Molecular testing and quality assuring the results

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Why do we need quality results?

- Patient diagnosis and management
- To relate patient results to prior results or to absolute values in clinical practice guidelines.
- Results need to be comparable over time and between methods
- Maintain staff morale and lab reputation

Summary

- Quality guide books
- Essential requirements for quality
- Issues encountered
- TGA - NPAAC requirements manufacturers
- Experiences on global scale
- Recommendations

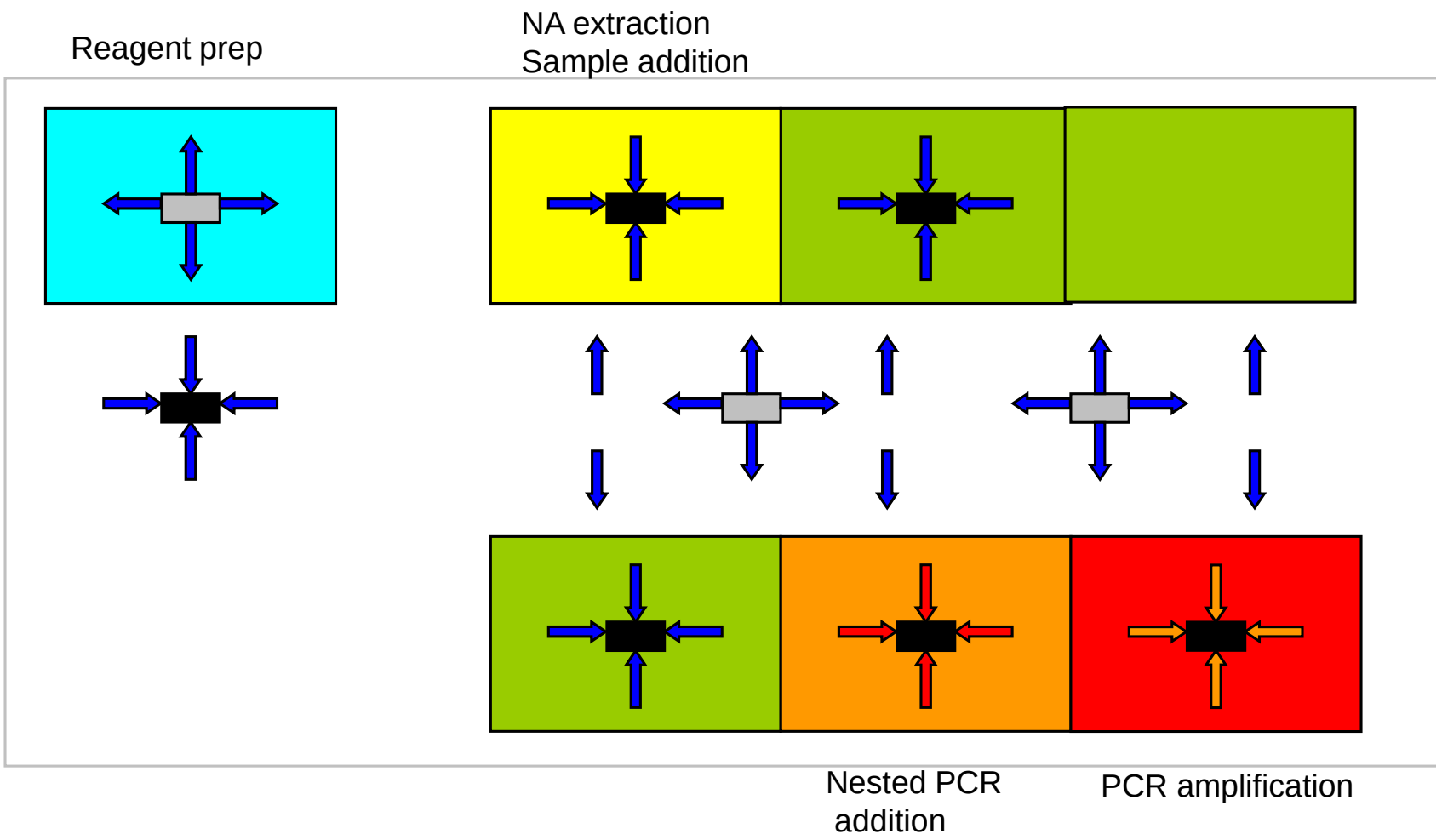
Quality guide books

- NPAAC: Requirements for the development and use of in-house IVDs (Third edition 2014)
- NPAAC: Requirements for medical testing of microbial nucleic acids (Second edition 2013)
- AS ISO 15189-2013: Medical laboratories – requirements for quality and competence.
- TGA: Regulatory requirements for in-house IVDs (ver 2.0 March 2016)
- NATA: Interpretation of NPAAC requirements and ISO 15189: Medical testing field application document (Nov 2013)

