Biological Monitoring

DAS 2004
DEZENFEKSİYON ANTİSEPSİ STERİLİZASYON DERNEĞİ
SOCIETY OF DISINFECTION ANTISEPSIS STERILIZATION

WORLD FEDERATION FOR WFHSS HOSPITAL STERILISATION SCIENCES

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Learning Objectives

The attandee will be able to

- Differantiate the biological indicators
- Describe the use and interpretation of BI
- Critique the reliability of the short incubation time of rapid readout indicators
- Describe and debate the Sterility Assurance Level concept
- Recognise the impact of excessive condensation on sterilization efficacy
- Analyse the factors effecting sterilization efficacy

Routine control of sterilization: Why?

- To detect PROBLEMS of a process IN A RELIABLE WAY and IN TIME by controlling one or more variables of a sterilization process

Problems

- A sterilizer malfunction that could account for failure to achieve the endpoint
- A change in the product and/or sterile barrier system
- A change in loading density
- A change in container/configuration
- Delayed or inappropriate sterilizer calibration and/or routine maintenance
- Wrong sterilizer process
- Inappropriate handling of chemical indicator
- Changes in the utilities supplied to the sterilizer that could materially affect cycle execution (pressure, flow rate, non-condensable gases in the steam supply, etc.)

ISO 17665-Routine control

10 Routine monitoring and control

10.1 Routine monitoring and control shall be performed on each operating cycle.

10.2 Evidence of successful maintenance and requalification (if applicable) shall be verified.

10.3 The operational status of the equipment (if applicable) shall be verified by evidence from periodic tests of factors such as (but not limited to) the following:

a) air leakage into the sterilizer chamber;

b) quality of saturated steam or heat transfer media admitted to the sterilizer chamber (which may include checks for non-condensable gas, conductivity of feed water, contaminant(s), moisture content);

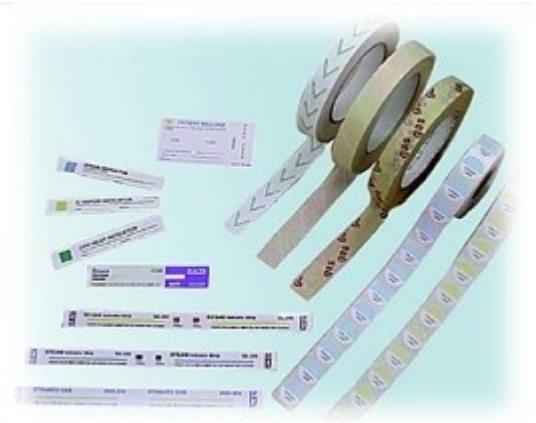
c) automatic control (e.g., a test to verify that the operating cycle continues to function correctly);

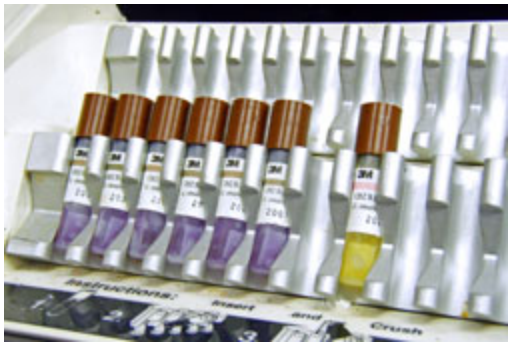
d) steam penetration;

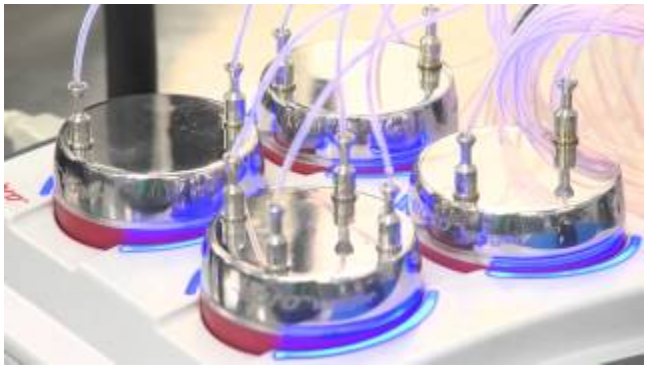
e) sterilization process (e.g., a test to verify that the sterilization process remains reproducible).

