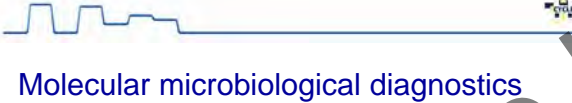


Impact of thermocycler variability on method validation and daily QC

Mary Span – Quality Manager
CYCLERtest




Molecular microbiological diagnostics

- Optimization
- Troubleshooting
- Validation
- Quality assurance


} reagents, focused

- Fundamental to PCR and qPCR – thermocycler

2




Metrology terminology



- **Calibration**
The total set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument and the corresponding values realized by an international traceable reference standard
- **Adjustment**
Adjustment of an indicated value of the instrument back within given specifications or tolerances
- **Validation**
Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled
- **Verification**
Provision of objective evidence that a given item fulfills specified requirements


3



Disclaimer

- All thermocycler thermal performance data as presented is exemplary and not necessarily representative for the total population of a particular thermocycler
- No statements are made about the performance of exemplary used thermocyclers and kits


4



Current situation most labs

- Thermocycler is calibrated
- Calibration results are compared to manufacturer specifications
 - If within specifications everything fine
 - If out of specifications call manufacturer for repair
- Stamp on report and archive

5



Current situation most labs


- Commercial pre-validated kit does not need validation (CE-IVD marked, FDA approved) only verification
- In house developed method is tested on the frequently used thermocycler for sensitivity, specificity and limit of detection on many samples to check for matrix effects

6

ESCMID Online Lecture Library © by author

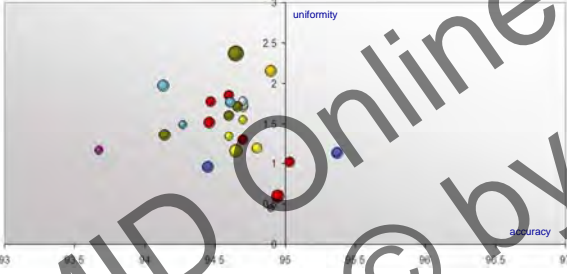
Current situation most labs

Although thermocycler is within specifications ...
... and kit is used according to instructions ...
... results show "phase of the moon" effects ... why?



