The Impact of Excessive Condensation on Sterility Assurance Level

Prof. Duygu PERÇİN, MD
Vice-president of DAS, Turkey
Department of Clinical Microbiology
Erciyes University Faculty of Medicine, Kayseri, TURKEY

Co-authors
Peter KOZIN & Wim RENDERS

Where is the idea coming from?

- Unfortunately it is coming from the real life situations:
  - Study for prolongation of shelf life of sterile packs in orthopedic hospital in Slovenia
  - Outbreak of surgical site infections due to inadequate sterilization in Turkey

Slovenian case

- Design qualification study to extend shelf life of sterile packs
- It was aimed to confirm sterility after one year or redesign the pack to prolong the shelf life
- Composition of the pack
  - Critical quantity of orthopedic surgical instruments (10kg metal instruments)

Simulation

- The instruments, which were cleaned but not used for a long time, were selected and used as a challenge pack
- Instruments were put in a metal tray
- The set was double wrapped
  - inner wrap: 60 gr non woven
  - outer wrap: 50 gr SMS

Sterilization and transfer of packs

- Simulation pack was sterilized in 134^{o}C for 7 min with validated steam sterilizer (MMM, 2012)
- After sterilization, packs were put into dust covers and plastic transport boxes, sealed and transported to National Institute of Public Health in Slovenia for accelerated ageing and microbiological testing.

Accelerated ageing and results

- Packs were sprayed for 3 weeks repeatedly with solution of *Bacillus subtilis* and kept at 56^{o}C for ageing
- For microbiologic analyses, instruments were immersed completely into broth
- There was growth!
- Confusion???
  - Growing bacteria was not *B. subtilis!*

Conclusions of this study

- Theoretical SAL was the same of **3.5 hours in 121ºC**
- Packs were not recontaminated but they were not sterile!
- Even overkill cycle of 7 min was not enough
- There is a need for a microbiological study to prove sterilization efficacy!

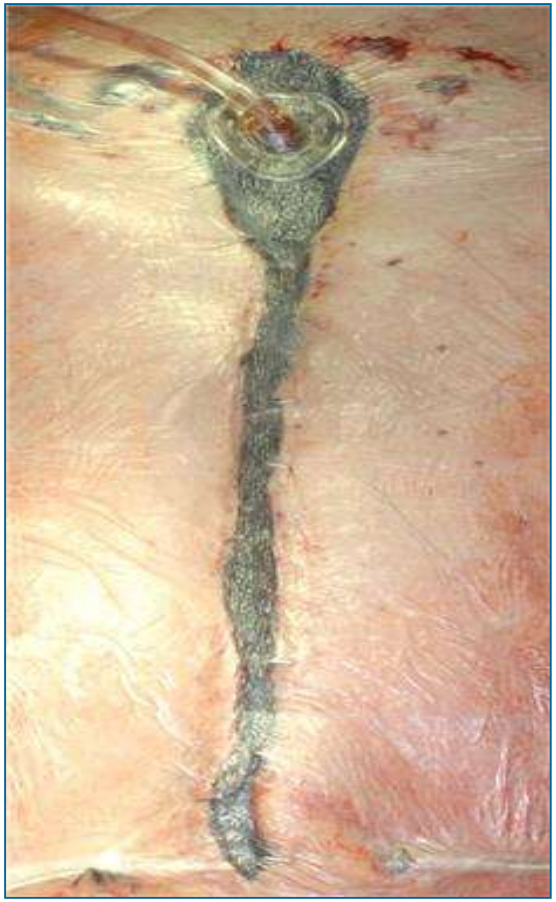
Turkish experience

An outbreak in a surgical intensive care unit
due to
inadequate sterilization

Duygu Esel (Percin), et al. J Hosp Infect 2002, 50 (3): 170-4

Evaluation of outbreak

- A case of polymicrobial ventriculitis
- An outbreak of *Serratia marcescens* mediastinitis in the intensive care unit of cardiovascular surgery
- 5 of 17 patients died



Duygu Esel (Percin), et al. J Hosp Infect 2002, 50 (3): 170-4

Molecular analysis of the strains

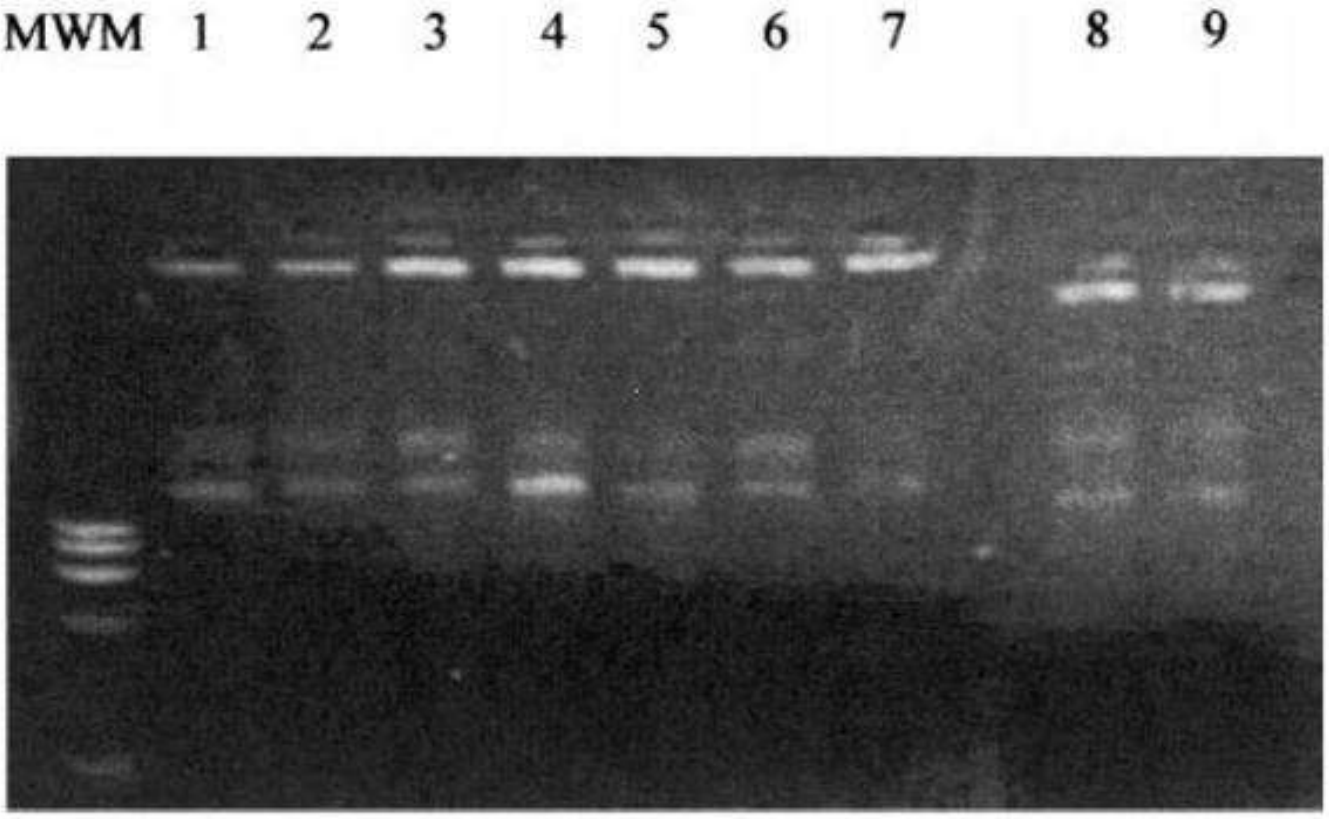


Figure 1 Plasmid profiles of nine *S. marcescens* isolates. MWM, molecular weight marker. 1–8, isolates from eight different patients (patient nos: 1, 2, 3, 4, 5, 7, 11, 13) (Table I); 9, isolate from sterilized drape (set no: 5).

Duygu Esel (Percin), et al. J Hosp Infect 2002, 50 (3): 170-4

In both cases:

There was something wrong with sterilization efficacy

The aims of present study

- To question the reason for low sterilization efficacy
- To evaluate if SAL theory is adequate enough to describe sterilization efficacy
- To evaluate the need for alternative methods, for evaluating efficacy of sterilization procedures

“STERILE” medical device

- For a terminally-sterilized medical device to be designated "STERILE“
  - the theoretical probability of there being a viable micro-organism present on/in the device must be equal to or less than $1x10^{-6}$
- Sterility assurance level (SAL)