Essential requirements

- Correct physical lab layout
- Correct workflow and practices
- Experienced staff
- Validated/ verified tests
- Staff training and competency
- Dedicated specimens
- Assay QC, QA and QAP
- Monitor and review

Ideal laboratory



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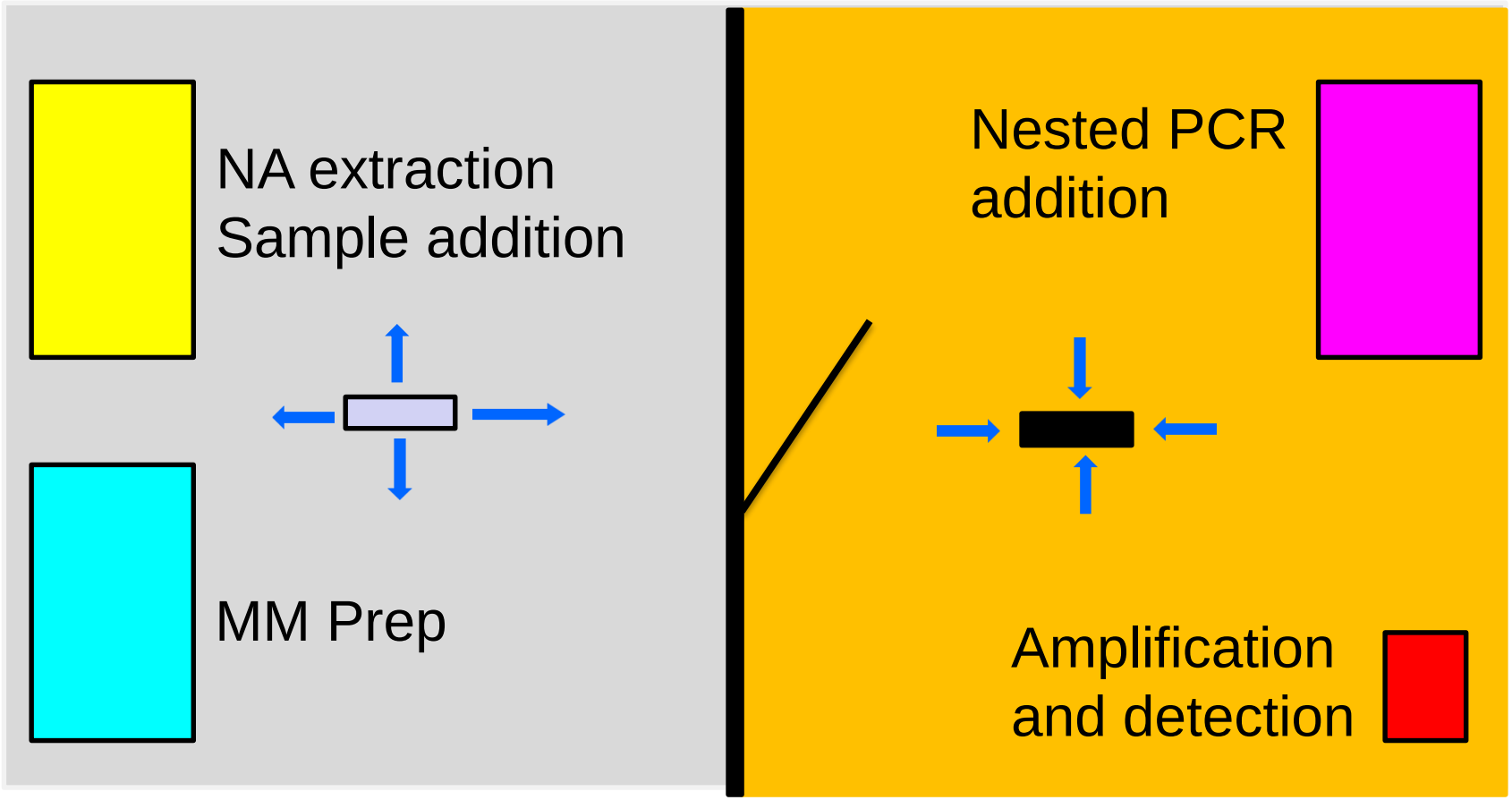
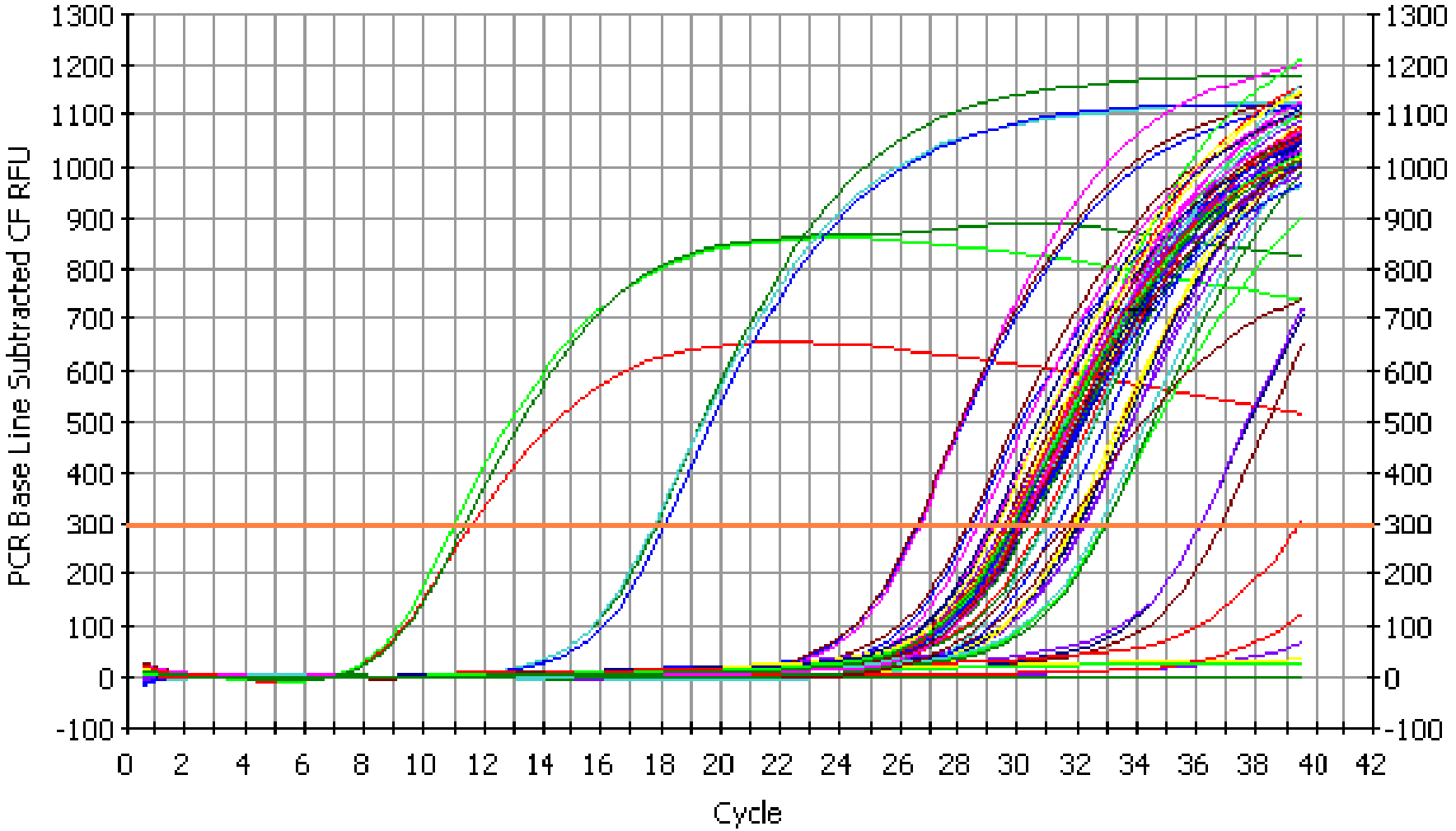
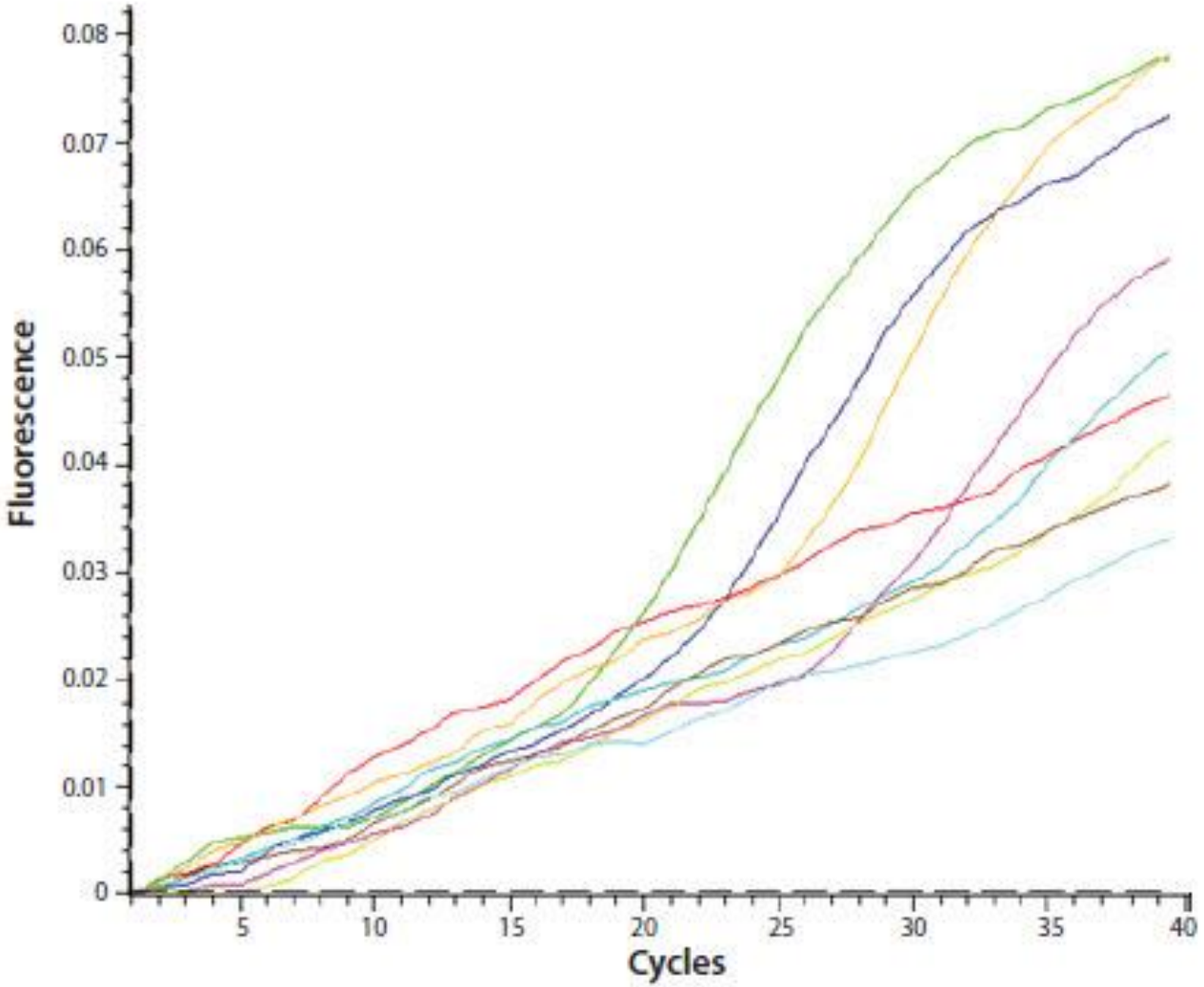
RT-PCR Lab

