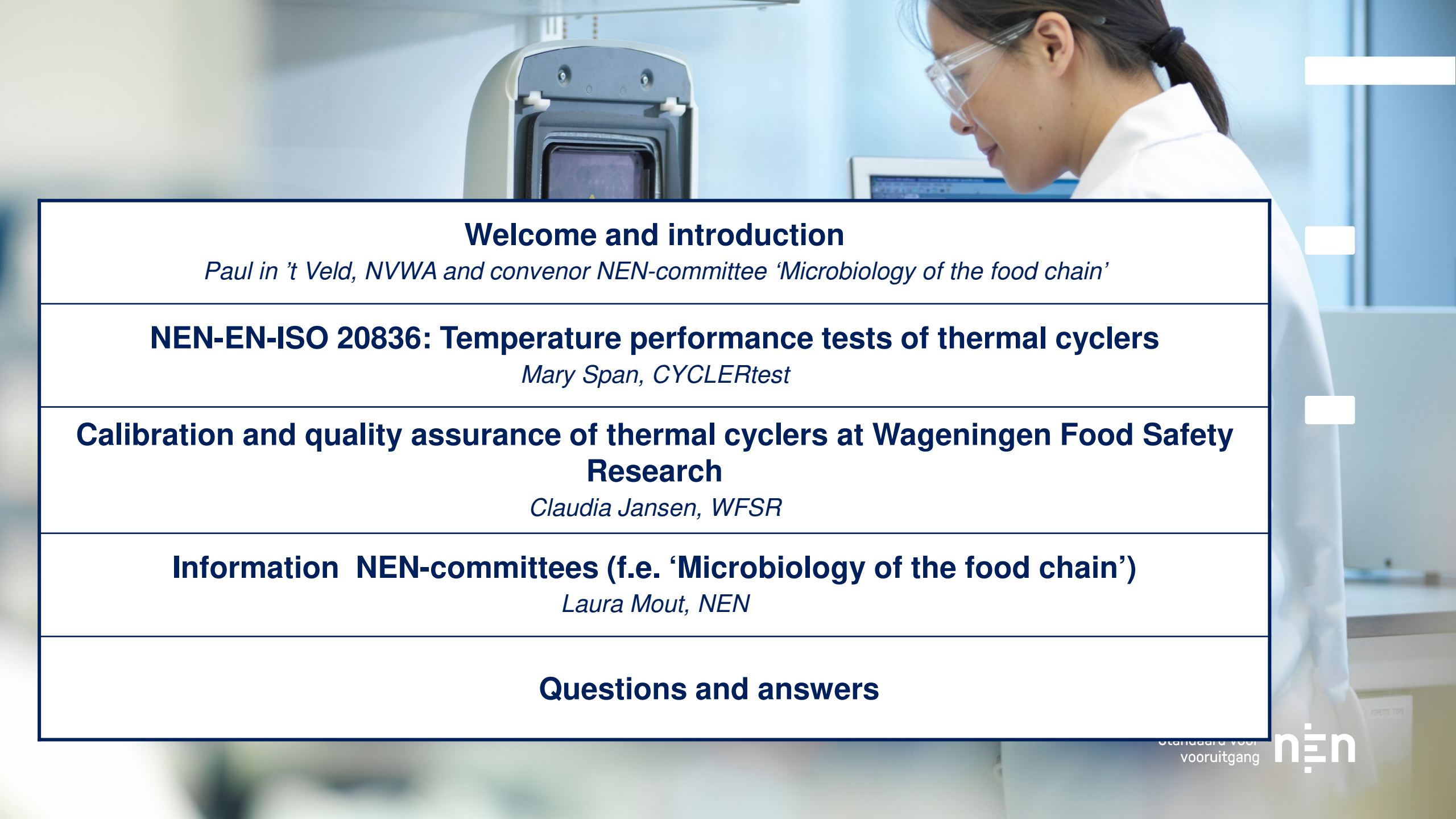
Webinar:
NEN-EN-ISO 20836 ‘thermal cyclers’

Mondag 14th february 2022

Standaard voor vooruitgang



Welcome and introduction

Paul in ’t Veld, NVWA and convenor NEN-committee ‘Microbiology of the food chain’

NEN-EN-ISO 20836: Temperature performance tests of thermal cyclers

Mary Span, CYCLERtest

Calibration and quality assurance of thermal cyclers at Wageningen Food Safety Research

Claudia Jansen, WFSR

Information NEN-committees (f.e. ‘Microbiology of the food chain’)

Laura Mout, NEN

Questions and answers

Introduction

- ISO 20836:2021 ‘thermal cyclers’ has been published by the global (ISO) standardisation network in collaboration with the European standardisation netwerk (CEN)

- The standard is under the responsibility of the international committees:
  - ISO/TC 34/SC 9 ‘Food products – Microbiology’
  - CEN/TC 463 ‘Microbiology of the food chain’

- Active input from the committee ‘Microbiology of the food chain’ during the development of the ISO 20836, for example:
  - Voting via NEN portal on formal voting rounds (incl. providing comments and improvements)
  - Dutch experts participate in CEN-working group
  - International project leader Mary Span

Related PCR standards

- Microbiology of food and animal feeding stuffs - Polymerase chain reaction (PCR) for the detection of food-borne pathogens:
  - ISO 22174:2005 ‘General requirements and definitions’
  - ISO 20837:2006 ‘Requirements for sample preparation for qualitative detection’
  - ISO 20838:2006 ‘Requirements for amplification and detection for qualitative methods’

- These three standards will be replaced by the following standard which is currently under developement:
  - ISO/CD 22174 ‘Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection and quantification of microorganisms - General requirements and definitions

- CEN/TC 463/WG 1 ‘General requirements relating to PCR methods’ is responsible for the development of these standards:
  - Working group of international experts from government, industry and laboratories

NEN-EN-ISO 20836: Temperature performance testing of thermal cyclers

Trust in test results

Mary Span

Standaard voor vooruitgang

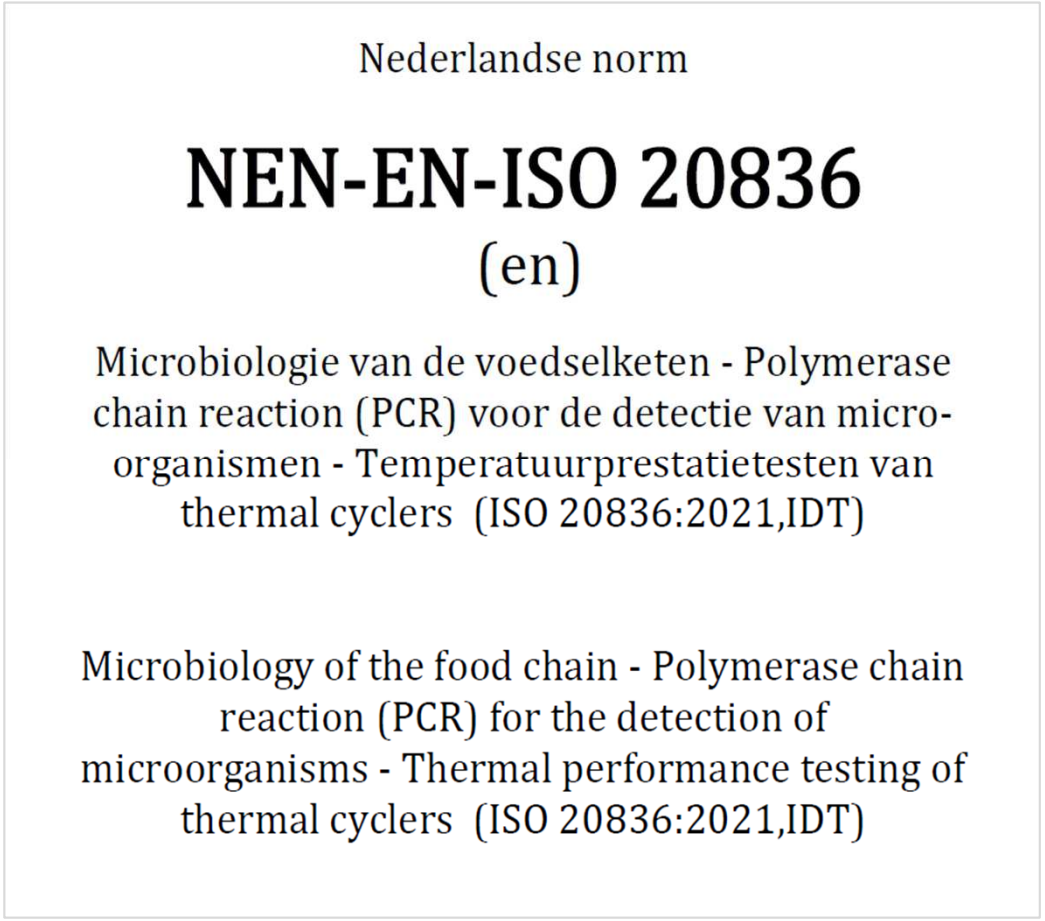
Introduction project leader

Mary Span

- Quality manager CYCLERtest
- Project leader ISO 20836
- Member NEN committee ‘Microbiology of the food chain’
- Member NEN-mirror group ‘ISO/IEC 17025’
- Member Technical Committee Temperature and Humidity (VSL)
- Expert in thermal cycler calibrations for over 15 years