SAL concept

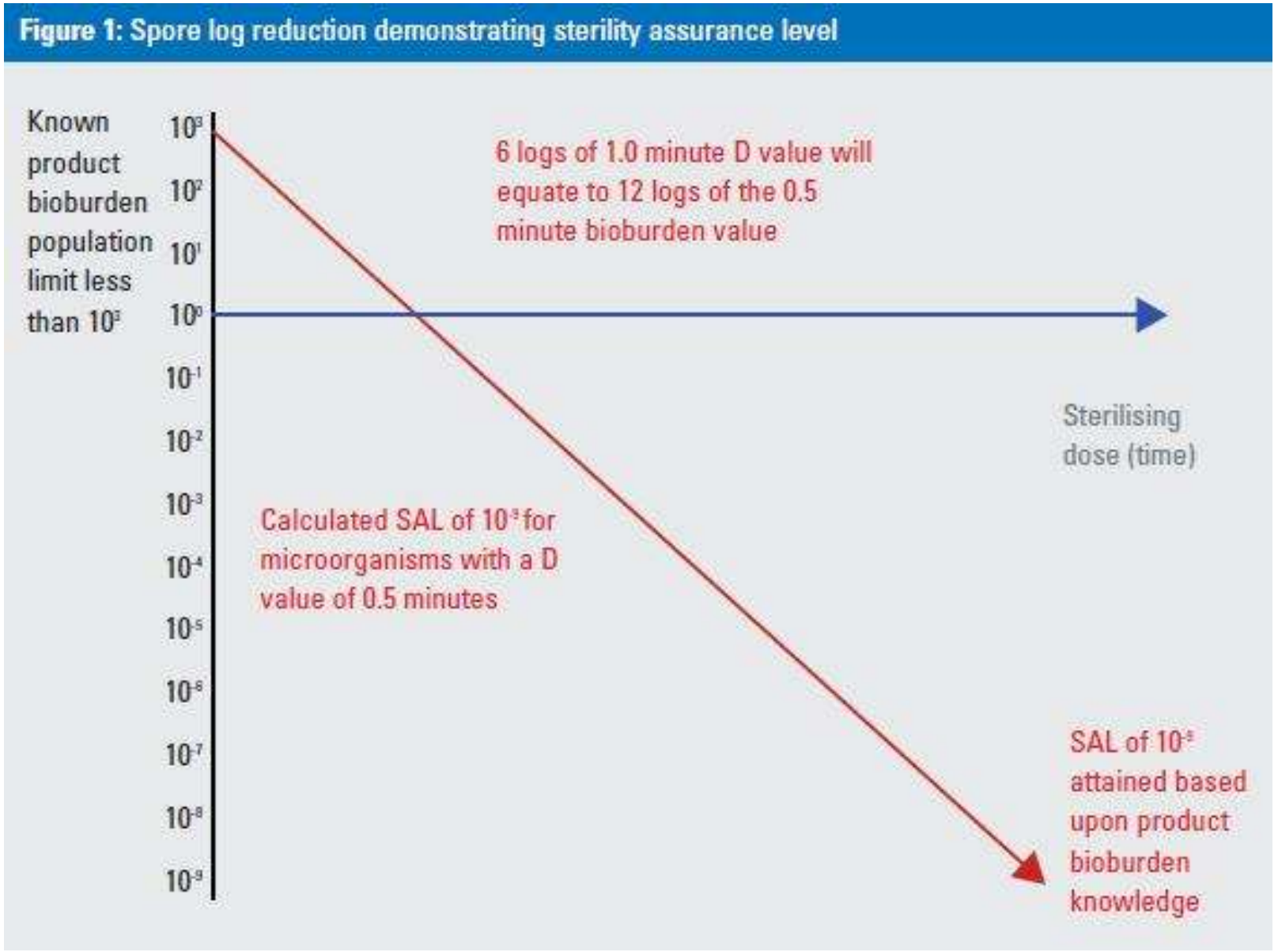
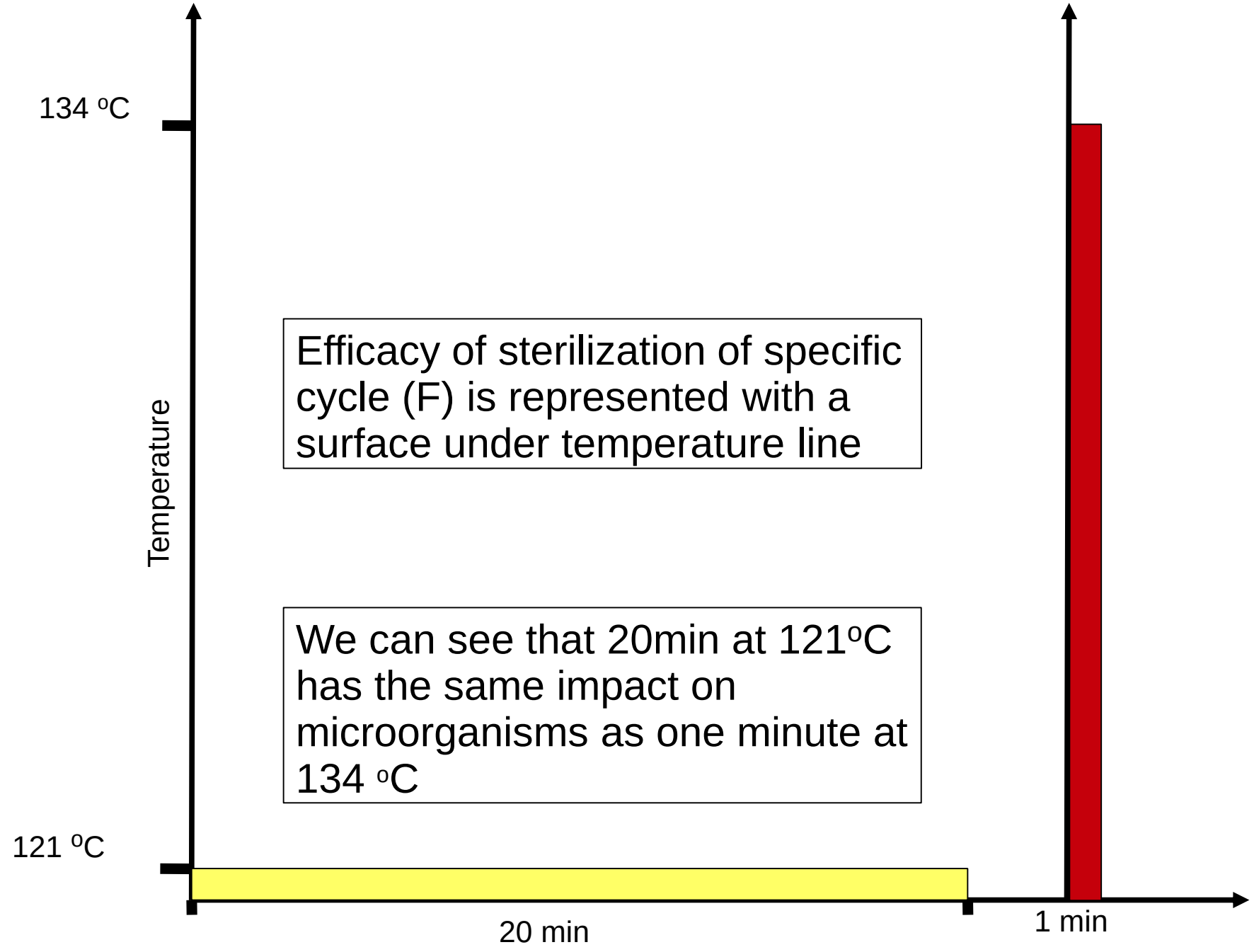


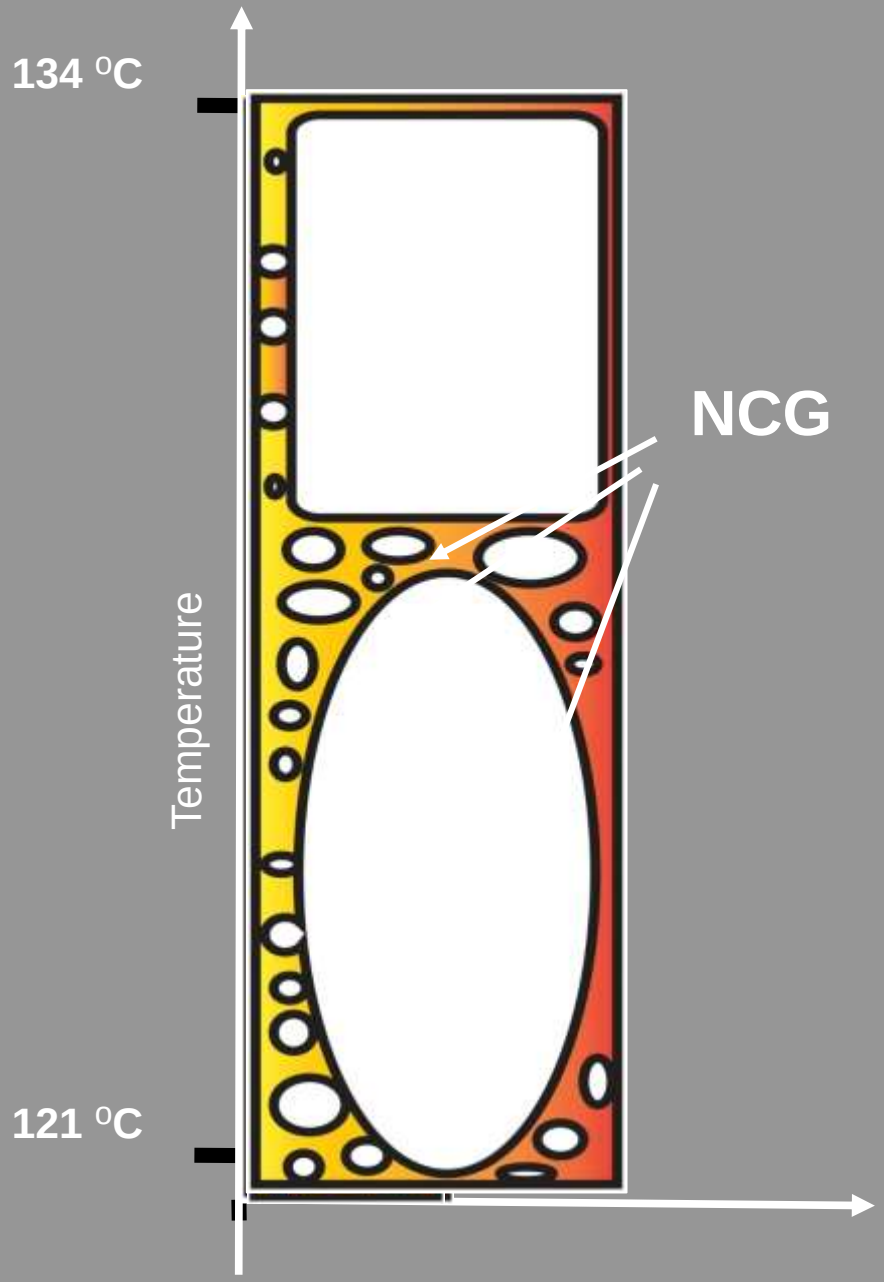
Figure 1: Spore log reduction demonstrating sterility assurance level
Known product bioburden population limit less than 10^3
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
6 logs of 1.0 minute D value will equate to 12 logs of the 0.5 minute bioburden value
Sterilising dose (time)
Calculated SAL of 10^{-3} for microorganisms with a D value of 0.5 minutes
SAL of 10^{-6} attained based upon product bioburden knowledge

Elimination of microorganisms

- A time-dependent process
- Influenced by
  - the intensity of treatment
  - the initial microbial contamination level
- Effect of some risks in CSSD
  - non condensable gases
  - improper cleaning
  - **excessive condensate**


134 °C
Temperature
Efficacy of sterilization of specific cycle (F) is represented with a surface under temperature line
We can see that 20min at 121°C has the same impact on microorganisms as one minute at 134 °C
121 °C
20 min
1 min

STERILIZATION EFFICACY AT 134ºC; WHAT IS GOING ON?