10.4 Delivery of the sterilization process shall be verified from the results of chemical indicators (see 8.8) or biological indicator systems (see 8.5 or 8.6), if used, and by confirming that within specified tolerances, recorded data from routine monitoring match data from validation.

Routine monitoring and control







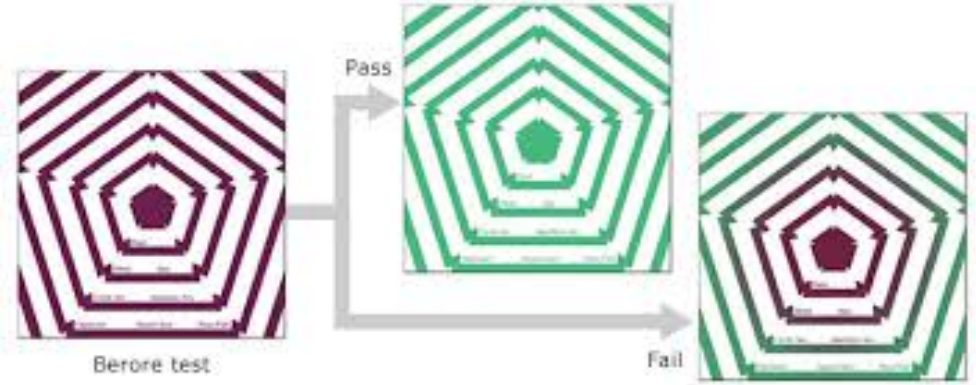


Table B.1 — Example of a schedule of tests for validation and periodic testing

Test and Monitoring	Installation qualification	Operational qualification	Performance qualification	Routine test of the sterilizer	Periodic test of the sterilizer	Comments
Type tests and safety checks: pressure vessel electrical plumbing environmental	×					
Biological challenge test pack		×			×	× in otherwise empty chamber
Air removal test		×		×	×	× in otherwise empty chamber
Air leak test						Sterilizer manufacturer test
Physical monitors		×	×	×	×	
Monitoring, recording, control		×	×	×	×	
Independent sensor/recorder						Optional
Biological indicators		×	×	×	×	
Chemical indicators		×	×	×	×	

× – tests that should be considered.

