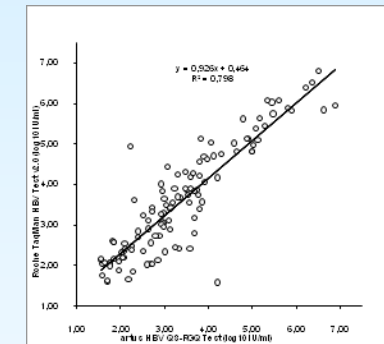
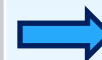


Kit performance

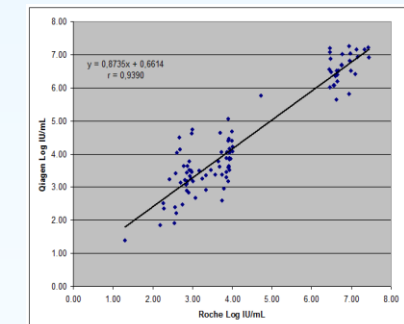
HIV	Gene Target(s)	LOD (c/ml)	Linear Range (c/ml)
QIAGEN <i>artus</i> HIV QS R6Q	5' LTR	34 (76.4 IU/ml)	45 - 45x10 ⁶ (100-100x10 ⁶ IU/ml)
Abbott RealTime HIV 1	<i>int</i>	40	40 - 10x10 ⁶
Roche CAP/CTM HIV 1 v2.0	<i>gag</i> + LTR	20	20 - 10x10 ⁶
Siemens HIV 1 kPCR	<i>pol</i>	37	37 - 11x10 ⁶



HBV	Gene Target	LOD (IU/ml)	Linear Range (IU/ml)
QIAGEN <i>artus</i> HBV QS R6Q	Core	10	32 - 20x10 ⁶
Abbott RealTime HBV	S gene	10	10 - 10x10 ⁸
Roche CAP/CTM HBV v2.0	Core	20	20 - 17x10 ⁷



HCV	Gene Target(s)	LOD (IU/ml)	Linear Range (IU/ml)
QIAGEN <i>artus</i> HCV QS R6Q	5' UTR	21	35 - 17x10 ⁶
Abbott RealTime HCV	5' UTR	12	12 - 100x10 ⁶
Roche CAP/CTM HCV v2.0	5' UTR	15	15 - 100x10 ⁶
Siemens HCV kPCR	5' UTR	15	15 - 100x10 ⁶



Requirements of a diagnostic systems

Optimized kits for extraction and real-time PCR amplification
Comparison with "gold standard" methods
Clinical validation in different reference laboratories
Kits with internal control and standard (WHO calibrated)
Instrumental system for check of process errors

❑ Reliability

From 1 to 96 samples per run
Continuous loading
Different protocols within the same run
Sample volumes ranging from 200 µl to 1 ml
For commercial and home-brew kits

❑ Flexibility

Complet compliance with 98/79/EC Directive on in vitro diagnostic medical devices
Optimized and tested protocols

❑ Standardization

Complete traceability of patient and reagent data
LIS interface
Export of reports and files

❑ Data management



Comparison of two nucleic acid extraction and testing systems for HCMV-DNA detection and quantitation on whole blood specimens from transplant patients

Cristina Costa^{a,*}, Samantha Mantovani^a, Cinzia Balloco^a, Francesca Sidoti^a, Fabrizio Fop^b, Rossana Cavallo^a

^a Microbiology and Virology Unit, Turin, Italy

^b Nephrology Unit, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Turin, Italy



SYSTEM 1

automated extraction
(Qiasymphony) + artus® CMV QS-
RGQ kit (Rotor-Gene Q, Qiagen)



SYSTEM 2

semi-automated extraction EasyMag
(Biomérieux) + Q-CMV Real Time
Complete kit (Nanogen, 7500 Real-
time PCR system, ABI)



Specimens - Features of the two systems

189 whole blood specimens	156 patients (90 SOT; 66HSCT) 1° year post-transplantation
QCMD 2012 proficiency panel	12 samples

FEATURES	SYSTEM 1	SYSTEM 2
Target	MIE	MIE
Extracted DNA volume (µl)	20	5
Final volume (µl) in assay	50	25
N _o . type of quantification standards	4, plasmid	4, plasmid
Analytical sensitivity	164,55 copies/ml	158 copies/ml
Specificity	100%	90%
Linear range	1x10 ³ -5x10 ⁷ copies/ml	20-1x10 ⁶ - copies/reaction

