

Real Time PCR Techniques and Laboratory Workflows

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Introduction

- Real-time PCR = PCR + fluorescent probe
- Fast - assay time ~ 45min
- Simple – less hands on compared to PCR + gel
- Closed tube – no amplicon contamination
- Excellent sensitivity, specificity
 - replace culture based methods
- Expensive test (yes), per patient diagnosis?

RT PCR instruments

Name	Samples	Volume	Comment
Roche LC2	32	10-20	Special tube
Roche LC480	96 384	10-100 5-20	Special plate? Special plate?
Cepheid SmartCyclerII	16	25-100	Special tube
Corbett Research Rotor-Gene 6000	72 100	10-100 5-50	Standard tube Gene Disc
ABI Prism systems(7500)	96	20-100	Standard tube or plate
BioRad iCycler iQ5	96	15-100	Standard tube or plate

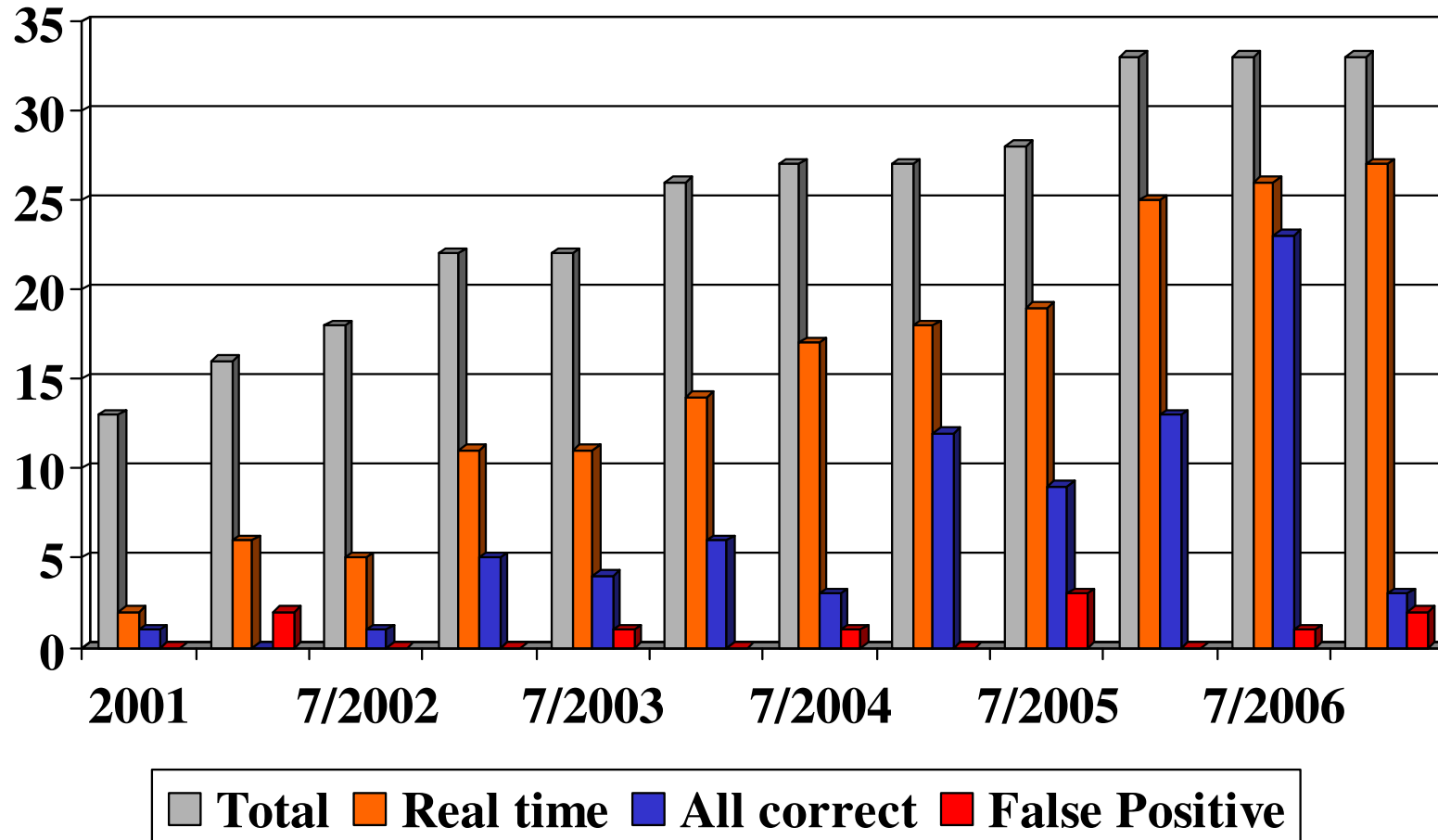
Which one?