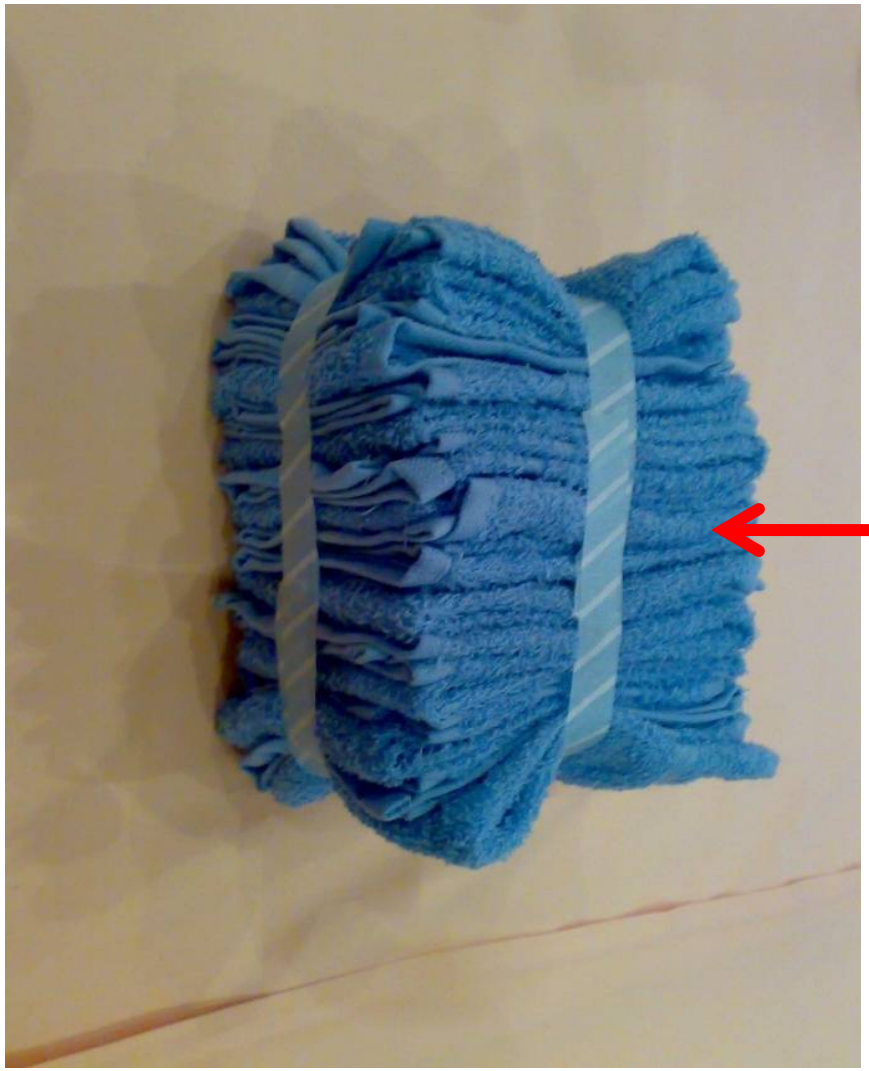
ISO 17665

Biological qualification of a sterilization process

- A sterility assurance level (SAL) of at least 10^{-6} should be demonstrated.
- When sterilizing heat-stable materials an overkill approach is normally utilized
- Acceptable results for three consecutive cycles of either half cycle or full cycle approach are required for each type of load
- The biological indicators used in testing should contain heat resistant spores, such as *Geobacillus stearothermophilus* spores and should comply with applicable standards

ISO 17665

Biological challenge



ISO 17665

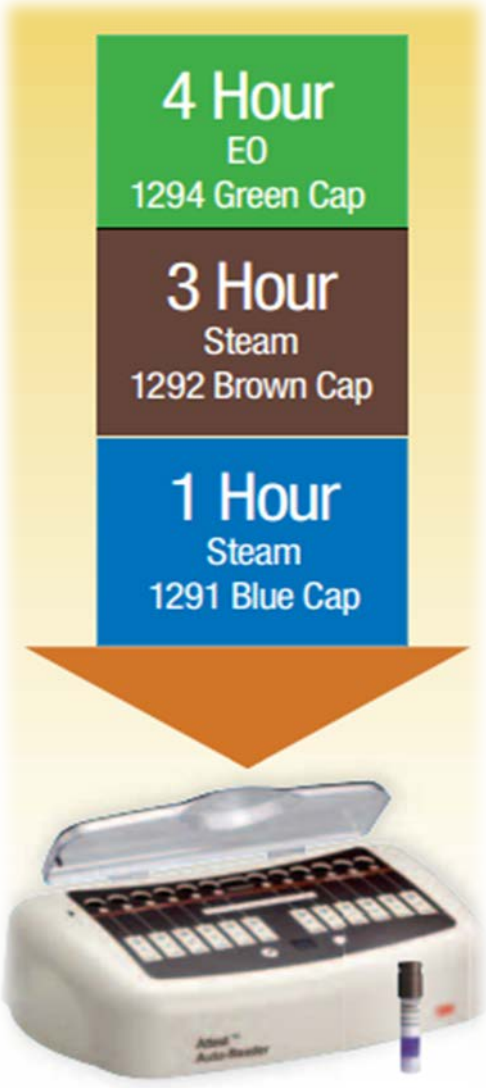
When to use BI

- In the first cycle of sterilizer after installation
- After every big repair of sterilizer
- Routinely once in a week
- In each cycle including implant

Biological monitoring of LTS methods

- According to ISO 11135 chemical indicators can not be used as the sole means of establishing sterilization process using ETO
- The only way to prove efficiency of gas concentration and exposure time on biological death is biological monitoring
- Every cycle of LTS methods must be monitored with BI and load must be released according to the BI result
- Parametric release with physical and chemical parameters is limited