Goal webinar

- Why has ISO 20836 been developed?
- To which laboratoria does ISO 20836 apply?
- How are thermal cycler performance tests executed?
- How are performance test results evaluated?


Nederlandse norm

NEN-EN-ISO 20836
(en)

Microbiologie van de voedselketen - Polymerase chain reaction (PCR) voor de detectie van micro-organismen - Temperatuurprestatietesten van thermal cyclers (ISO 20836:2021,IDT)

Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection of microorganisms - Thermal performance testing of thermal cyclers (ISO 20836:2021,IDT)

Metrology definitions

Calibration:

operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

Verification:

Provision of objective evidence that a given item fulfils specified requirements

Adjustment:

set of operations carried out on a measuring system so that it provides prescribed indications corresponding to given values of a quantity to be measured

Bron: ISO/IEC GUIDE 99:2007 ‘International vocabulary of metrology - Basic and general concepts and associated terms (VIM)’

Why has ISO 20836 been developed?

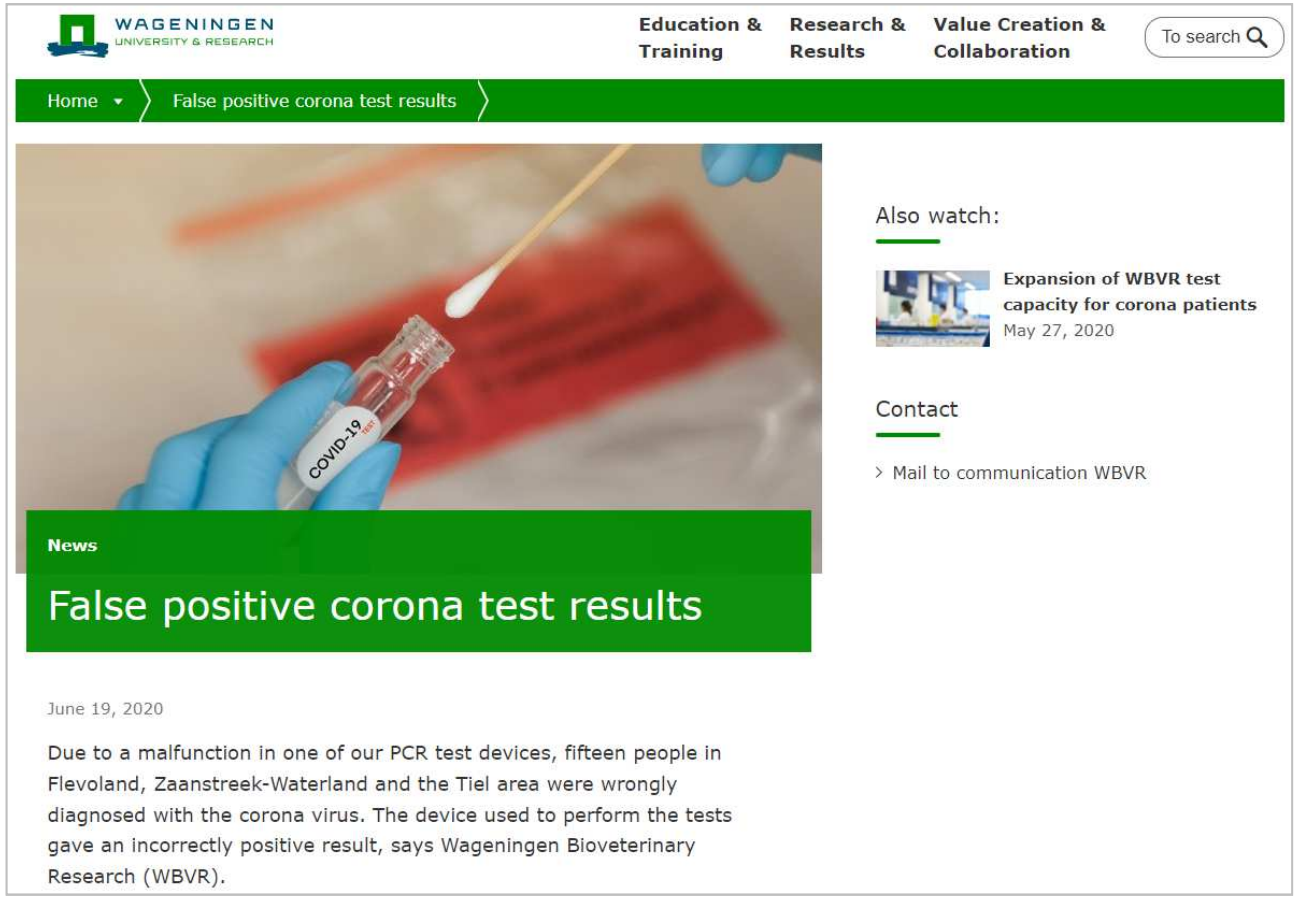
- Metrological traceable calibration of critical equipment is a requirement of:
  - ISO/IEC 17025:2017 ‘General requirements for the competence of testing and calibration laboratories’
  - ISO 15189:2012 ‘Medical laboratories - Requirements for quality and competence’

- No international standard for calibration of thermal cyclers

- Increased need for standardized calibration method of thermal cyclers due to increased application of PCR based tests

- Assure that thermal cycler functions correctly and produces reliable results

What do you wish to avoid?



Source: https://www.wur.nl/nl/nieuws/Onterechte-positieve-corona-testuitslagen.htm

To whom does the standard apply?