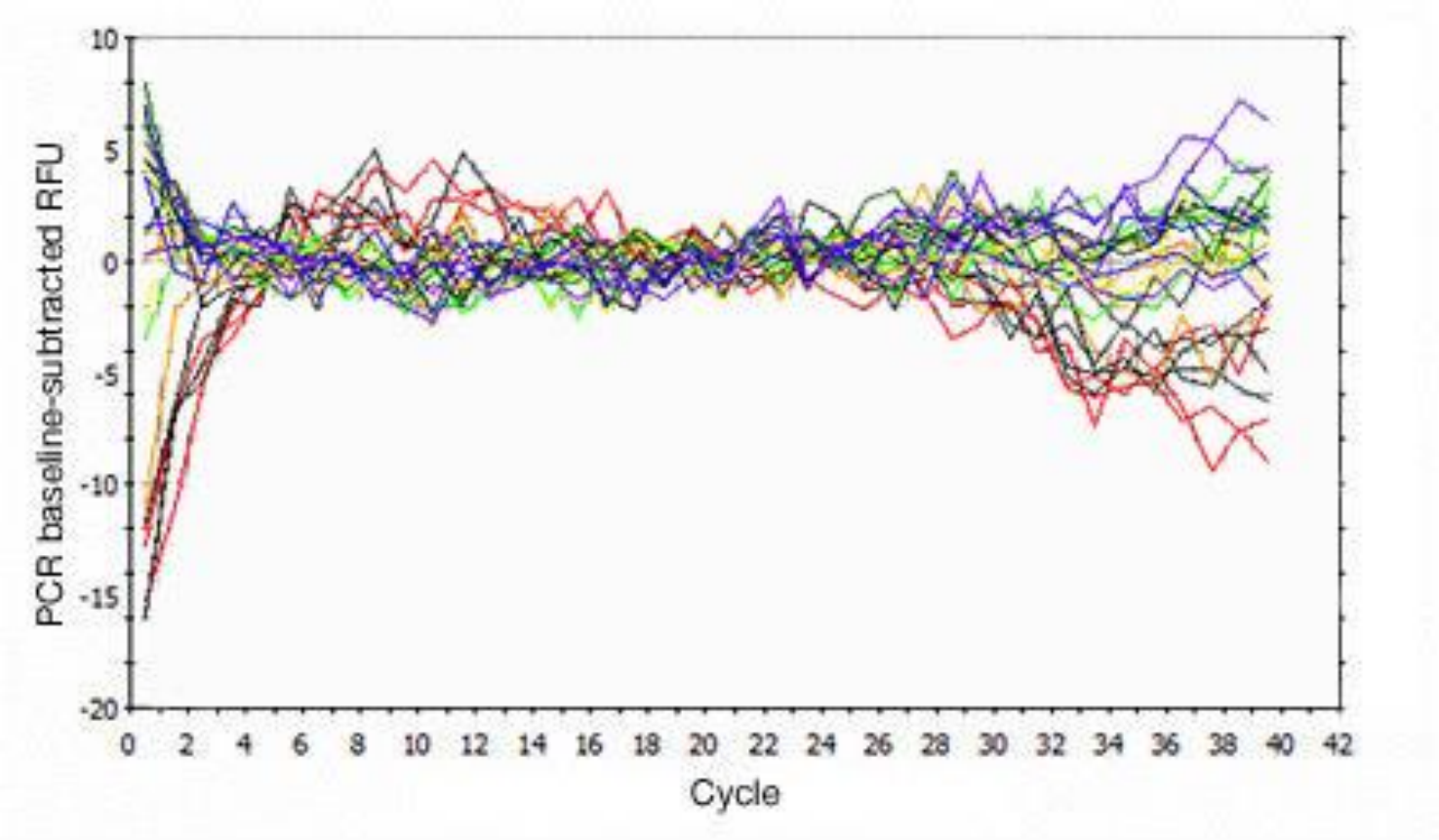

NA extraction
Sample addition
MM Prep
Nested PCR
addition
Amplification
and detection