- Turn around time - all fast – small capacity special tubes faster (LC2 and SmartCyclerII)
- Specimen number
 - Individual tests (SmartCyclerII)
 - Large numbers – plate
- Software - all easy to use – upgrade cost
- Cost – instrument, consumables, service
- Available commercial assays/ reagent kits
- Flexibility – kits + in-house
- Foot print
- Automated specimen processing
- Link to laboratory information system

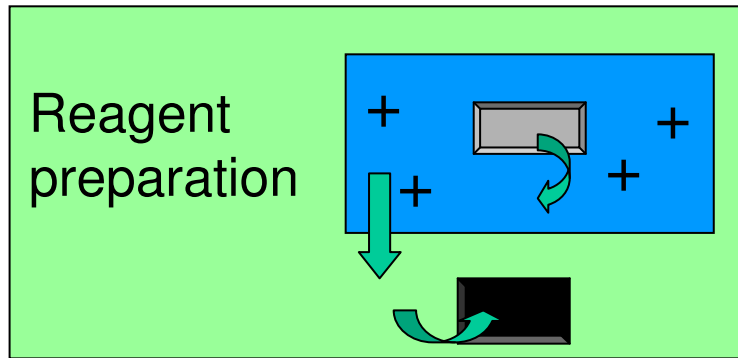
PCR product analysis

System	Quantify	Melt curve	Specific	Multiplex	Measure
SYBR Green	++++	Product	-	+ Tm	Extension
TaqMan probe	++++	-	+++	++++	Any
FRET probes	++	Probe	++++	++++	Annealing
Molecular beacon probe	++	Probe	+++	++++	Annealing