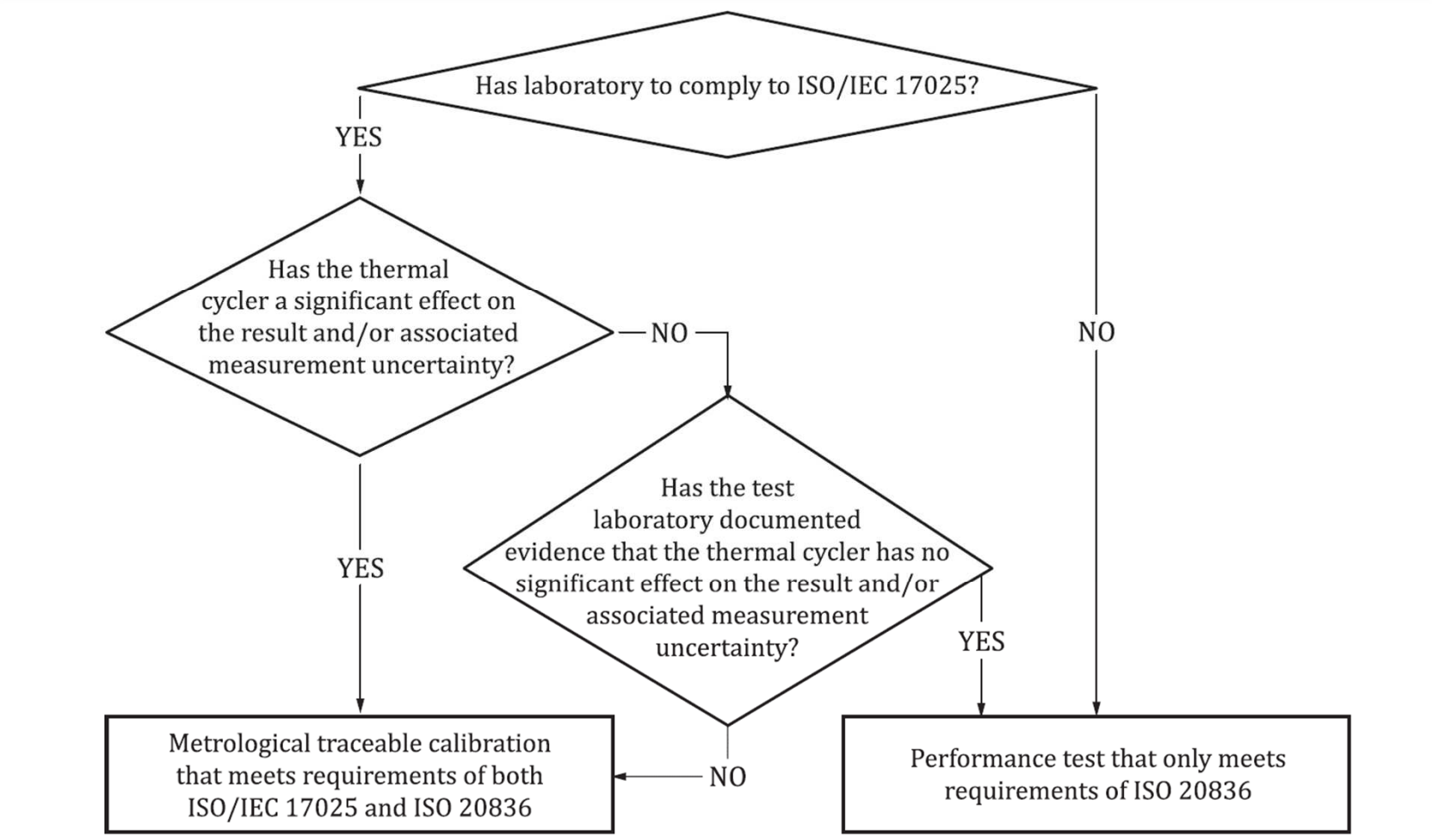
Target audience:

- ISO 17025 accredited test laboratories
- ISO 17025 accredited calibration laboratories
- ISO 15189 accredited medical laboratories
- Non-accredited laboratories
- Manufacturers of thermal cyclers
- Manufacturers of test kits

Application of PCR methods in:

- Agrofood sector
- But also environmental, medical, veterinary and forensics sector

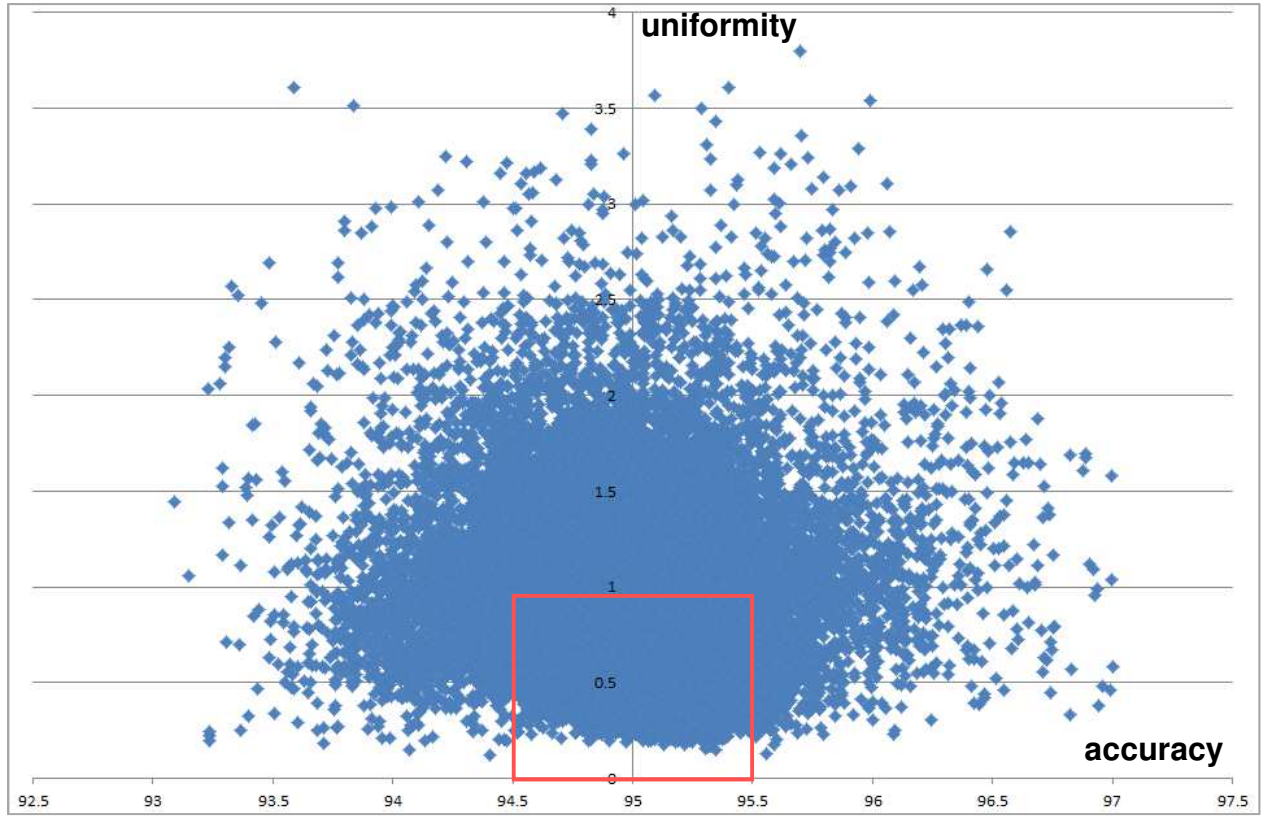
Decision chart



ISO 20836:2021 replaces ISO/TS 20836:2005

ISO/TS 20836 is first edition:

- Pulished in 2005 als ‘Technical Specification’, not as complete standard
- First step towards an international normative document
- Many practical issues with implementation, for example caused by absolute specification of ± 0,5 °C
- Many thermal cyclers out of specifications, although PCR ran without troubles

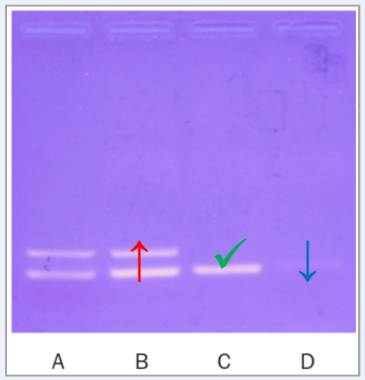


Methods

ISO/TS 20836:2005

Biochemical method

- Systematic errors
- Deviation can not be quantified
- Results reagents dependent
- Limited reliability



In-tube physical method

- Systematic errors
- Uncertainty ± 4 °C
- Results tube dependent
- Limited reliability

ISO 20836:2021

In-well physical method

- No systematic errors
- Deviation can be quantified
- Uncertainty < 0,15 °C
- Traceable to ITS-90
- High reliability

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Foreword

Introduction

1 ‘Scope’

2 ‘Normative references’

3 ‘Terms and definitions’

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5 ‘Maintenance of thermal cyclers’

6 ‘Performance testing of thermal cyclers’

Annex A ‘Sensor locations’