In fact we are prolonging sterilization cycles to be sure to achieve SAL 10^{-6}

BUT…

ARE WE ALSO INCREASING OUR MISTAKES WITH IT ???


Condensation

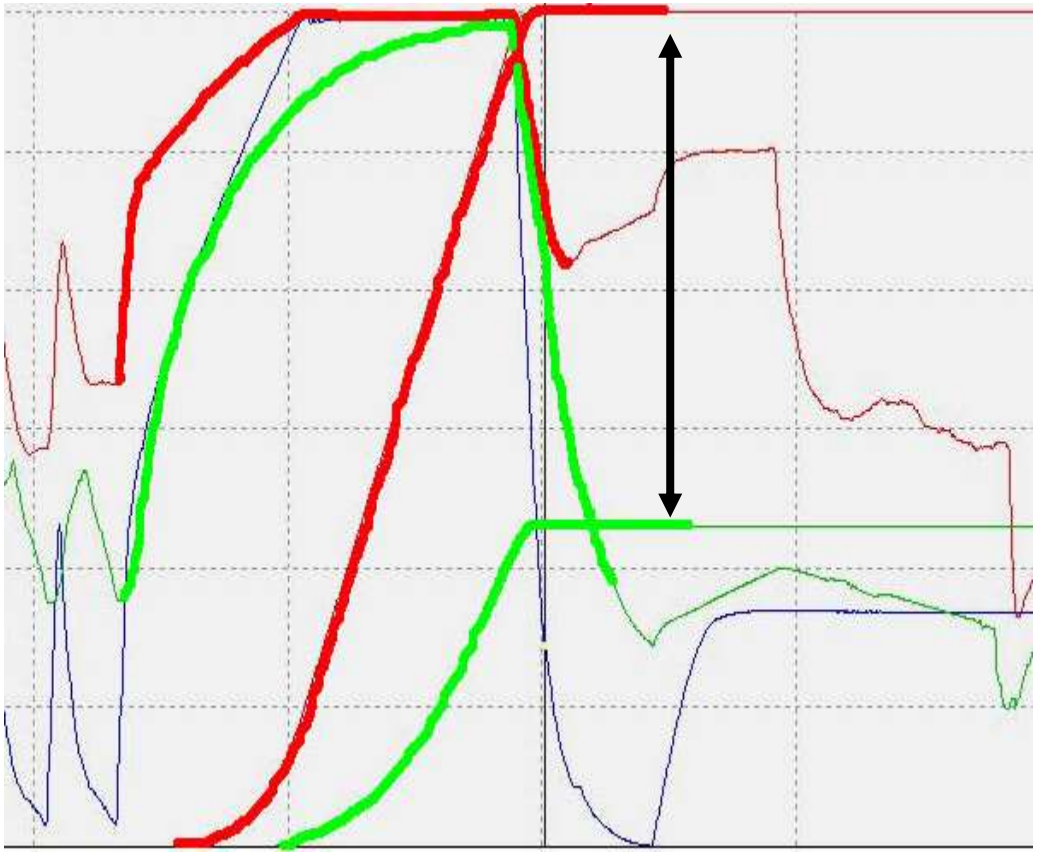
Excessive condensate (1)

- At steam sterilization cycle, we have to heat up our surgical instruments to 134^{o}C to achieve sufficient sterilization
- To achieve this we are using condensation
- During condensation saturated steam is transformed into condensate
- The heavier our sterilization packs are, the more condensate we are generating at heating up

Excessive condensate (2)

- For every kilogram of metal we are generating a couple of deciliters of condensate
- If this condensate is trapped into sterilization pack it does not gain temperature as fast as metal surfaces in the load
- It means that preset temperature of sterilization cycle is reached much slower in condensate than on exposed surfaces

Effect of excessive condensate on sterilization efficacy



Difference in F value

Condensate (green)

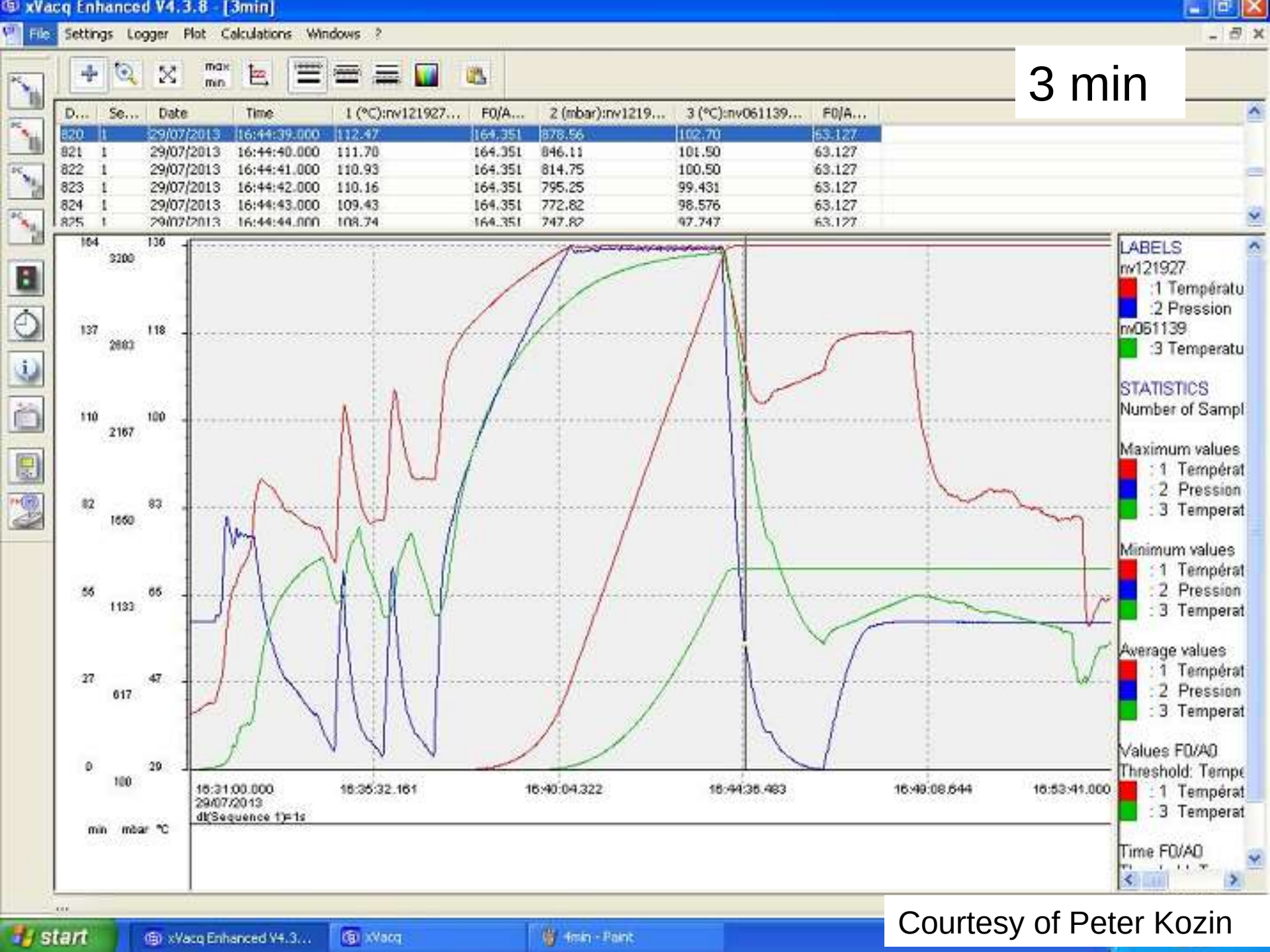
Without condensate

(red)

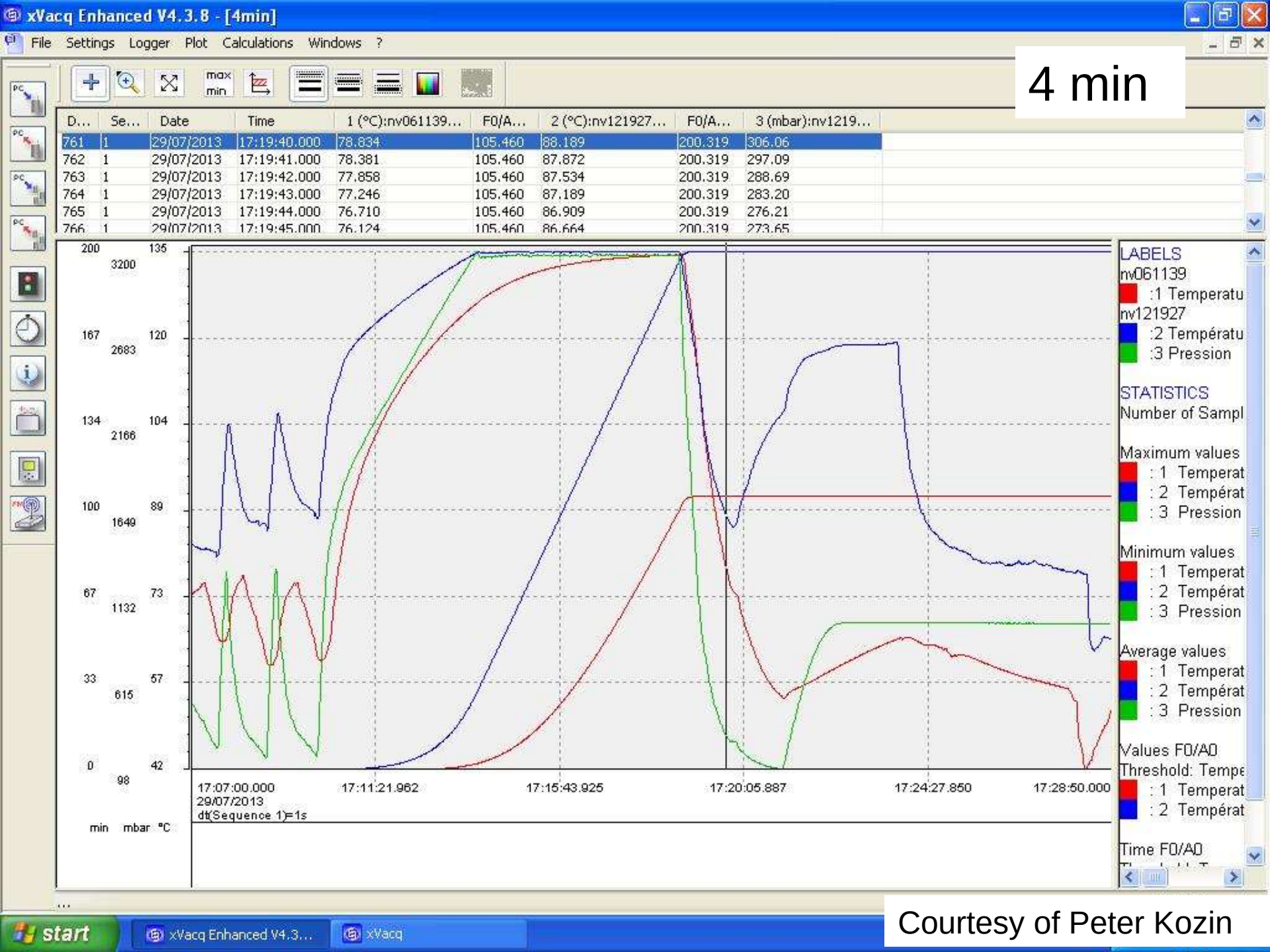
Up to:

-60%

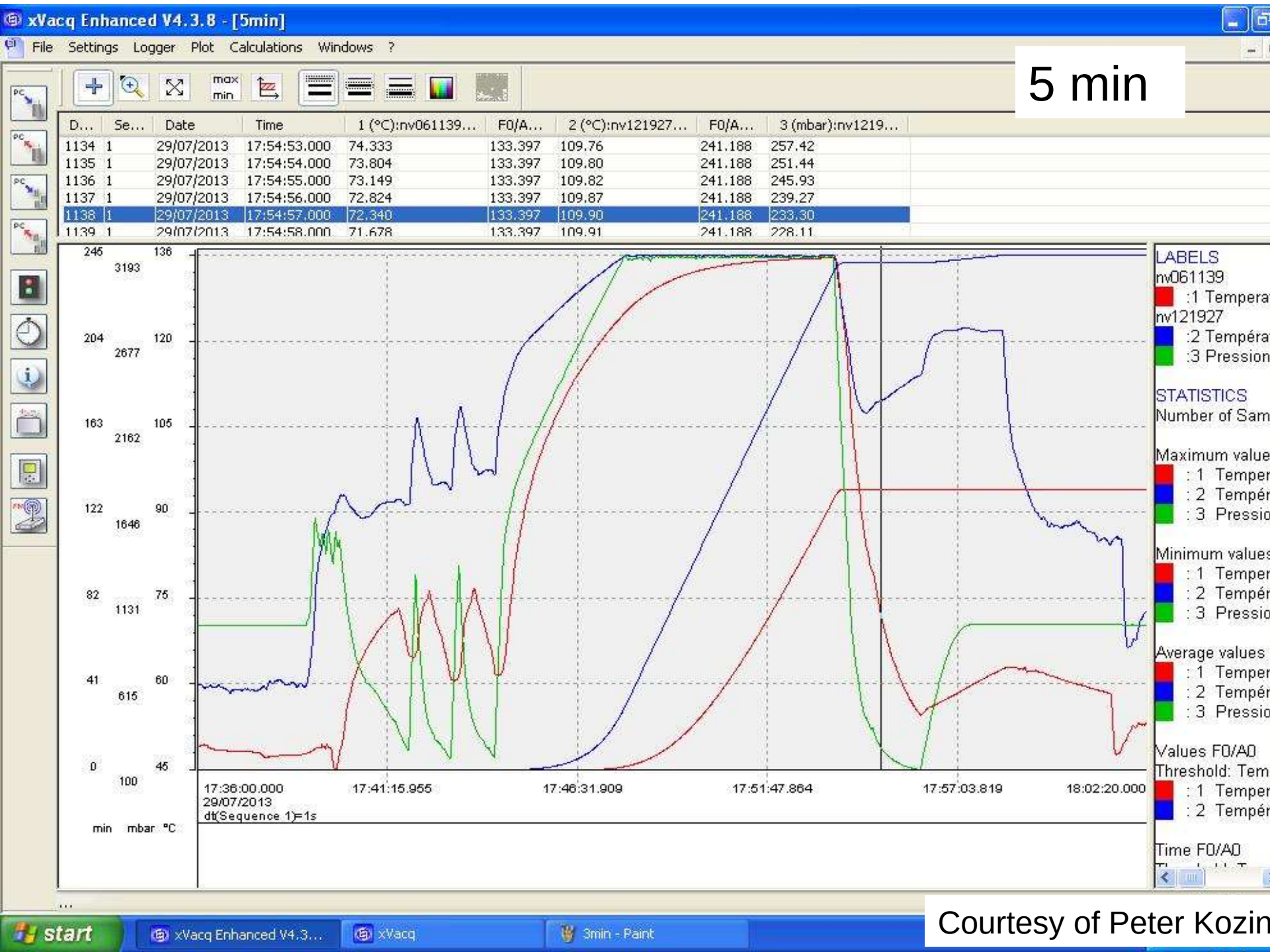
…at short cycles



Courtesy of Peter Kozin



Courtesy of Peter Kozin


xVacq Enhanced V4.3.8 - [5min]
File Settings Logger Plot Calculations Windows ?
5 min
D... Se... Date Time 1 (°C):nv061139... F0/A... 2 (°C):nv121927... F0/A... 3 (mbar):nv1219...
1134 1 29/07/2013 17:54:53.000 74.333 133.397 109.76 241.188 257.42
1135 1 29/07/2013 17:54:54.000 73.804 133.397 109.80 241.188 251.44
1136 1 29/07/2013 17:54:55.000 73.149 133.397 109.82 241.188 245.93
1137 1 29/07/2013 17:54:56.000 72.824 133.397 109.87 241.188 239.27
1138 1 29/07/2013 17:54:57.000 72.340 133.397 109.90 241.188 233.30
1139 1 29/07/2013 17:54:58.000 71.678 133.397 109.91 241.188 228.11
245 3193 136
204 2677 120
163 2162 105
122 1646 90
82 1131 75
41 615 60
0 100 45
min mbar °C
17:36:00.000
29/07/2013
dt(Sequence 1)=1s
17:41:15.955
17:46:31.909
17:51:47.864
17:57:03.819
18:02:20.000
LABELS
nv061139
:1 Tempera
nv121927
:2 Tempéra
:3 Pression
STATISTICS
Number of Sam
Maximum value
: 1 Temper
: 2 Tempér
: 3 Pressio
Minimum values
: 1 Temper
: 2 Tempér
: 3 Pressio
Average values
: 1 Temper
: 2 Tempér
: 3 Pressio
Values F0/A0
Threshold: Tem
: 1 Temper
: 2 Tempér
Time F0/A0
start
xVacq Enhanced V4.3...
xVacq
3min - Paint
Courtesy of Peter Kozin

Materials and methods

- Preparation of *Geobacillus stearothermophilus* (ATCC 7953) spores from 10^5 to 10^9
- Inoculation of screws
- Steam sterilization
- Device for generation of condensate
- Culture and incubation
- Microbiological results
- Electron microscopic evaluation

Spore production

(Writz-Conklin staining)