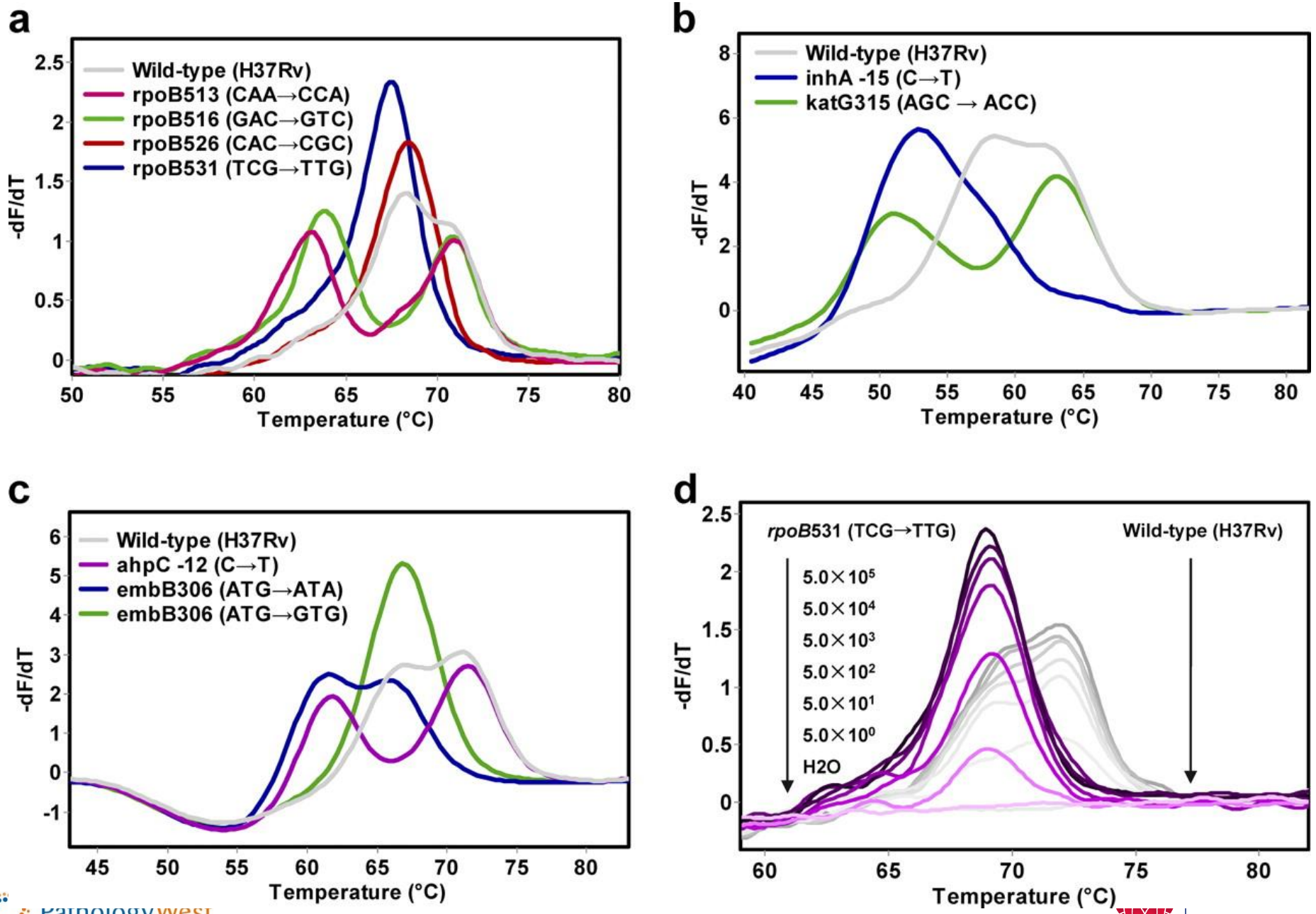
Experienced staff

- No mention in; NPAAC: Requirements for medical testing of microbial nucleic acids (Second edition 2013)
- NPAAC: Requirements for the development and use of in-house IVDs (Third edition 2014) – S3.4 states “Senior staff must have significant diagnostic and research experience”


PCR Base Line Subtracted CF RFU
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42
Cycle


Fluorescence
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
5
10
15
20
25
30
35
40
Cycles


PCR baseline-subtracted RFU
10
5
0
-5
-10
-15
-20
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
Cycle



Validated and verified tests

NPAAC: Requirements for the development and use of in-house IVDs (Third edition 2014)

- General requirements
- Design
- Production and contracted services
- Analytical performance
- Scientific validity
- Clinical performance
- Clinical utility
- Multivariate index analysis
- Monitoring, analysis and improvement
- Adverse event reporting
- Documentation