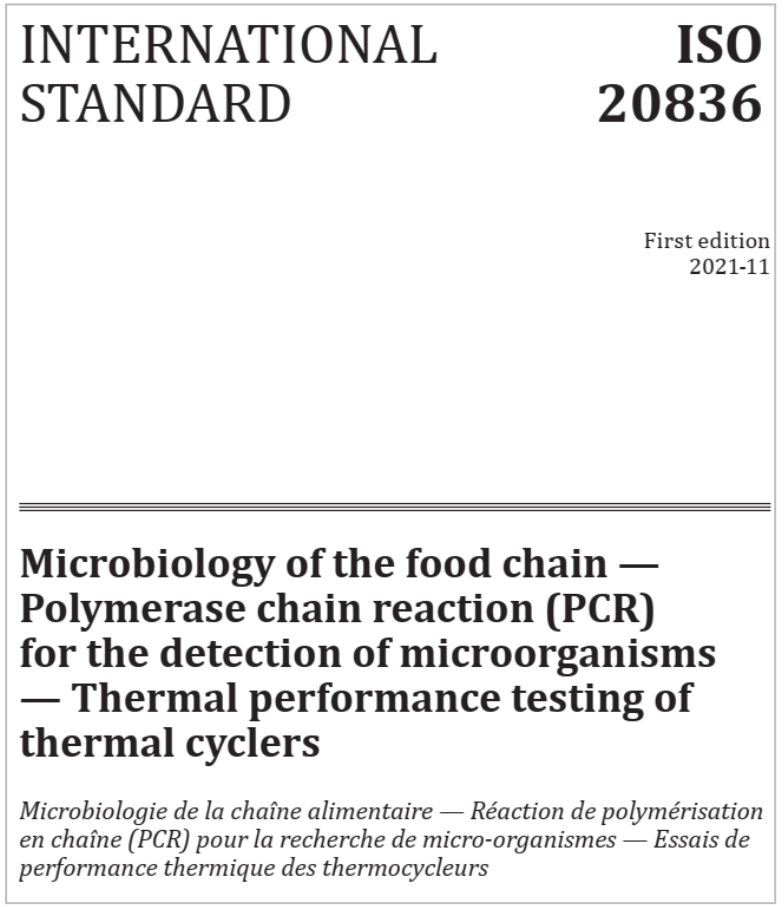
Annex B ‘Universal temperature protocol’

Annex C ‘Compliancy testing’

Annex D ‘Example of a thermal cycler temperature profile’

Annex E ‘Example of performance test and compliancy test’

Bibliography


INTERNATIONAL STANDARD

ISO 20836

First edition
2021-11

Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of microorganisms — Thermal performance testing of thermal cyclers

Microbiologie de la chaîne alimentaire — Réaction de polymérisation en chaîne (PCR) pour la recherche de micro-organismes — Essais de performance thermique des thermocycleurs

Goal of performance test

If metrological traceable calibration, conformity test or reference method:

- Measure in at least 12,5 % of the wells
- Metrological traceability up to level of thermal cycler *(this is a requirement of the ISO/IEC 17025, ISO 15189 and RvA-T018 'Acceptable traceability')*

If other purpose *(for example preventative maintenance by manufacturer)***:**

- Measure in at least 8% of the wells
- Metrological traceability up level of measurement system

Measurement system

Sensor based system:

- Multisensor
- Heated lid sensor
- Sample frequency at least 1 Hz
- Temperature range corresponding to PCR temperature range
- Metrological traceable in case of calibration
- Resolution ≤ 0,1 °C
- Uncertainty ≤ 0,15 °C
- Mass sensor head about equal to filled PCR-plate
- Response time sensors ≤ 1 s

Sensor locations

If metrological traceable calibration

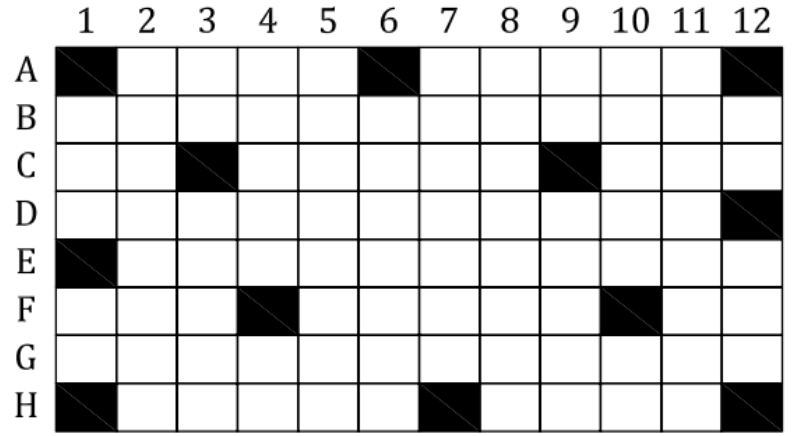
- Number of sensors equal to at least 12,5 % of the wells
- Sensors on corners, edges and central positions

If other purposed:

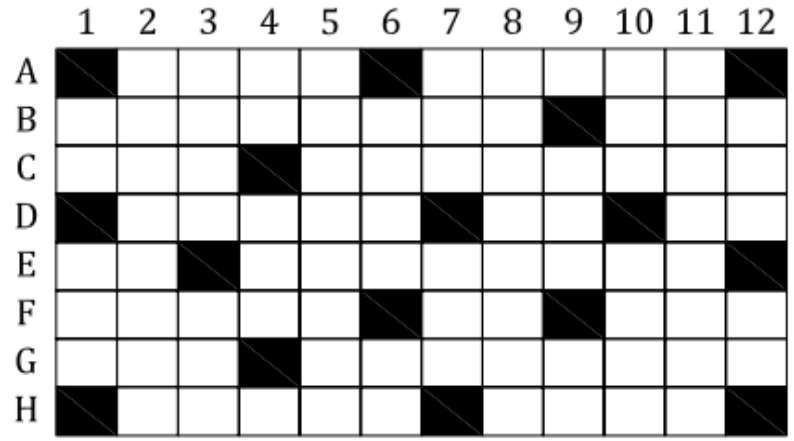
- Number of sensors equal to at least 8 % of the wells

‘Black box’-approach:

- Risk based



12 sensor layout - minimal

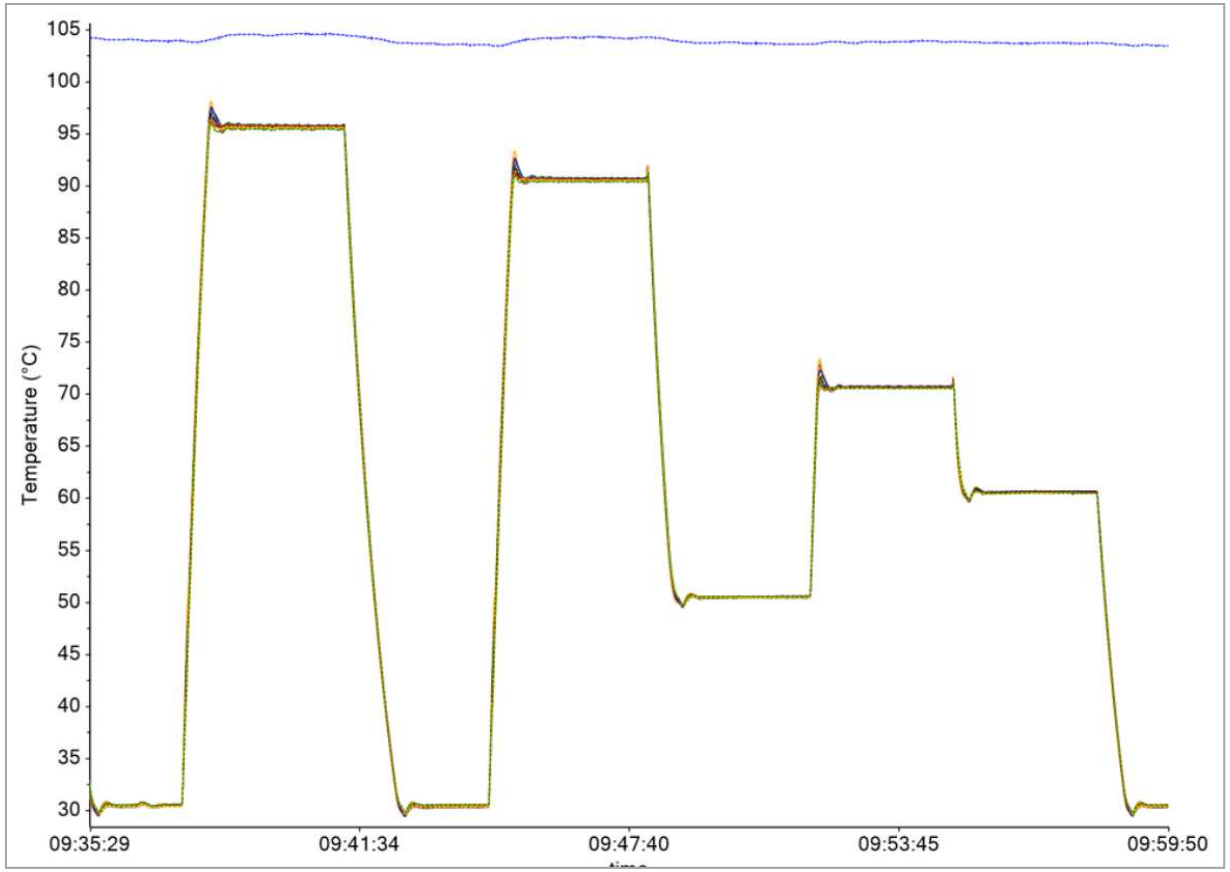


16 sensor layout - optimal

Measurement protocol

Representative of PCR:

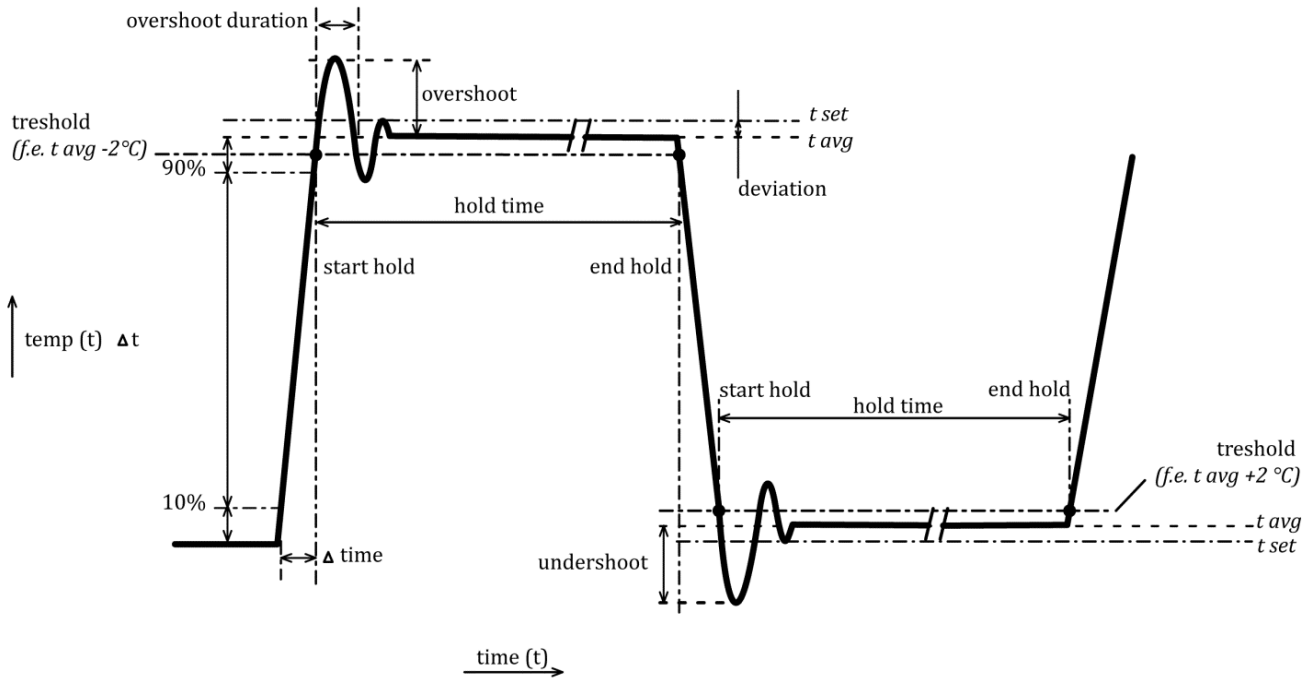
- Pre-heat
- PCR temperature range:
  minimum, middle and maximum temperature
- Measure denaturation temperature in heating mode, annealing temperature in cooling mode and elongation in heating mode
- Minimum 30 s per temperature step
- Heated lid hotter than block
- Universal protocol for all thermal cyclers



Results

Calculated results:

- Uniformity at 30 s
- Average temperature at 30 s
- Temperature deviation at 30 s
- Average overshoot
- Maximum overshoot
- Duration overshoot
- Uniformity during maximum overshoot
- Average heat/cool rate
- Maximum heat/cool rate
- Hold time



Conformity testing

ISO/IEC 17025 and ISO 15189:

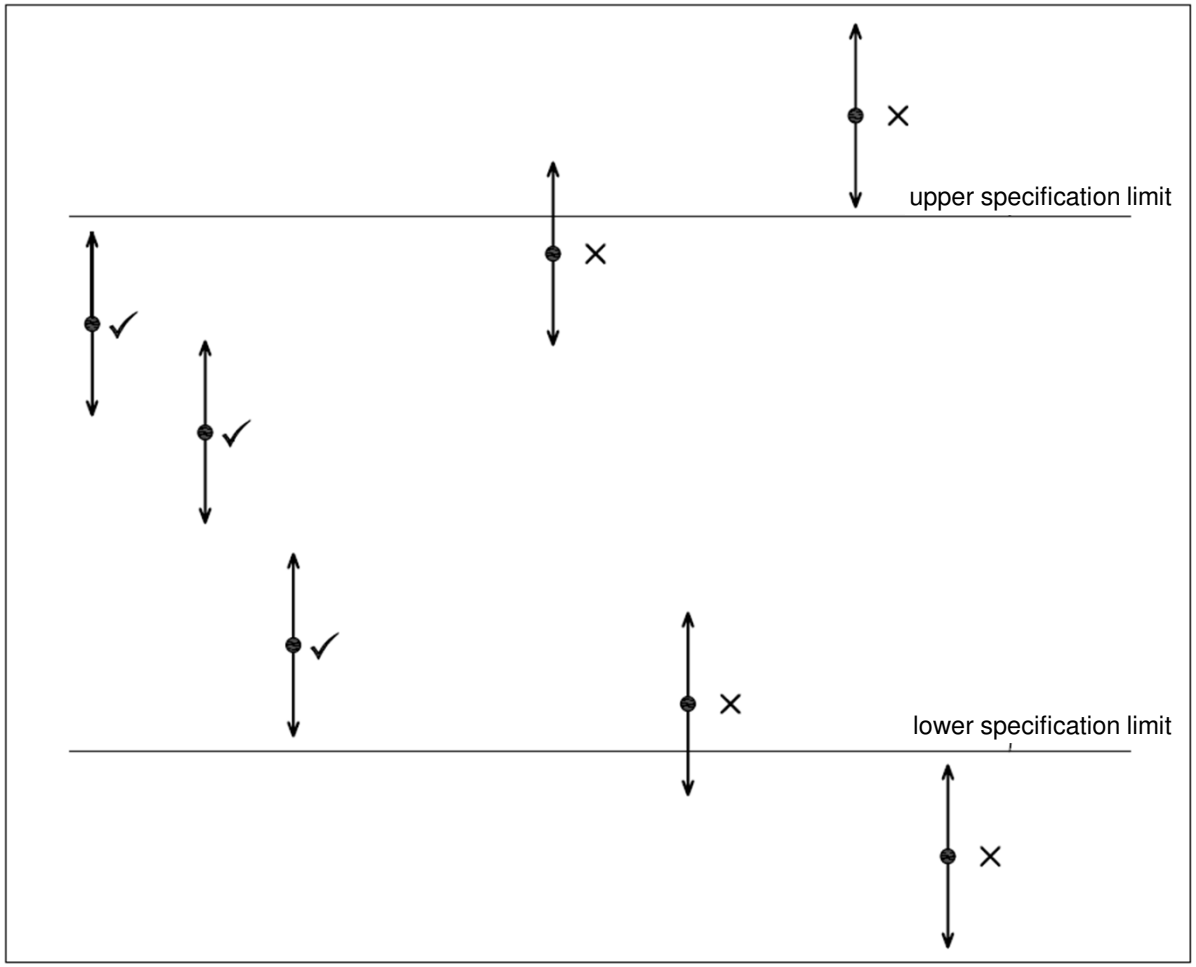
- Evaluate if instrument is suitable for intended use