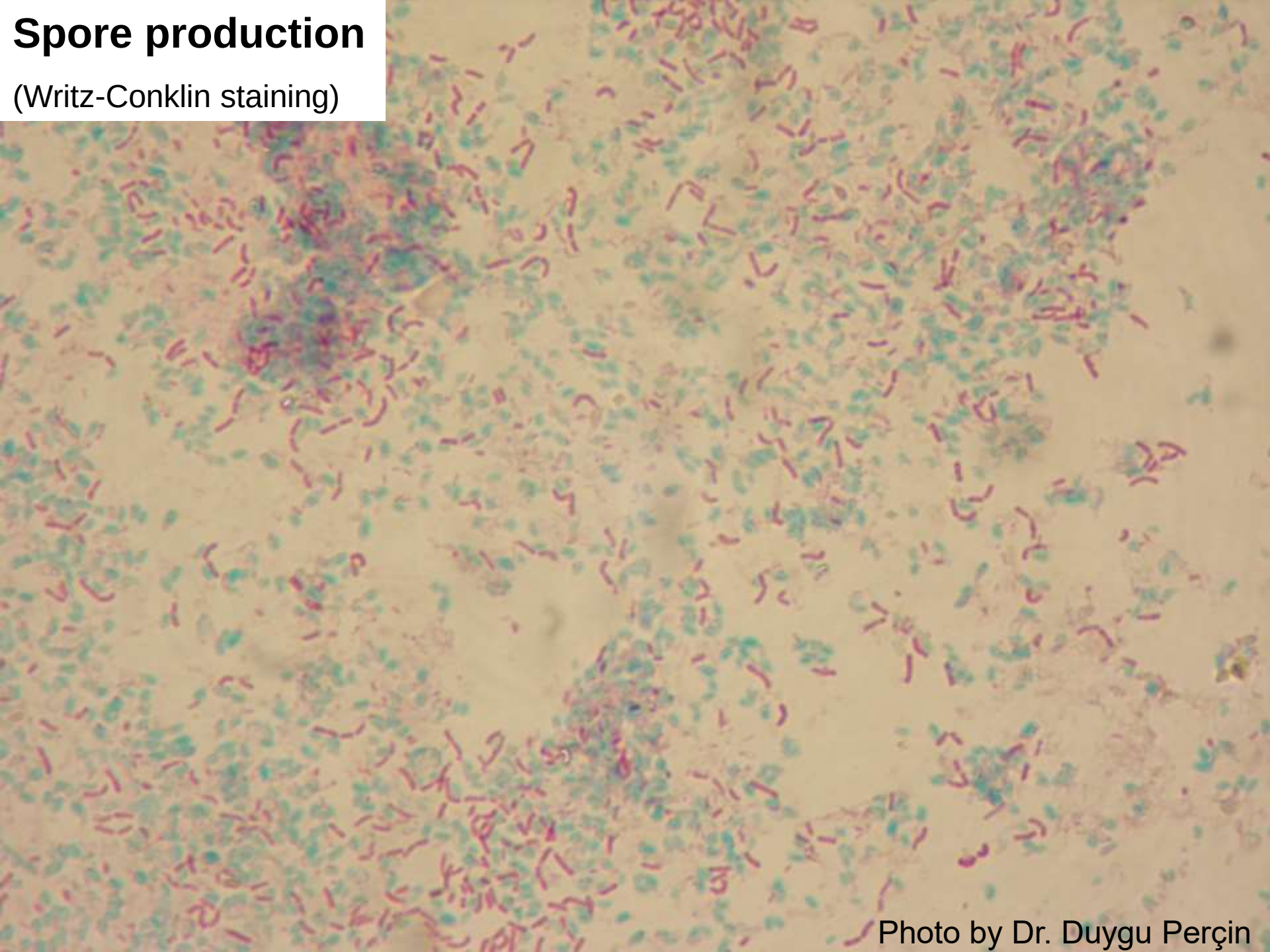
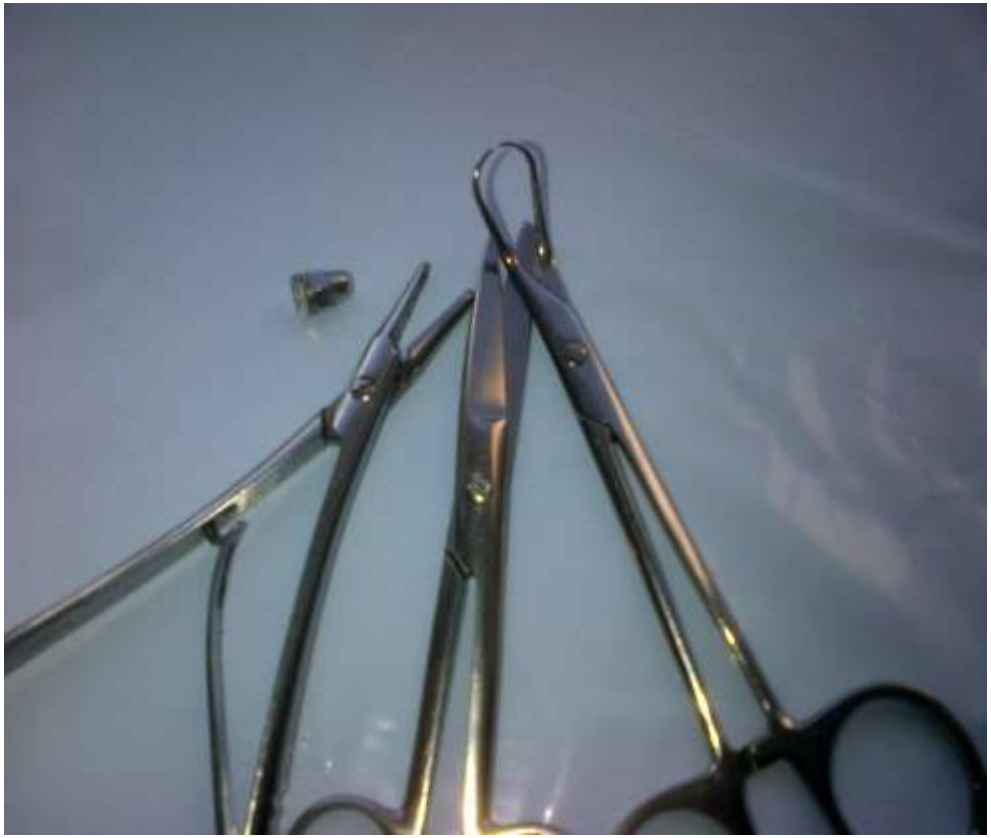


Photo by Dr. Duygu Perçin

Screws

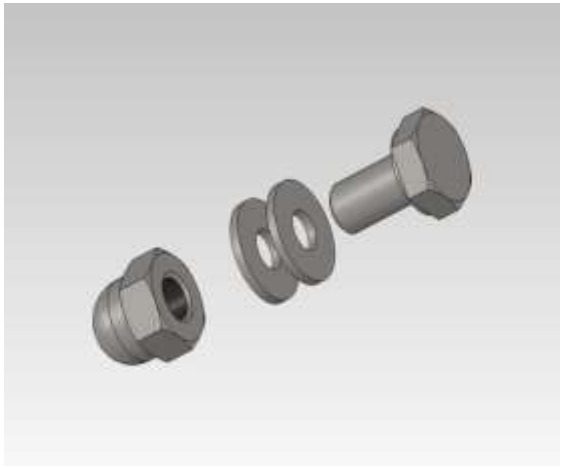
Photo by Peter Kozin

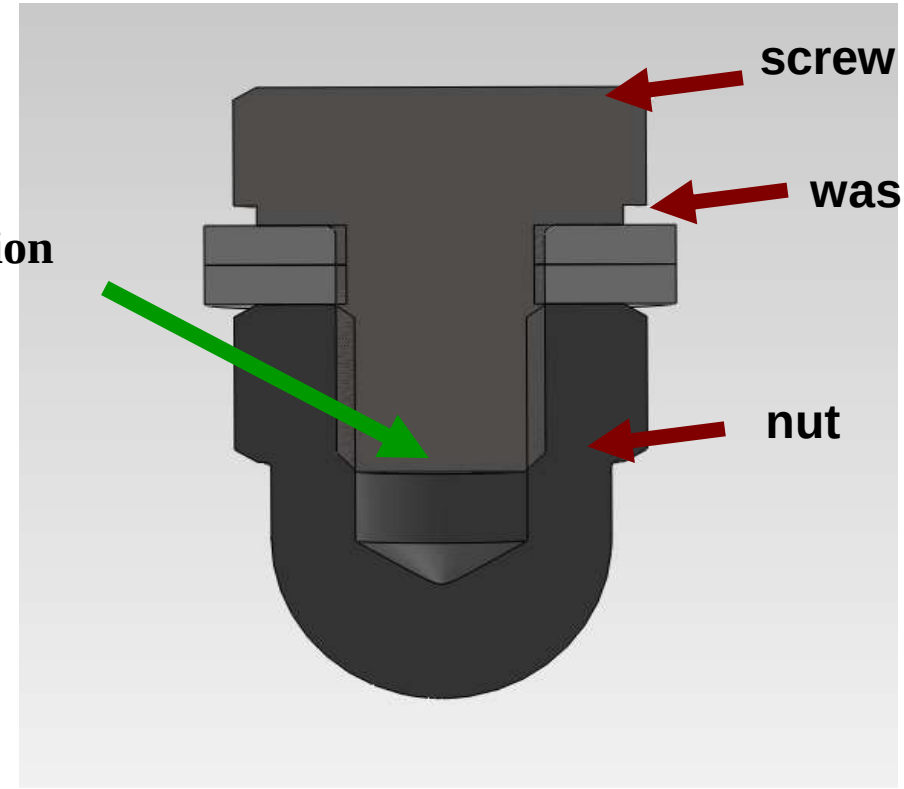
Correlation:
Testing device vs. Real life instruments



Similar shape
and size

Screws





Spore inoculation

Steam sterilization aparatus and cycle

Steam sterilizer

Getinge Ge336c

Validated cycle

-Temperature 135,5^{o}C

-3 transatmospheric pulses for air removal

-Different holding times

-Short vacuum drying time


Device for production of condensate during sterilization cycle
Photo by Peter Kozin

Photo by Peter Kozin

Photo by Peter Kozin

Transfer into broth and incubation

Results

- Microbiologic results
  - Step 1-5

- Microscopic results
  - Gram staining
  - Scanning Electron Microscopy

STEP 1: Results of screws inoculated with 10^9 spores

Sterilization time	Sample size	Cycle (134°C)	Growth
3 min	6	correct	+
	6	condensate	+
4 min	6	correct	+
	6	condensate	+
5 min	6	correct	+
	6	condensate	+