Results by the two systems

	SYSTEM 1 / POS	SYSTEM 1 / NEG
SYSTEM 2 / POS	90 (47.6%)	9 (4.8%)
SYSTEM 2 / NEG	28 (14.8%)	62 (32.8%)

Overall concordance: 80.4%

Concordantly positive: mean $\pm$ SD

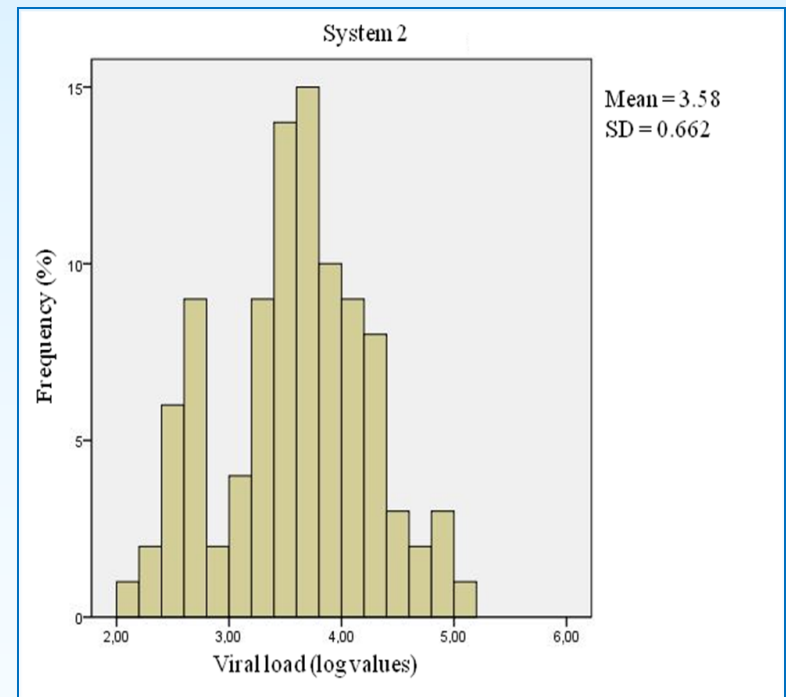
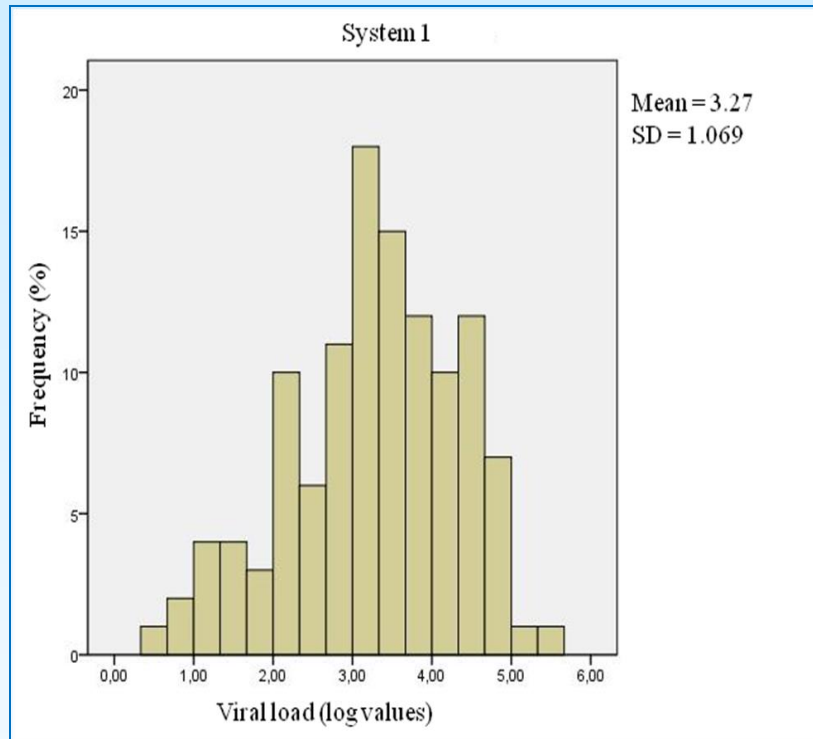
SYSTEM 1 $4.25 \pm 4.58 \log_{10}$ copies/ml