New biological indicators



★*New FDA approval*

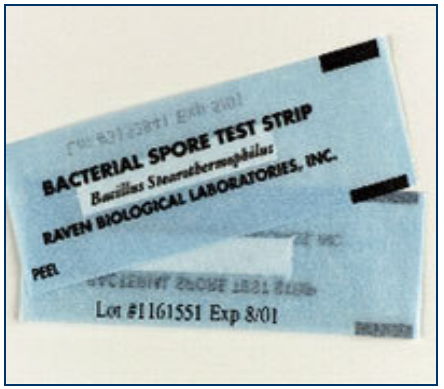
- 3M Attest Rapid Readout BI 1295 & Autoreader 490H
- Routine monitoring of vaporized hydrogen peroxide sterilization processes in STERRAD® NX and 100NX systems

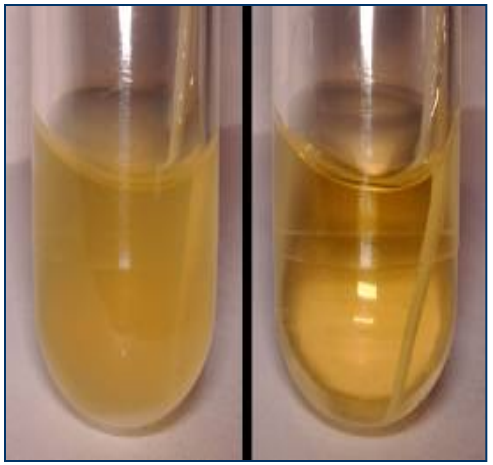
If a BI is positive

- Check the indicator with negative and positive controls
- Check the cycle parameters
- Check the load
- Check the packaging material (esp. For ETO and H_2O_2)
- Check the sterilizer
- Inform IC team for the items if they are used on the patients

First generation indicators

- Spore suspensions inoculated on paper strips (log5-6)
- These strips are placed within a sterile liquid medium under aseptic conditions following the sterilization cycle
- Microbial growth = any turbidity in the medium
- Negative result requires an incubation period of 24-168 hours





Second generation indicators

- Contain the medium required for the growth of the spores together with the paper strips containing spores (log5-6)
- No risk of contamination during the inoculation of the medium
- Growth is seen within 24-48 hours by way of the visible change of color of the medium due to the change of pH as a result of growth

Third generation indicators

- Rapid readout indicators
- Log 5-6
- Capable of giving the results within a short period of incubation like 3-10 hours???
- Give CSSDs the opportunity of seeing the results before the sterilized items are used on patients ???

Rapid readout indicators that are widely used

- Attest 1292 Rapid Readout Biologic Indicator
  - (3M, USA)
  - based on showing the alfa-glucosidase enzyme produced by the active spore with fluorescent radiation
  - within 3 hours

- Bright-Check Rapid Readout Indicators
  - (Etigam, The Netherlands)
  - capable of showing the change of color related to pH change
  - within 10 hours

Super Rapid Readout Indicators

Question 1:

Is there a relationship between

the number of surviving spores on a biological indicator

and

the overall grow-out time or detection time of indicator?