Turbidity in broths in 72 hours

Photo by Duygu Perçin


Gram staining of turbid broth
Photo by Duygu Perçin

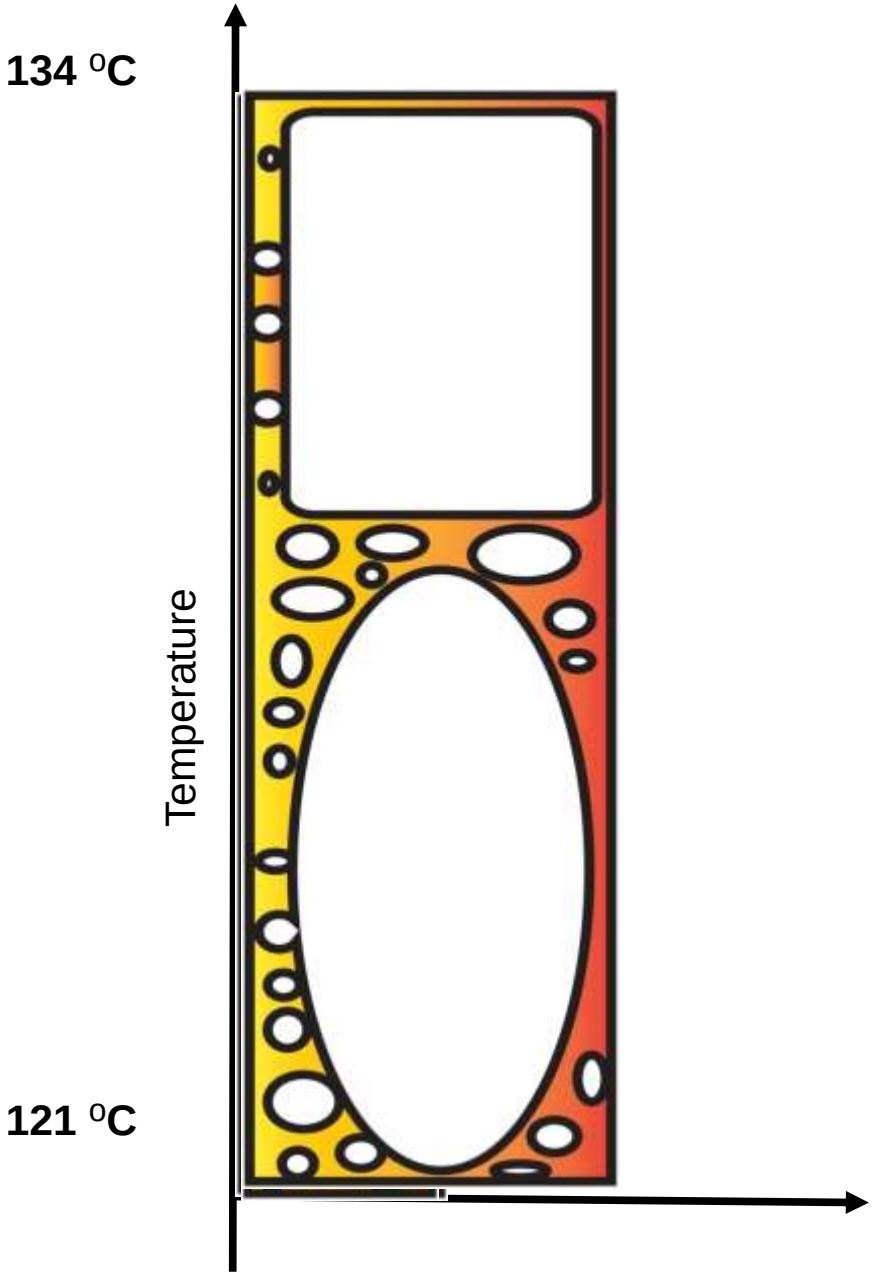
STEP 2: Results from screws with less load and metal plates ($2cm^2$)

Sterilization time	Cycle (134^0C)	Sample size / type / load	Growth
3 min	Correct	6 / Screws / 10^6	No
	Condensate	6 / Screws / 10^6	No
3 min	Correct	2 / Screws / 10^7	No
	Condensate	4 / Screws / 10^7	No
4 min	Condensate	4 / Screws / 10^7	No
3 min	Correct	6 / Plates / 10^6	No
	Condensate	6 / Plates / 10^6	No

STEP 3: Effect of condensation and sterilization time on screws carrying 10^9 spores

Sterilization time	Cycle (134°C)	Growth
7 min	Correct	No
	Condensate	Growth +
10 min	Correct	No
	Condensate	Growth +
18 min	Correct	No
	Condensate	Growth +

STERILIZATION EFFICACY AT 134ºC; WHAT IS GOING ON?



EVEN IF WE PROLONG THE CYCLE WE ALSO INCREASE OUR MISTAKES TOGETHER WITH IT

STEP 4: Effect of inoculum

(sterilization in 134°C for 3 min)

Inoculum	**Cycle**	**Result**		
		24 h	**48 h**	**72 h**
10^5-10^6-10^7	Correct	No	No	No
	Condensate	No	No	No
10^8	Correct	No	No	No
	Condensate	**No**	**No**	**Yes**
10^9	**Correct**	**No**	**Yes**	**Yes**
	Condensate	**Yes**	**Yes**	**Yes**


G.stearothermophilus
before sterilization
Photo by Duygu Perçin

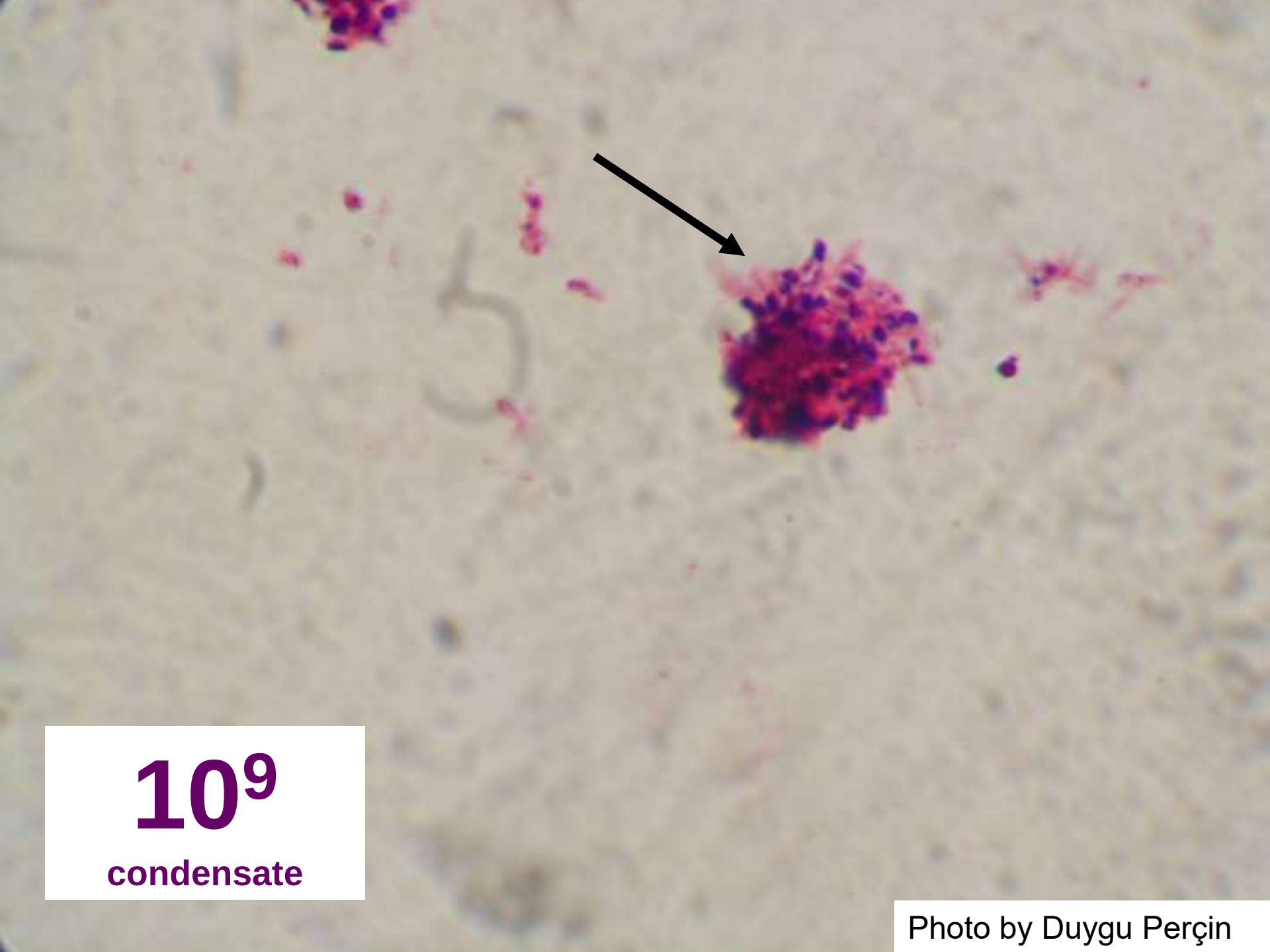
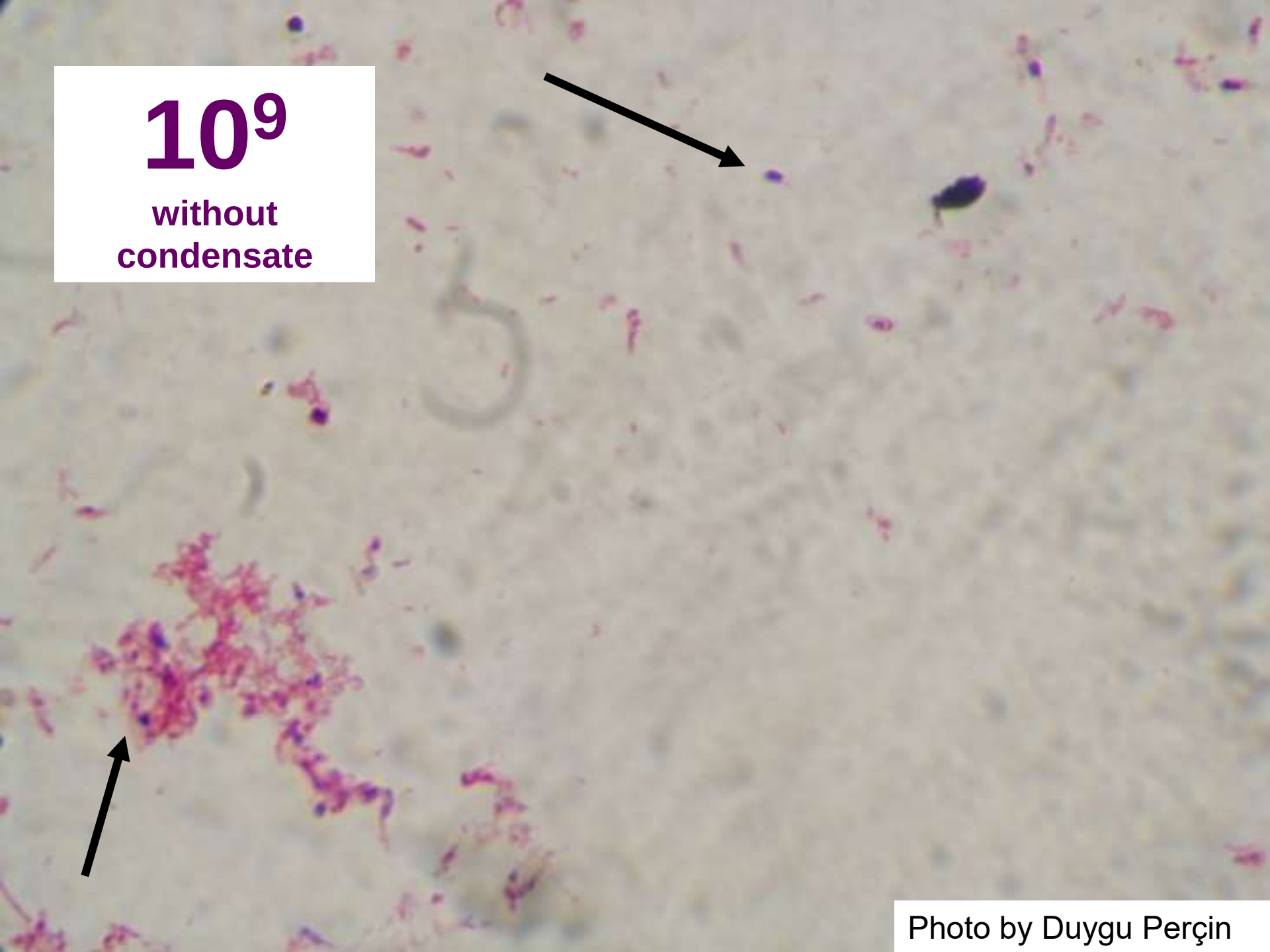
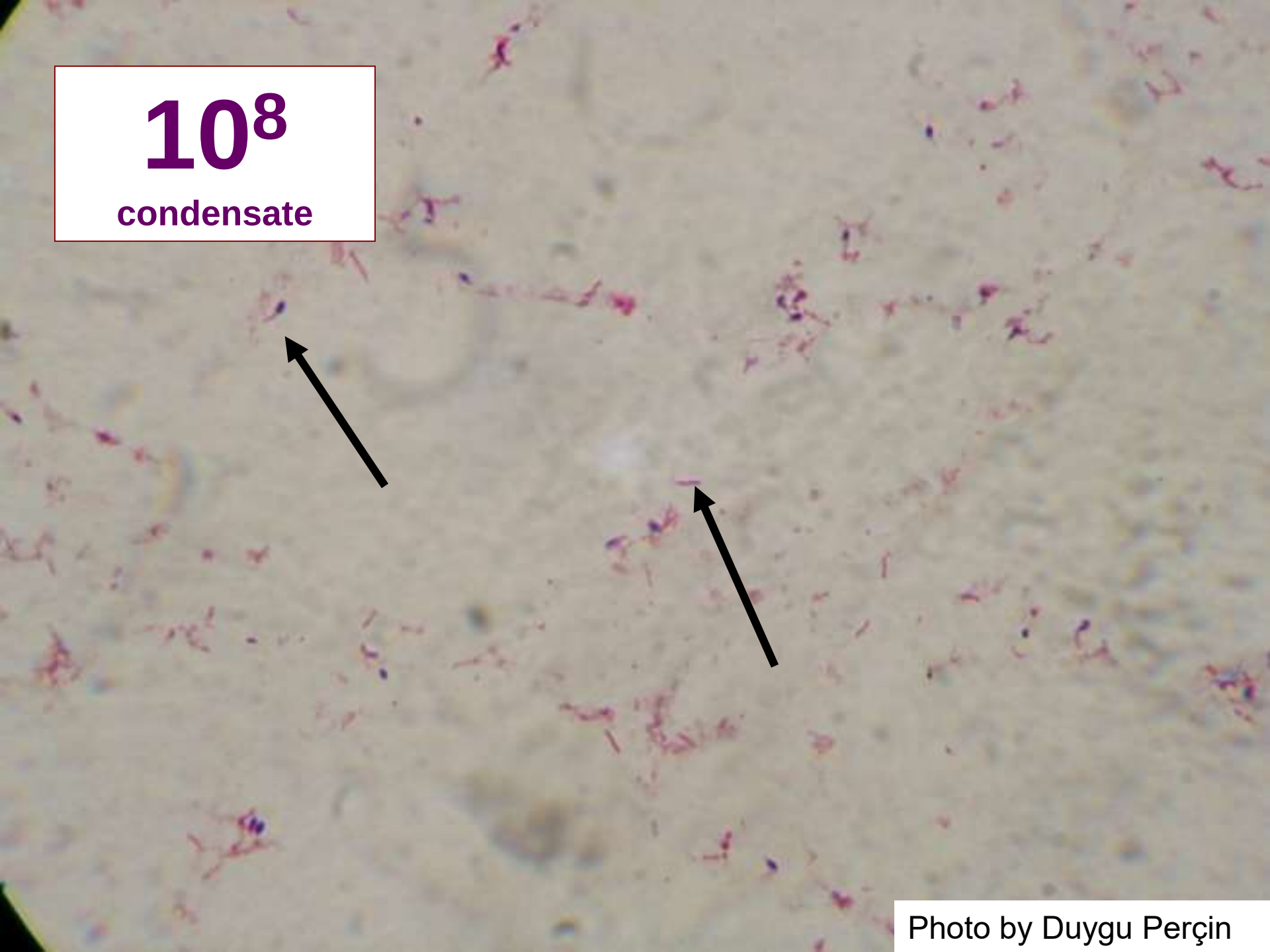