Specifications:

- Thermal cycler manufacturer specifications
- PCR-kit manufacturer specifications
- PCR-method based specifications

Functional test:

- Hottest and coldest position



Calibration and quality assurance of thermal cyclers at Wageningen Food Safety Research

Vertrouwen in laboratoriumresultaten

Claudia Jansen

Standaard voor vooruitgang

Introduction

Claudia Jansen,

Researcher at the National Reference Laboratory (NRL) virusses in food,
Wageningen Food Safety Research (WFSR), Wageningen University & Research
Method development, method validation, quality at department of microbiology

WFSR >135.000 microbiological tests/year for source detection, legal standards and complaints

→ Large share of tests done on instruments with a heating block

Calibration through the years

2008: start of annual calibration of 11 instruments with a heating block *(according to ISO/IEC 17025)*

→ Qualitative evaluation versus manfacturer specifications
→ Historical comparison (f.e. total run time)

: disposal of deviating instruments or adjustment of temperature profile of the PCR protocol

→ Most of our thermal cyclers do not meet manufacturer nor market specifications

NEN-EN-ISO 20836 *(Annex C, C.1.1)*:

The thermal cycler can be qualified as suitable for intended use in different ways:

a) comparison to manufacturer specifications; ✖

b) comparison to PCR-method-based specifications; ✔ Drafting of our own specifications

c) if no specifications can be obtained, by a functional test with low positive controls in the wells with the most extreme temperatures.

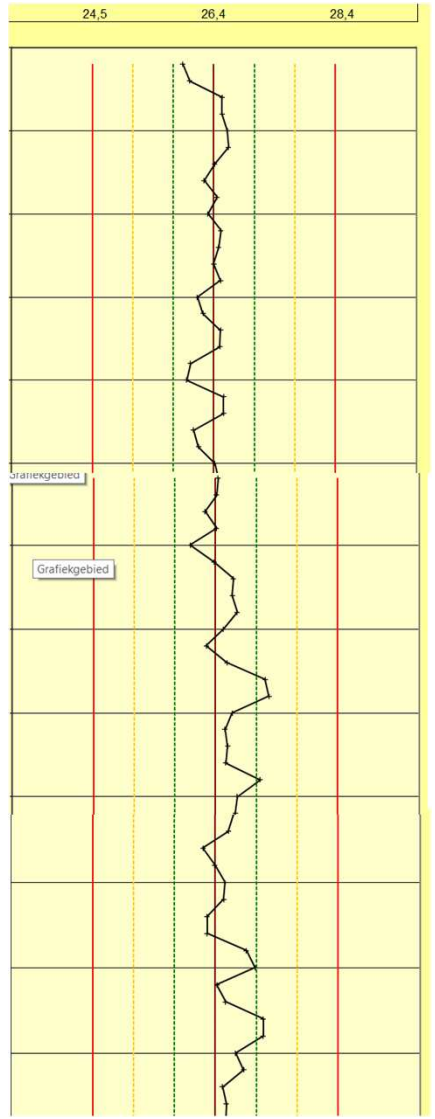
Drafting specifications

Is equipment suitable for intended use?

Method performance criteria during development method and validation:

- Gradiënt for cDNA-synthesis and/or annealing
- LOD, LOQ
- Reproducibility, repeatability, robustness
- Selectivity, specificity
- Precision

Shewhard-Westgard chart



Drafting specifications

Functional test

Temperature

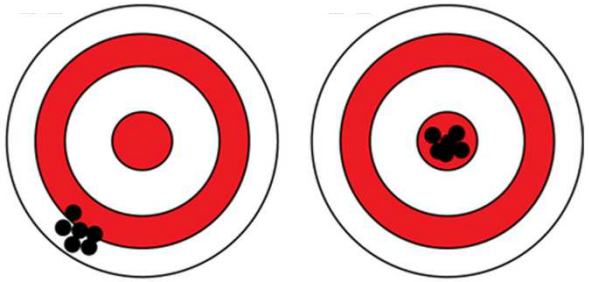
@95C na 15 seconden

	1	2	3	4	5	6	7	8	9	10	11	12
A	95,21			95,49			95,47			95,57		95,31
B												
C												
D	95,39						95,7					95,37
E				95,7						95,71		
F												
G												
H	95,22			95,46			95,45			95,5		95,27

→ **Equipment suitable for intended use**

Drafting specifications

→ **Criteria:**
- Uniformity
- Accuracy



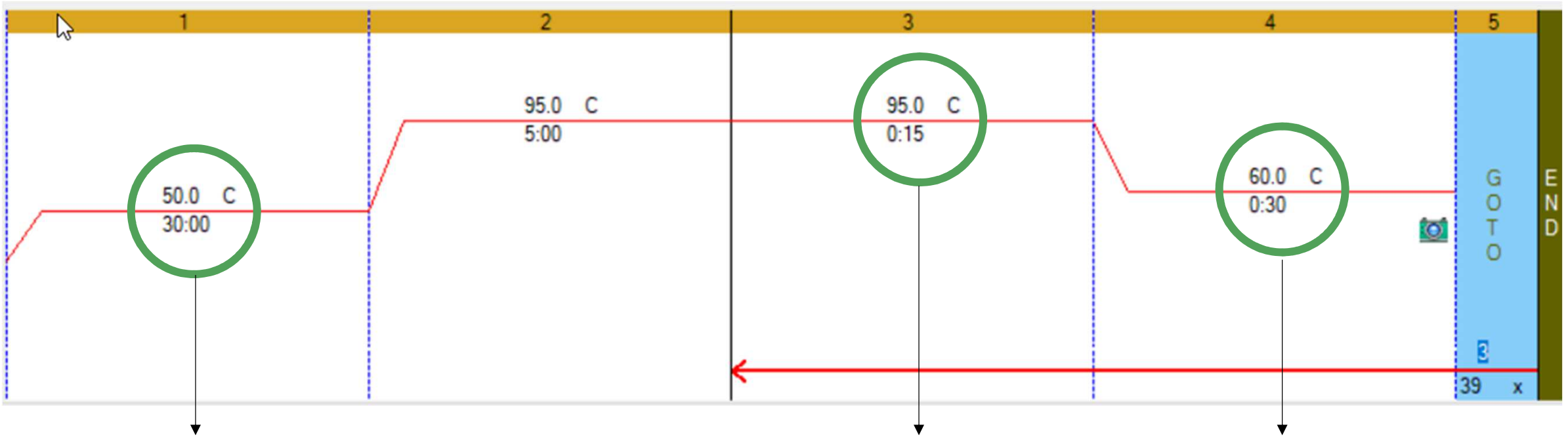
→ **Based on calibration results:**
Specifications based on data from 2010-2018
(since 2016 also measurement uncertainty calculated)