When sterilization fails

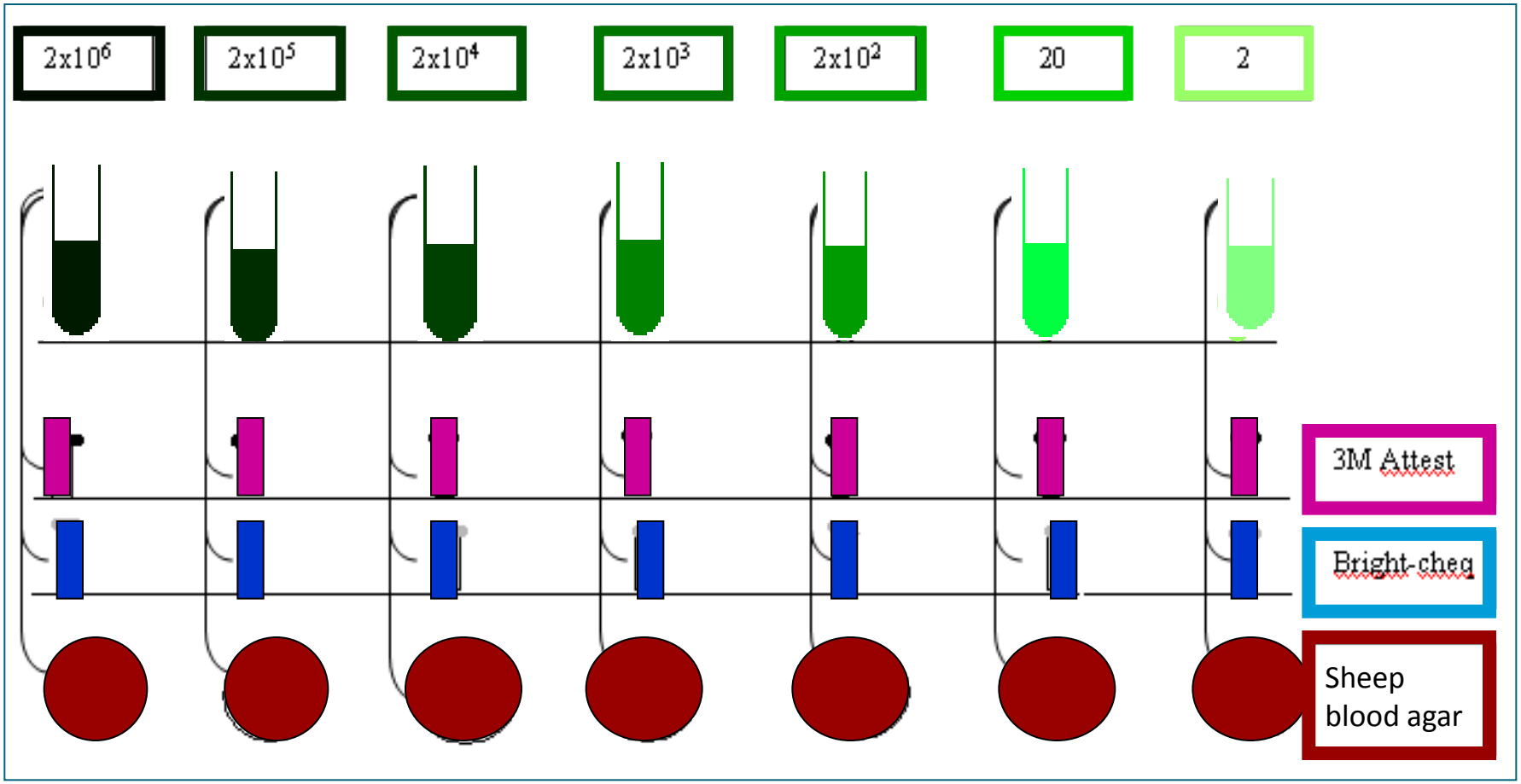
- Due to the adverse effects of sterilization on the spore structure, germination time can be delayed
- Surviving but damaged spores can cause prolonged grow-out times
- Rapid readout biological indicators should also be able to detect these cases to ensure the efficacy of sterilization process