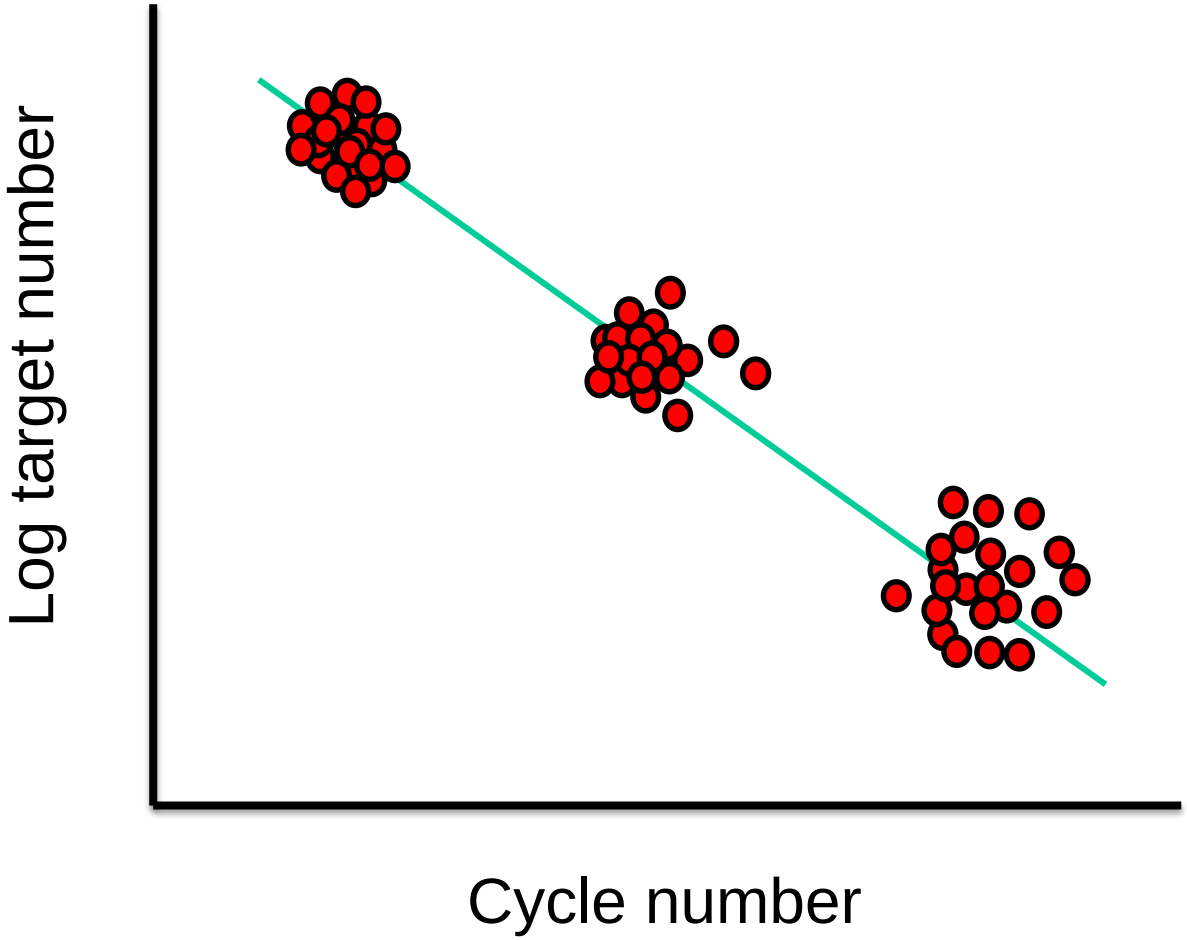
Meaningful validation

- Include critical elements
- Must represent test population
- Elements should be measured - multiplex
- Data must be analysed correctly
- Specimens should be stored correctly
- QC samples should be determined and aliquoted for long term storage and monitoring
- Validation true to end of lot and assay life

Commercial assays

- TGA, CE, FDA, WHO registered
- Varying assessment criteria
  - Design – all relevant targets?
  - Components – suppliers***
  - Production – batch acceptance – QC bias
  - Stability – in use and transport - customer
- Varying levels of validation evidence
  - Clinical validations

Clinical validation



NPAAC assay QC

- Confirm NA extracted = Pos C and IC - includes RNA reverse transcription – inhibition (FN)
- Pos C's may be 'spiked'
- IC homologous – identical primer
- IC heterologous – housekeeping gene
- Controls tested over a cycle
- NDC's added as validated – contamination (FP)

Meaningful QC

- Monitor the whole system
- QC ingredients and test lots
- Pos C's near LOD for each target
- IC's compete with target – calibrated
- Commercial assays - External Run Control (ERC) near LOD for each target
- ERC per cycle/ lot no.
- Reduced sensitivity – UNG, primer dimer

External QAP

- Essential element - proof of performance
- Meaningful QAP samples
- Multiplex assays challenge
- Competing market

Recommendations

- Best lab layout and workflow - monitor
- Assay system design – get it right
- Suppliers – monitor – lot release QA
- Validation – meaningful for application and population
- QC – samples that test/ monitor the system
- QAP – tests the system, compares performance

Thank you

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