7

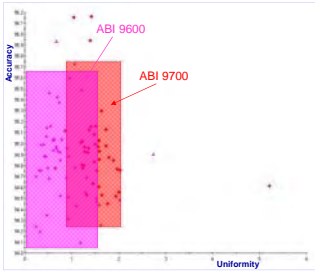
Thermocycler variability



Dots of different color are different models of the same brand

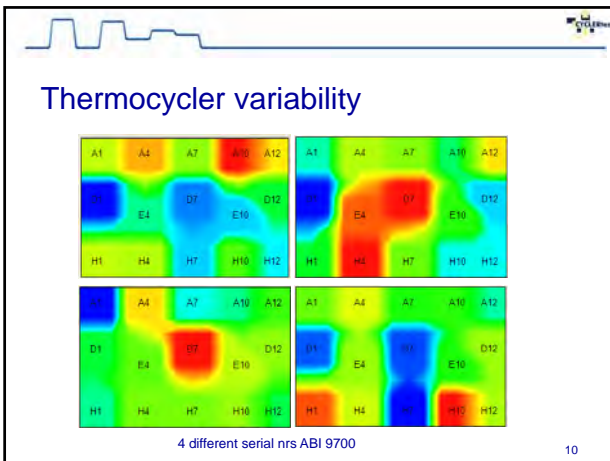
8

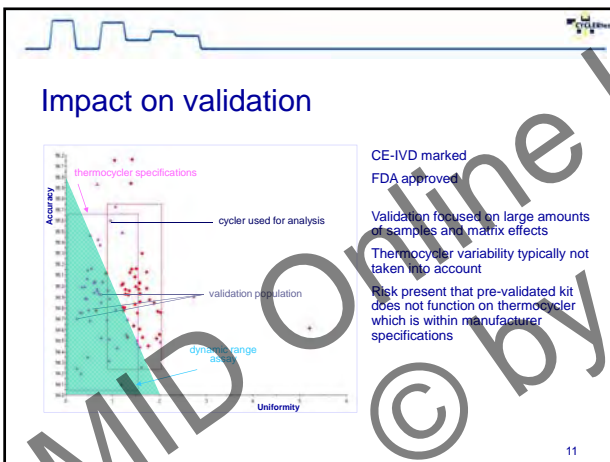
Thermocycler variability

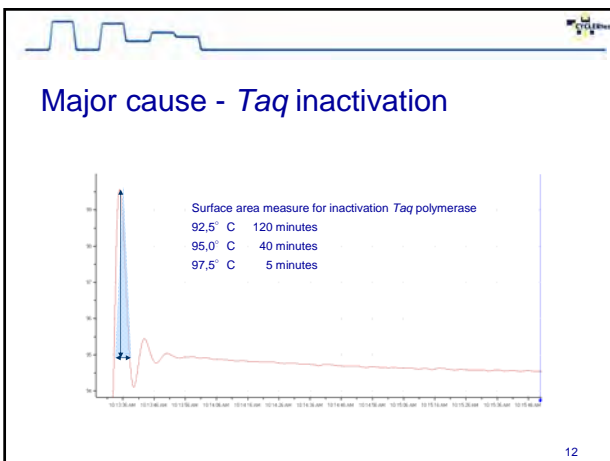


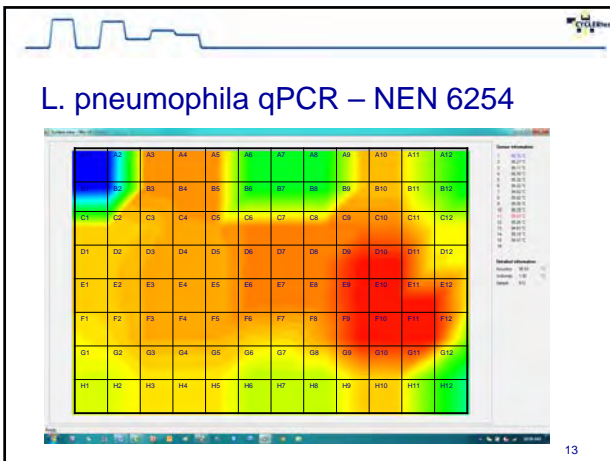
Each dot is a unique serial number

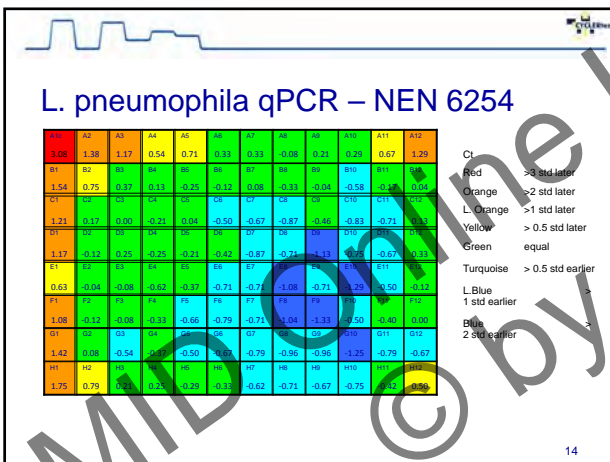
9










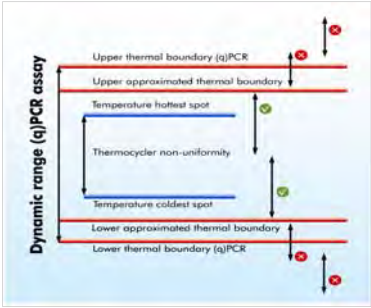


ISO deals with this issue

- ISO 15189 requires
 - Traceable calibration of equipment before putting in use, after being out of control of lab, after repair and after relocation
 - Check if equipment meets specifications as set by laboratory
 - Method validation - method is suitable for intended use
 - Standard method only needs verification
 - In house developed method needs full validation
 - Daily quality controls
 - Quality assurance via reference materials and participation in external quality assessments



Setting lab specifications



Dynamic range (q)PCR assay

Upper thermal boundary (q)PCR

Upper approximated thermal boundary

Temperature hottest spot


Thermocycler non-uniformity

Temperature coldest spot

Lower approximated thermal boundary

Lower thermal boundary (q)PCR


16



IVD directive deals with issue incompletely


- IVD directive 98/79/EC describes criteria for assessing if product meets EU safety, health and environmental protection requirements to enable free trading within the European Union before placing it in the market.
- The CE IVD mark is an economical mark, not a quality mark
- IVD directive describes kit should be validated and validation should be documented
- IVD directive does not describe on how many samples and thermocyclers a kit should be validated
- Validation of List A products is verified by a notified body
Validation of List B products is not verified by a notified body and is therefore a self certification
- Therefore CE IVD mark is no guarantee the kit will produce correct results in the hands of the medical lab

17



Conclusions

- Take thermocycler variability into account during assay verification and validation
- Check if thermocycler meets assay based specifications, not manufacturer specifications
- Be critical towards self certified CE-IVD kits
- More info: Chapter 9



18