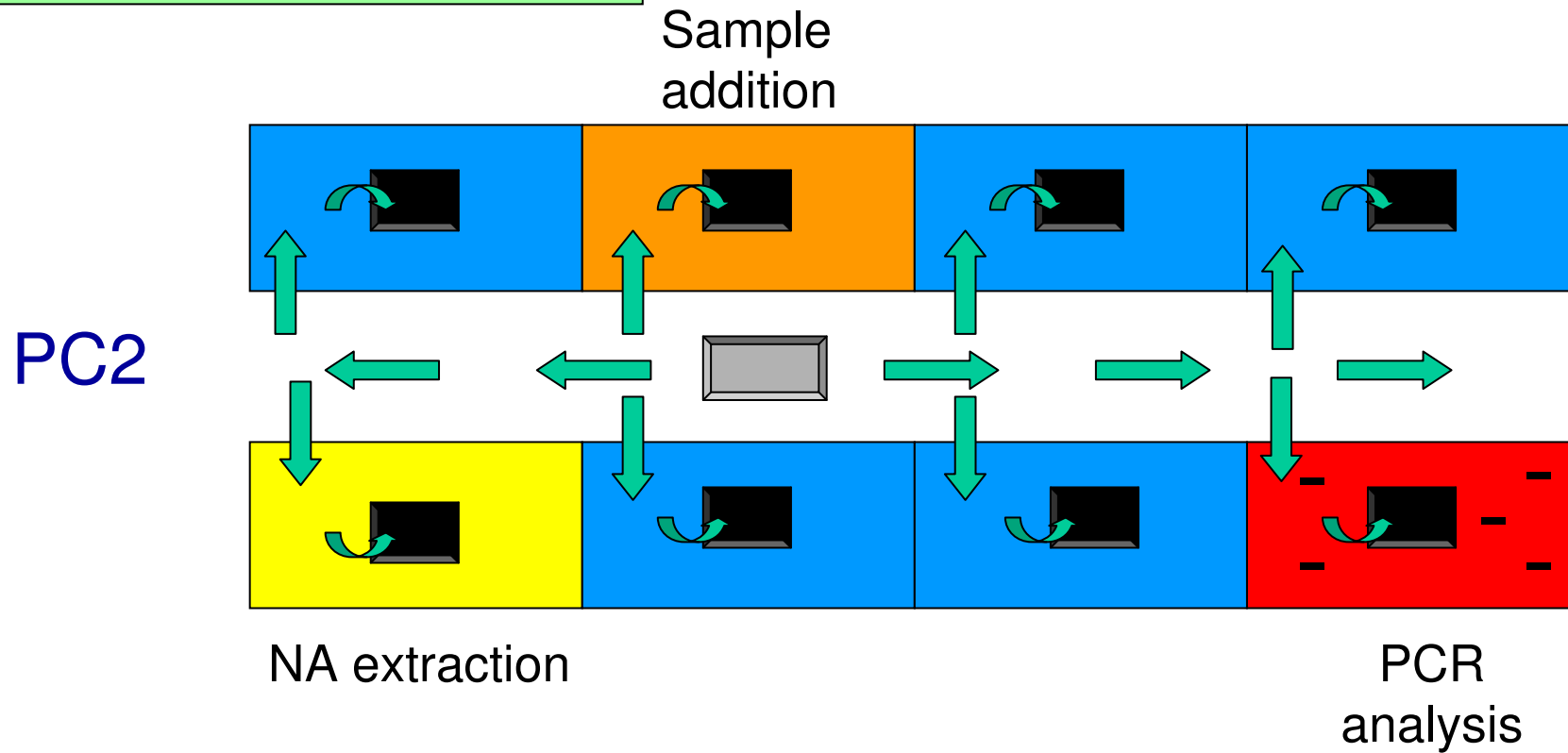
Use of RT PCR in Micro Labs



Ideal lab layout and work flow - PCR

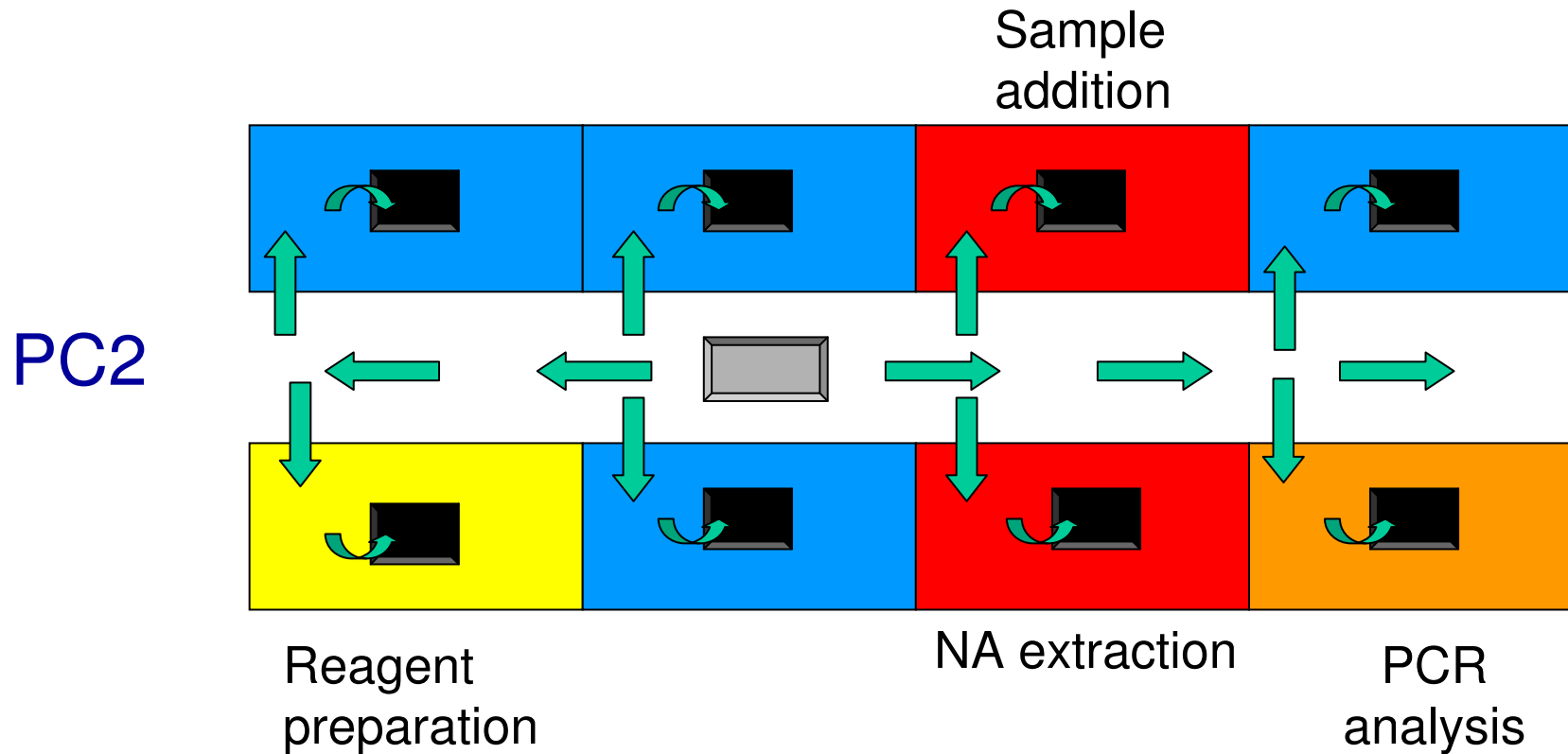


Compartmented dedicated equipment + consumables
One way



Ideal lab layout and work flow – RT PCR

Compartmented – dedicated equipment + consumables
One way



Work flow

- Order tests - Specimen collection - transport
- Reception – enter tests LIS – deliver to lab
- Split into samples
- Process - liberate/ extract NA
- PCR reagent preparation/ storage
- Sample NA addition
- PCR analysis
- Result report
- Sample storage