SYSTEM 2 $4.06 \pm 4.29 \log_{10}$ copies/ml

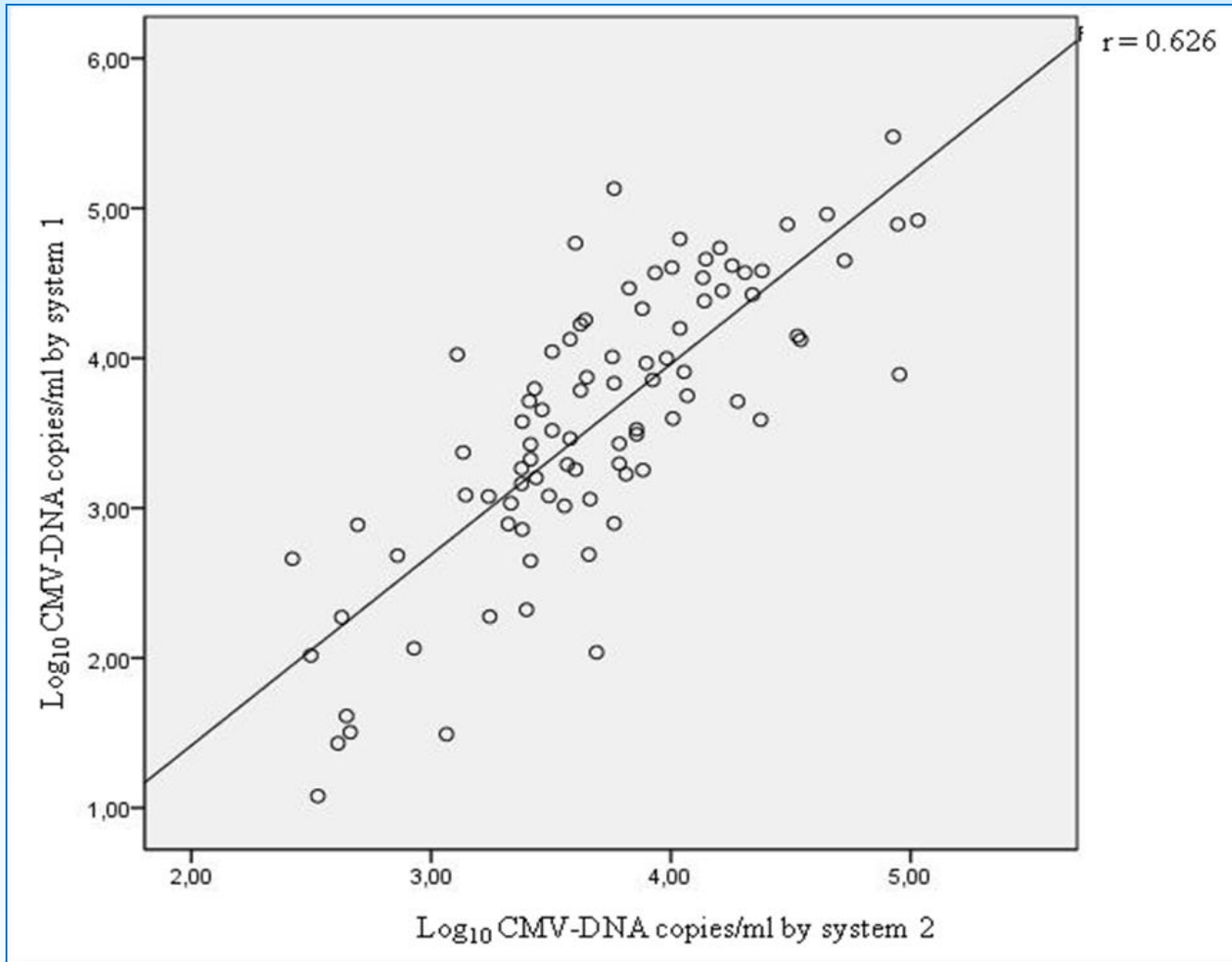
Difference $0.19 \log_{10}$ copies/ml

Qualitatively discordant: mean viral load $\leq 3 \log_{10}$ copies/ml

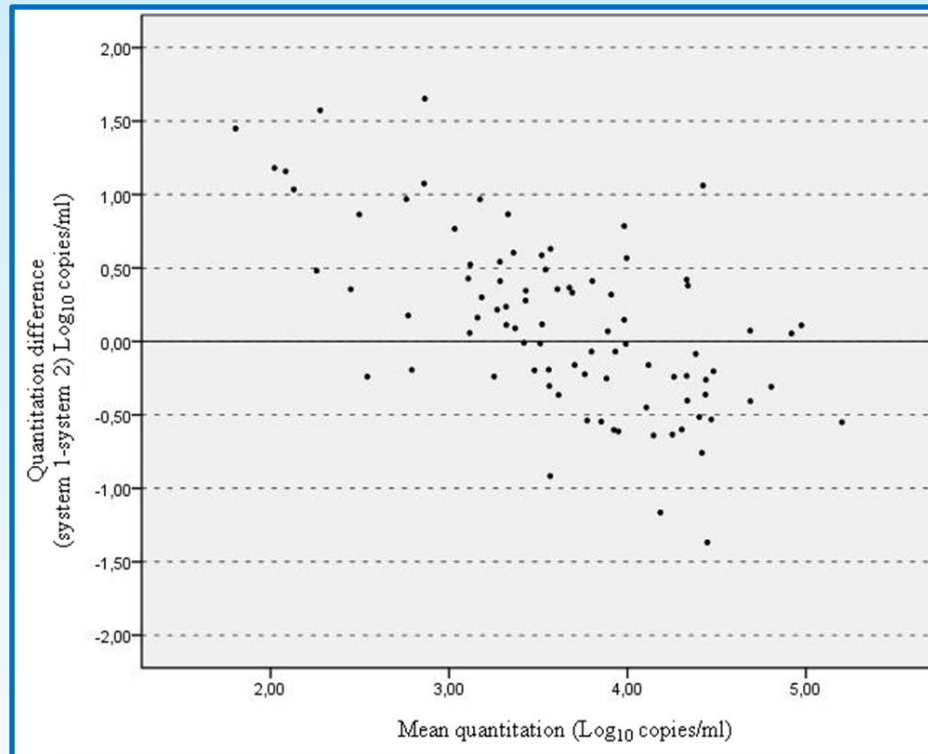
Frequency distribution of log values of viral load by the two systems



Linear regression



Bland-Altman analysis (mean differences in CMV-DNA quantitation)



Differences between the two systems entro ± 1 $\log_{10}$ copies/ml of the averaged $\log_{10}$ results for 88.9% of the tested specimens

Results and variability of the two systems for the QCMD 2012 proficiency panel

Samples	System 1 results $\log_{10}$ (copies/ml)	System 2 results $\log_{10}$ (copies/ml)	QCMD results $\log_{10}$ (copies/ml)	Difference system 1 / QCMD $\log_{10}$ (copies/ml)	Difference system 2 / QCMD $\log_{10}$ (copies/ml)
CMV12-01	4.36	4.46	4.30	0.06	0.16
CMV12-02	3.89	3.86	3.74	0.15	0.12
CMV12-03	2.30	2.14	2.24	0.06	-0.10
CMV12-04	1.43	1.47	2.07	-0.64	-0.60
CMV12-05	2.99	3.40	2.90	0.09	0.50
CMV12-06	3.33	3.51	3.30	0.03	0.21
CMV12-07	3.50	3.50	3.32	0.18	0.18
CMV12-08	negative	negative	negative	/	/
CMV12-09	3.62	3.82	3.67	-0.05	0.15
CMV12-10	2.88	2.69	2.73	0.15	-0.04

For positive specimens, difference was $<0.7 \log_{10}$ copies/ml for both the systems
(mean $\Delta\log_{10}$: 0.1566 for system 1 and 0.2288 for system 2)

Potential clinical impact

Cut-off for guiding pre-emptive therapy (guide lines Italian Society virology - SIV)

100.000 copie/ml in SOT
10000 copie/ in HSCT



SOLID ORGAN TRANSPLANTATION:

1 patient treated by system 1 and not system 2

HEMATOPOIETIC STEM CELL TRANSPLANTATION:

5 patients treated by system 1 and not system 2

1 patient treated by system 2 and not system 1

Conclusions

- Both systems are reliable: 80.4% overall concordance
- Early identification of patients at risk (frequency distribution of viral load)
- Automation levels, indispensable in high-throughput laboratories
- Comparison studies only on amplification step
- Studies on viral load kinetics



Azienda Ospedaliera
Città della Salute e della Scienza di Torino
SC Microbiologia e Virologia