Hurst A and Gould GW. *The Bacterial Spore 2*. 1983

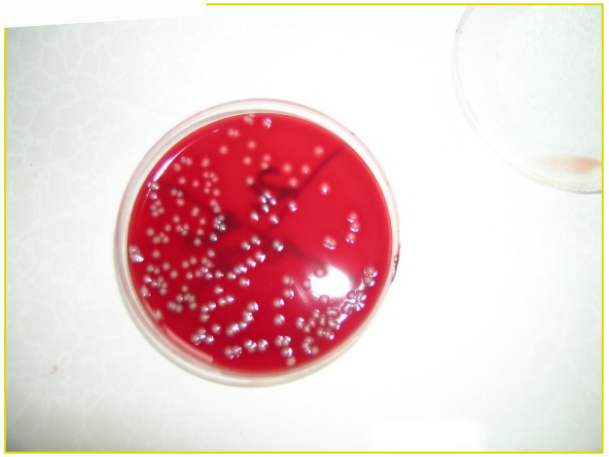
Aim of the study

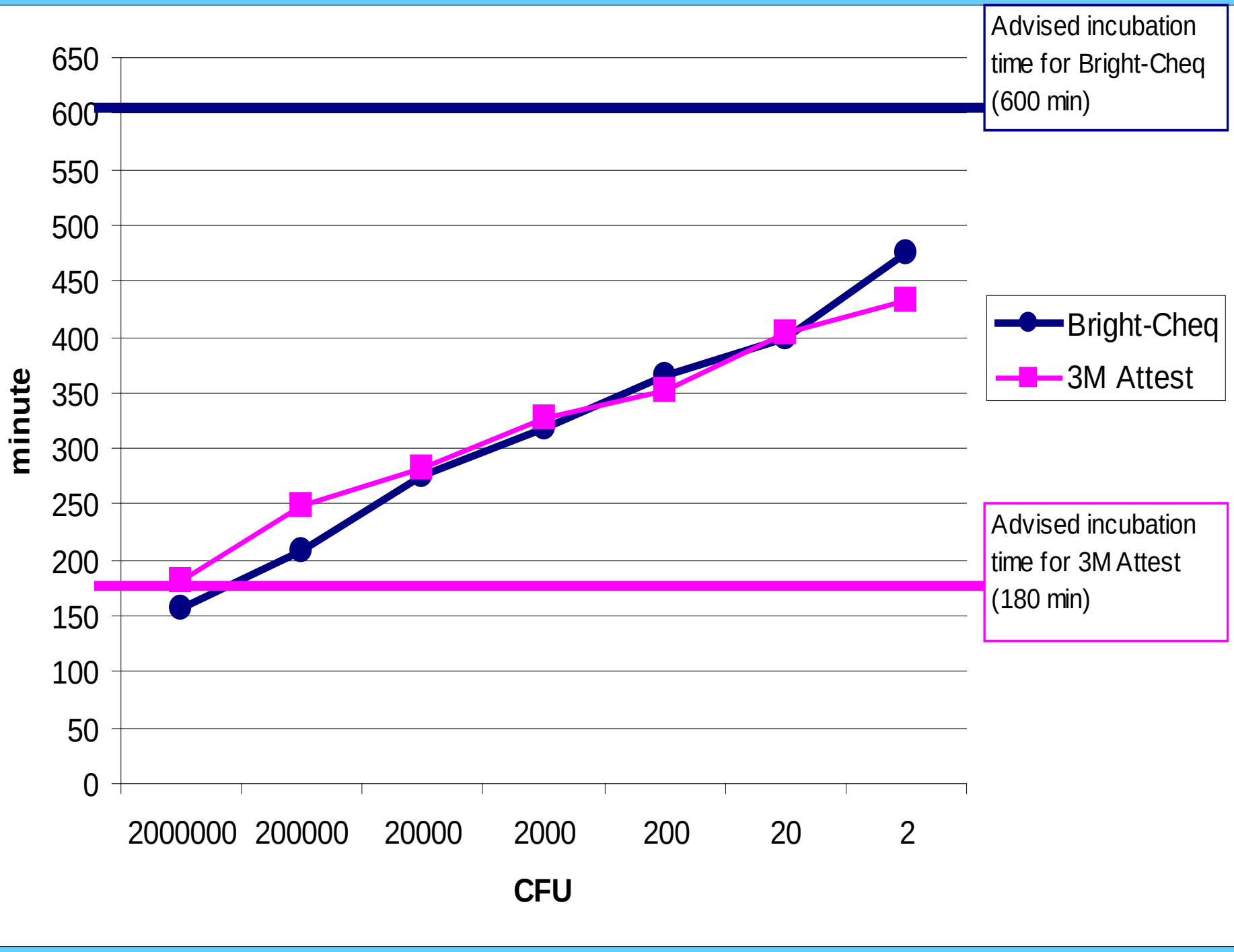
- To evaluate the reliability of the short incubation time of these rapid readout indicators
- by using a simulative model including different numbers of spores

Inoculation scheme



Incubation




Advised incubation time for Bright-Cheq (600 min)
Bright-Cheq
3M Attest
Advised incubation time for 3M Attest (180 min)
minute
650
600
550
500
450
400
350
300
250
200
150
100
50
0
2000000
200000
20000
2000
200
20
2
CFU

Conclusions from the study

- The less inoculum is in the indicator the longer detection time is achieved for rapid readout indicators
- Minimal incubation time to report negative result for both rapid readout indicators cannot be shorter than **9 hours**

spore germination time (2 h)

+

min incubation time (7 h)

ISO 14161

- BIs are used to test the effectiveness of a given sterilization process and the equipment used, by evaluating microbial lethality according to the concept of sterility assurance level
- BIs do not prove if the load is sterile!