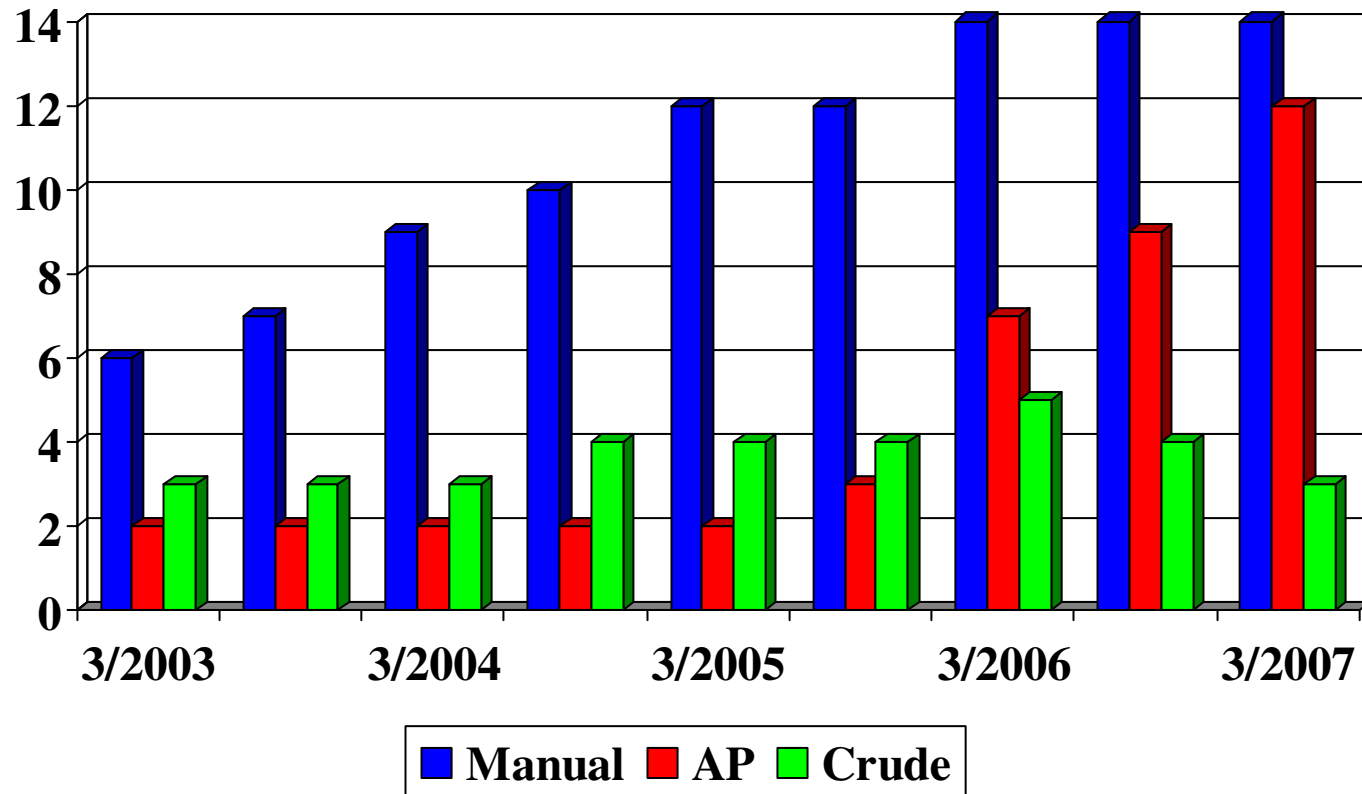
Specimens

- Right test ordered
- Correct specimen taken appropriately
- Labelling and documentation
- Storage – room temp, 4°C, -20°C
- Transport conditions/ time
- Specimen reception – deliver to lab

In Lab

- Split specimen if required
- Link assay frequency to specimen arrival
 - One off tests – SmartCyclerII
- Extract NA – inhibition + contamination risk
 - Crude – boiling (RT PCR suitability?)
 - Manual – chemical, magnetic particle, column
 - Automated
 - Test efficiency, inhibition and contamination rate
- Store samples (4°C, - 20°C, -80°C)

RT PCR Processing methods



RT PCR Reagents

- Reputable manufacturer – QC supplied
- Oligonucleotides & Probes – purified + QC
- Commercial master mix – GMP – QC
- In-house – QC all ingredients
- Storage conditions – MM aliquots
 - 4°C – daily use
 - -20°C – Do not cycle freeze / thaw
- Monitor performance

RT PCR assay

- Commercial kit or validated in-house
- Sample addition – contamination risk
- Closed tube – amplicon contamination eliminated – cleanup 5% sodium hypochlorite
- Result interpretation – QC + Clin diagnosis
- Monitor performance each assay
- Quality assurance program

National Pathology Accreditation Advisory Council

Laboratory accreditation standards
and guidelines for nucleic acid detection
and analysis

Implementation date 1st August 2006

Main points

- Dedicated sample for PCR
 - Declaration on report if samples used prior to PCR
- Controls to assess collection and NA extraction
 - HHKG + efficiency control
- Validated assay – Commercial or In-house.
 - **NPAAC – Requirements for validation of in-house IVD's**
- Contamination - document event and resolution
 - Hygiene, equipment maintenance, compartmented
 - Weak positive - adequate negative controls - NDC's
- Inhibition – monitor with IC, HHKG, spike
- Product certification – RT = probe, melt curve

Cost effective use RT PCR instruments

- Good validation and monitoring of performance = less controls, more samples
- Design assays with same profile – multiple assays in same run
- Multiplex – multiple targets in one tube
- Consumable cost greater than culture
 - RT PCR - reduced labour cost + faster diagnosis – shorter hospital stay – lower patient cost to health system

Thank you