Specifications

Certain temperatures and times are crucial for RT-qPCR assays

RT | Activation enzym | PCR

Denaturation | Annealing & extension

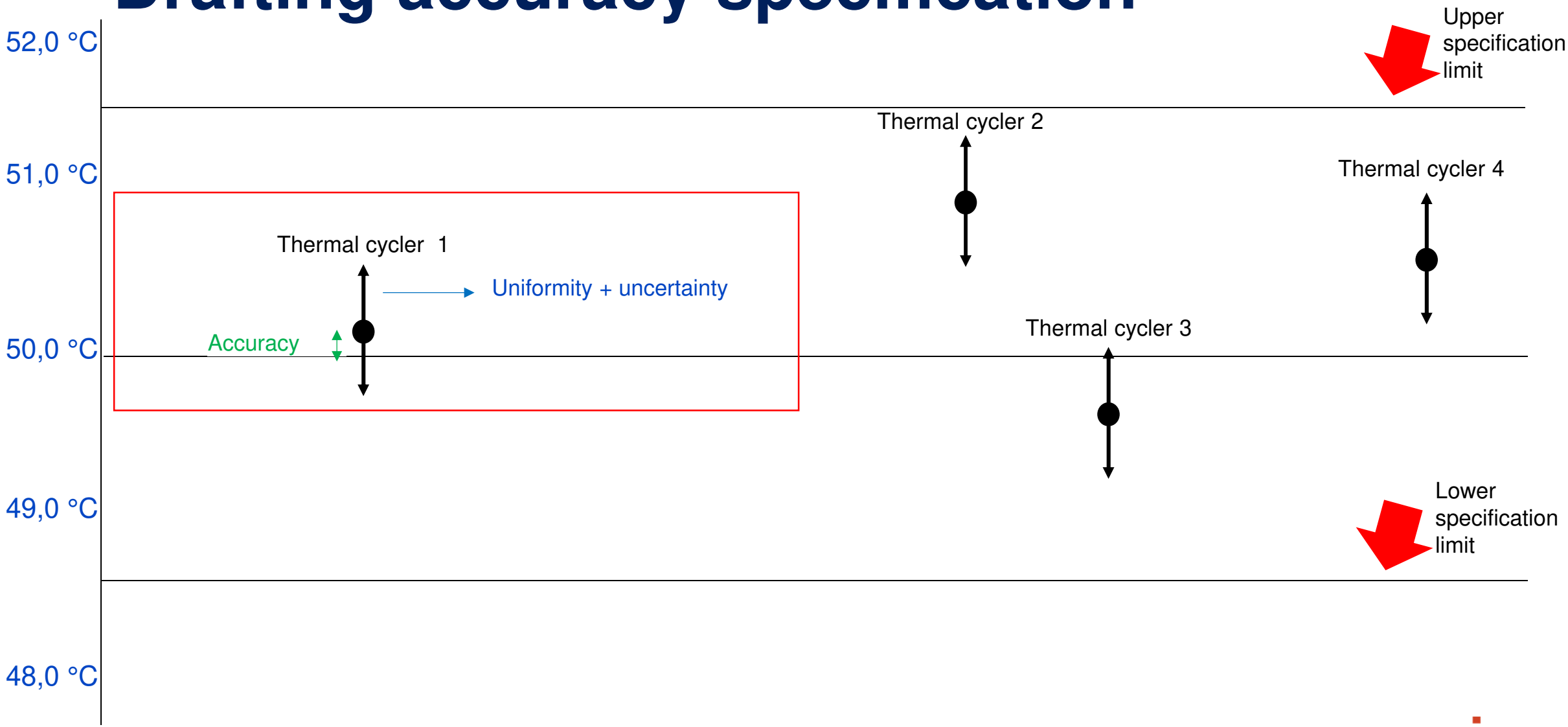


Accuracy, uniformity
@50 °C, 90 s

Accuracy, uniformity
@95 °C, 15 s

Accuracy, uniformity
@60 °C, 30 s

Drafting accuracy specification



Drafting accuracy specification

Apparaat

50°C	1	2	3	4	5	6	7	8	9	10
2010	nda	nda	nda	49,36	nda	nda	nda	nda	nda	nda
2011	50,18	49,51	49,63	49,28	nda	nda	nda	nda	nda	nda
2012	49,52	50,12	49,59	49,26	nda	nda	nda	nda	nda	nda
2013	49,76	50,37	49,84	49,54	nda	nda	nda	nda	nda	nda
2014	49,80	50,42	50,19	49,56	50,44	50,40	49,85	50,22	nda	nda
2015	49,80	50,41	50,16	49,53	50,58	50,44	49,87	50,20	50,33	50,48
2016	49,83	50,45	50,22	49,58	50,62	50,45	49,92	50,27	50,38	50,51
2017	49,88	50,48	50,22	49,58	50,60	50,47	49,94	50,27	50,43	50,51

nda= no data available

$AVG_A = 50{,}06$ °C
$ST\ DEV_u = 0{,}40$

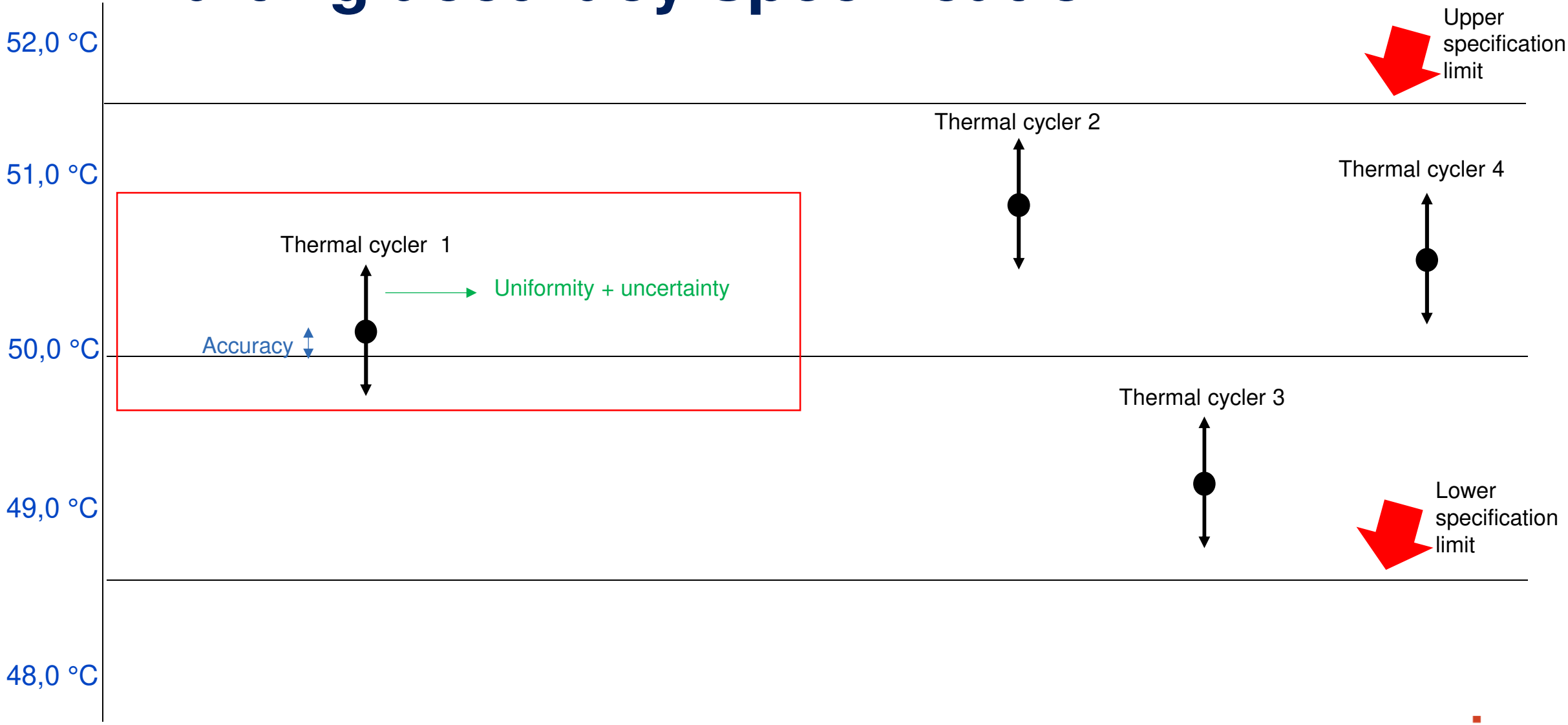
$MAX_A = 50{,}06 + (3 \times 0{,}40) = 51{,}25$

$MIN_A = 50{,}06 - (3 \times 0{,}40) = 48{,}87$

Deviation: 51,25 - 50,0 (MAX_A - 50 °C) = 1,25

50,0 - 48,87 (50 °C - MIN_u) = 1,13

Drafting accuracy specification



Specification accuracy: uncertainty

Apparaat

50°C	1	2	3	4	5	6	7	8	9	10
2016	0,34	0,35	0,40	0,32	0,33	0,33	0,36	0,36	0,36	0,34
2017	0,34	0,40	0,39	0,32	0,34	0,34	0,35	0,33	0,35	0,33
2018	0,33	0,35	0,38	0,34	0,35	0,33	0,36	0,34	0,36	0,34