Photo by Duygu Perçin


10^9
without condensate
Photo by Duygu Perçin


10^8
condensate
Photo by Duygu Perçin

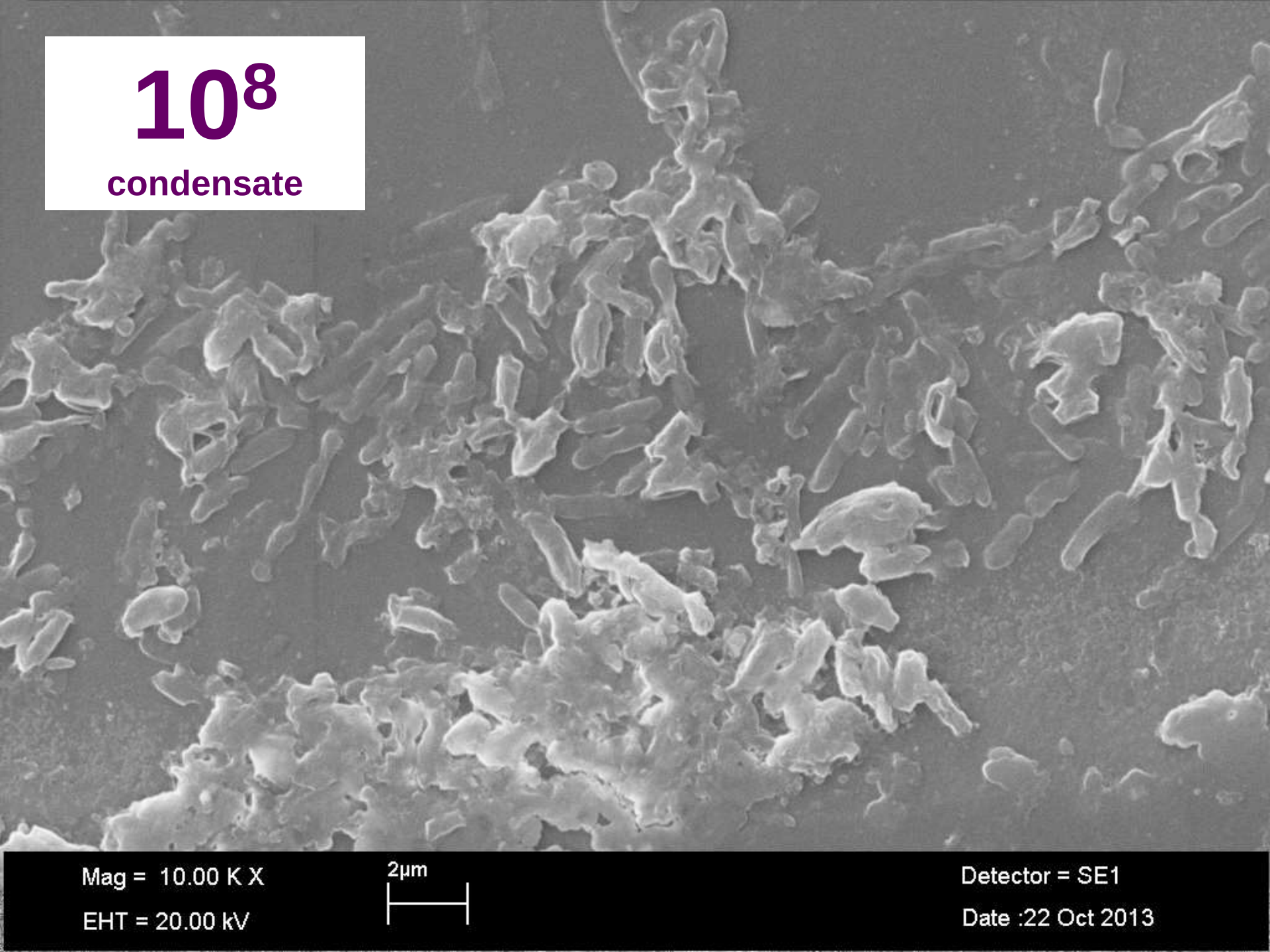

10^8
without condensate
No growth
Photo by Duygu Perçin


G.stearothermophilus
before sterilization
2 µm*
EHT = 16.82 kV
WD = 8.5 mm
Signal A = VPSE G3
Mag = 6.37 K X
Date :26 Sep 2013
Time :16:30:53
ernam


10^9
condensate
2 µm*
EHT = 20.55 kV
WD = 10.0 mm
Signal A = VPSE G3
Mag = 12.51 K X
Date :30 Sep 2013
Time :10:16:27
ernam


10^9
without condensate
2 µm*
EHT = 20.55 kV
WD = 10.0 mm
Signal A = VPSE G3
Mag = 12.51 K X
Date :30 Sep 2013
Time :10:37:41
ernam