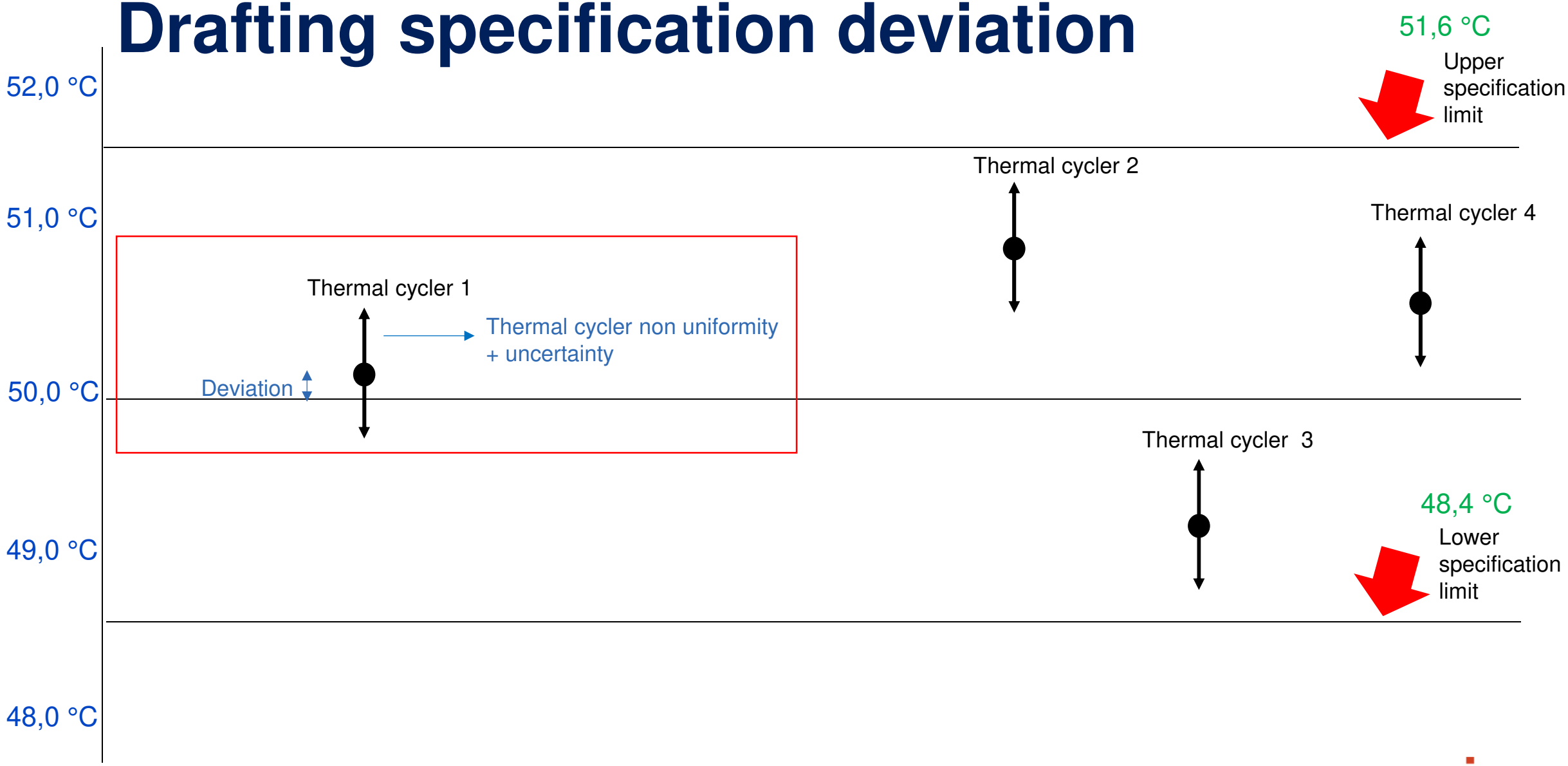
GEM. MO_A = 0,35

MAX_U Value based on historical data (8 years) measured at 15 well positions of the thermal cycler: 1,25

MO_U Value based on measurement uncertainty last 3 years: ± 0,35

Specification qPCR thermal cycler @ 50 °C ±1,60 °C

Drafting specification deviation



Uniformity thermal cycler

@50C na 90 seconden

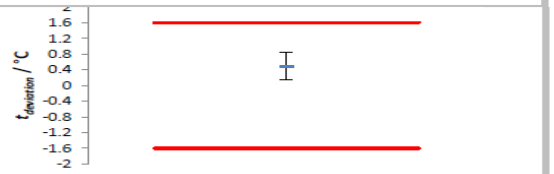
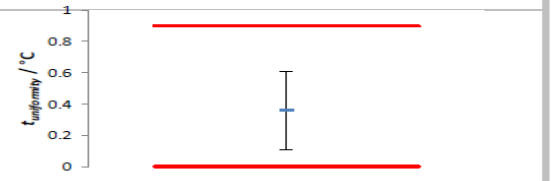
	1	2	3	4	5	6	7	8	9	10	11	12
A	50.48			50.34			50.34			50.38		50.43
B												
C												
D	50.38						50.24					50.35
E				50.29						50.31		
F												
G												
H	50.41			50.36			50.37			50.43		50.44

→ All thermal cyclers

→ All years

→ All relevant temperatures

Specification temperature	Specification time	Specification limits accuracy	Specification limits uniformity
50 °C	90 s	±1,6 °C (1,25+0,35)	< 0,9 °C (0,68+0,25)

specification item[1] t_{set} / °C		specification limits / °C	reported value / °C	reported uncertainty ($k=2$) / °C	compliance to specification[2]	graphical
50.0	$t_{deviation}$	-1.6 ≤ t ≤ 1.6	0.49	0.34	COMPLIANCE	
	$t_{uniformity}$	0.0 ≤ t ≤ 0.9	0.36	0.25	COMPLIANCE	

Conclusion

→ Annual metrological traceable calibration of all thermal cyclers

Fail on manufacturer specifications, but still suitable for intended use!!

NEN-EN-ISO 20836 *(Annex C, C.1.1)*:

The thermal cycler can be qualified as suitable for intended use in different ways:

a) comparison to manufacturer specifications; ✗

b) comparison to PCR-method-based specifications; ✓

c) if no specifications can be obtained, by a functional test with low positive controls in the wells with the most extreme temperatures.

→ Draft PCR methode based specifications based on realistic performance for all thermal cyclers for all relevant temperatures used

NEN-standardization committees

370 009 ‘Microbiology of the food chain

Laura Mout

Standaard voor vooruitgang

NC ‘Microbiology of the food chain’

Membership:

- → Expert group of specialists and generalists: +/- 40 organisations
- → Members form goverment, laboratories, companies, scientific community
- → NEN independent secretary

Access to:

- → National network
- → Internationa groups of experts
- → Concept standards incl several commenting cycles
- → Determining dutch point of view

Standardization committee

Reference methods, f.e.:

Campylobacter

Listeria

Salmonella

Yeast, moulds, virusses

General standards, f.e.:

Challenge tests

PCR

Sample preparation

Validation and verification of methods

Whole Genome Sequencing (WGS)

Potentially relevant committees

- **Microbiology of the food chain** *(370 009)*
  - NEN-EN-ISO 22174 Ontw. ‘Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection and quantification of microorganisms - General requirements and definitions’
  - NEN-EN-ISO 7218 Ontw. ‘General requirements and guidance for microbiological examinations’

- **Water microbiology** *(390 020 06)*
  - ISO/TS 16099 ‘Water quality - Polymerase chain reaction (PCR) for the detection and quantification of microorganisms - Quality control and validation of molecular methods’

- **Ecology** *(390 020 05)*
  - NEN-EN 17805 Ontw. ‘Water sampling for capture of macrobial environmental DNA in aquatic environments’

- **In Vitro Diagnostics** *(301 086)*
  - NEN-EN-ISO 15189 Ontw. ‘Medical laboratories - Requirements for quality and competence’

Questions

Standaard voor vooruitgang

Standaard voor
vooruitgang