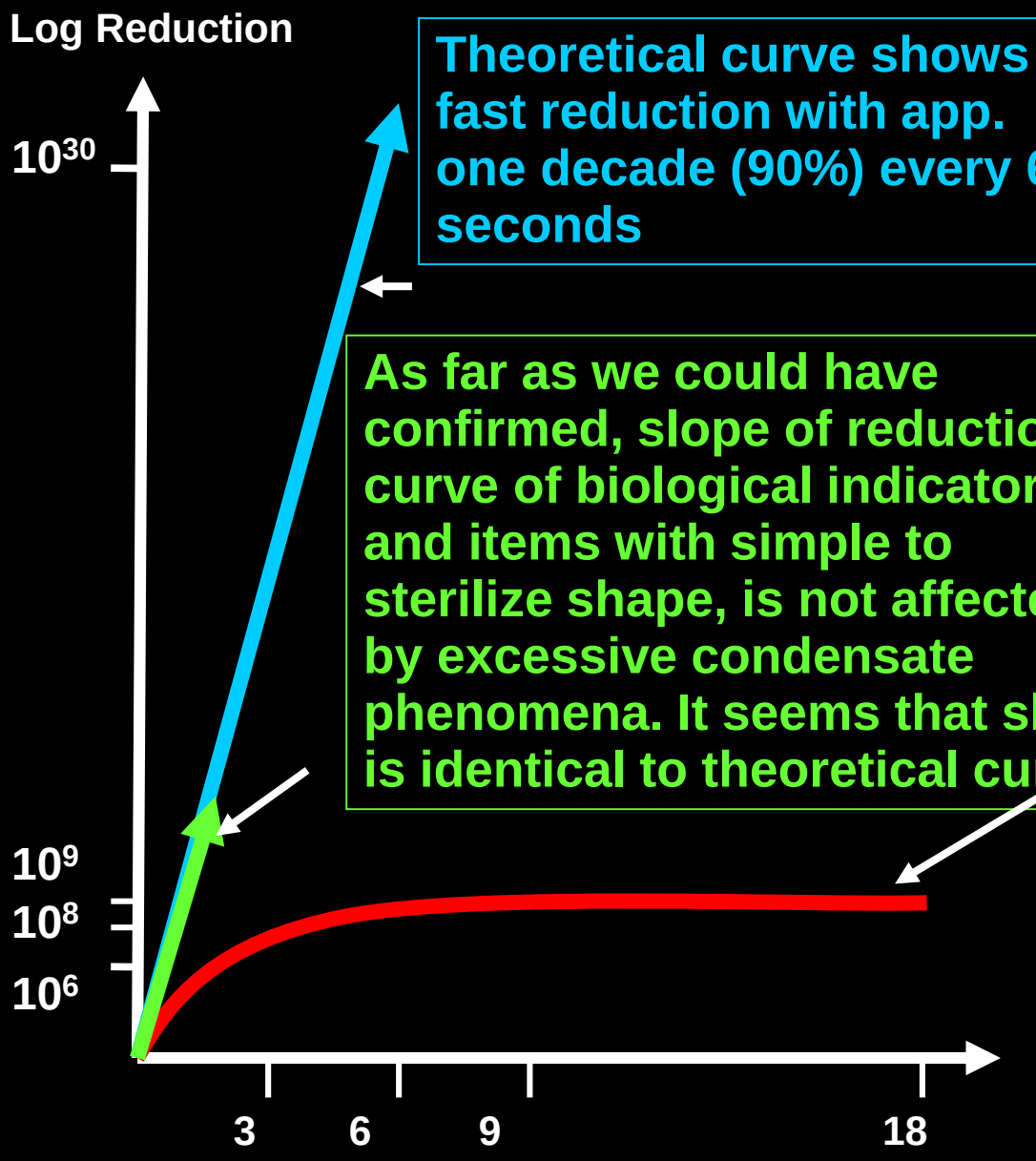

10^8
condensate
3 µm*
EHT = 17.95 kV
WD = 10.0 mm
Signal A = VPSE G3
Mag = 9.89 K X
Date :27 Sep 2013
Time :14:34:19
ernam


10^8
condensate
Mag = 10.00 K X
EHT = 20.00 kV
2µm
Detector = SE1
Date :22 Oct 2013

STEP 5: Effect of sample type and sterilization time

Sterilization time	Sample type 10^9	Cycle (134ºC)	Growth
3 min	Nuts only	Correct	Growth +
		Condensate	Growth +
3 min	Screws	Correct	Growth +
		Condensate	Growth +
4 min	Nuts only	Correct	No
		Condensate	No
4 min	Screws	Correct	No
		Condensate	Growth +

Reduction at 134 °C



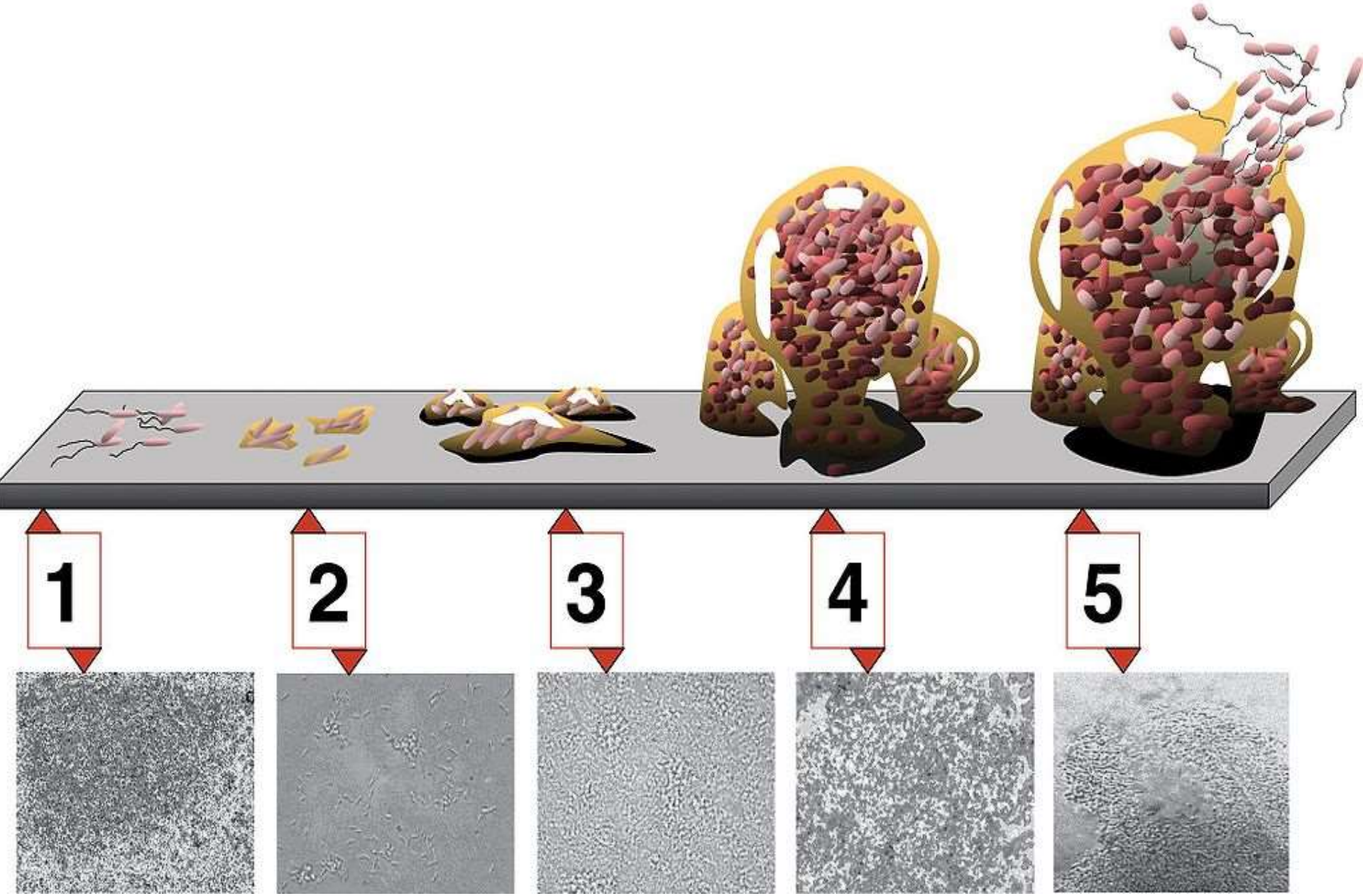
Theoretical curve shows fast reduction with app. one decade (90%) every 6 seconds

As far as we could have confirmed, slope of reduction curve of biological indicators and items with simple to sterilize shape, is not affected by excessive condensate phenomena. It seems that slope is identical to theoretical curve.

If instruments with difficult structure are immersed in condensate, it seems that we are unable to sterilize them if bioburden is higher than 10^8 CFU

Conclusions

- Inoculum has a big effect on sterilization efficacy
  - impresses the importance of cleaning
- Condensation lowers the sterilization efficacy
  - impresses the importance of proper loading of packs and sterilizer
- Instrument shape has a big impact on sterilization efficacy
  - impresses the importance of challenging structure of instruments and packaging



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Today’s sterilizers

- Time based
- Simple
- They use overkill aproach
  - Different conditions inside the load are not monitored
  - Phenomenas as excessive condensate are not recognized

Good example already in use at present

Liquid sterilizers with probe (time based)

Future solutions

- Move from time-based steam sterilizers to F_{value} based ones
  - Autoclaves integrated with a real-time F calculation function
  - Electronic indicators that are able to communicate with sterilizer with capability of calculating F_{value} real-time in the package and noticing threads for sterilization like NCG, excessive condensate, etc.

Synthesis

- Microorganisms do not follow first-order kinetics when they die especially when the bioburden is too high!
- In case of immersion in excessive condensate it is not possible to reach the preset values during sterilization!
- We should follow empirical results of detailed studies related to inactivation of microorganisms.
- We must stay away from mathematical models when sterilization is the subject, at the time being…
- Or we must teach mathematics to microorganisms or to our sterilizers!


THANK YOU!