ISO 17665

- A BI is a microbiological challenge of known resistance that is used to confirm sterilization process lethality at locations on or in product where it is placed.
- The physical parameters measured during the sterilization process must be used to verify that the defined sterilization process has been carried out

SAL concept

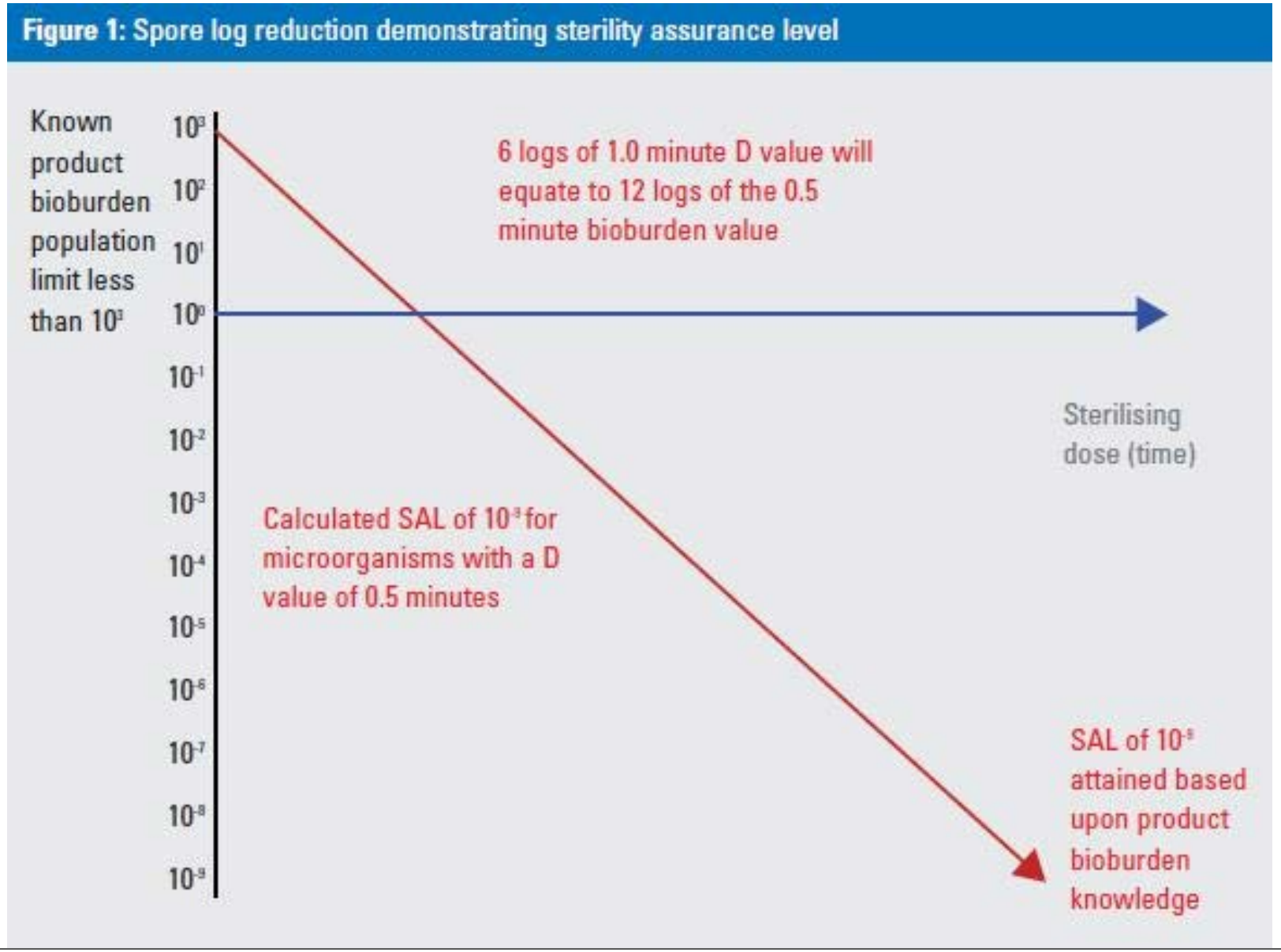
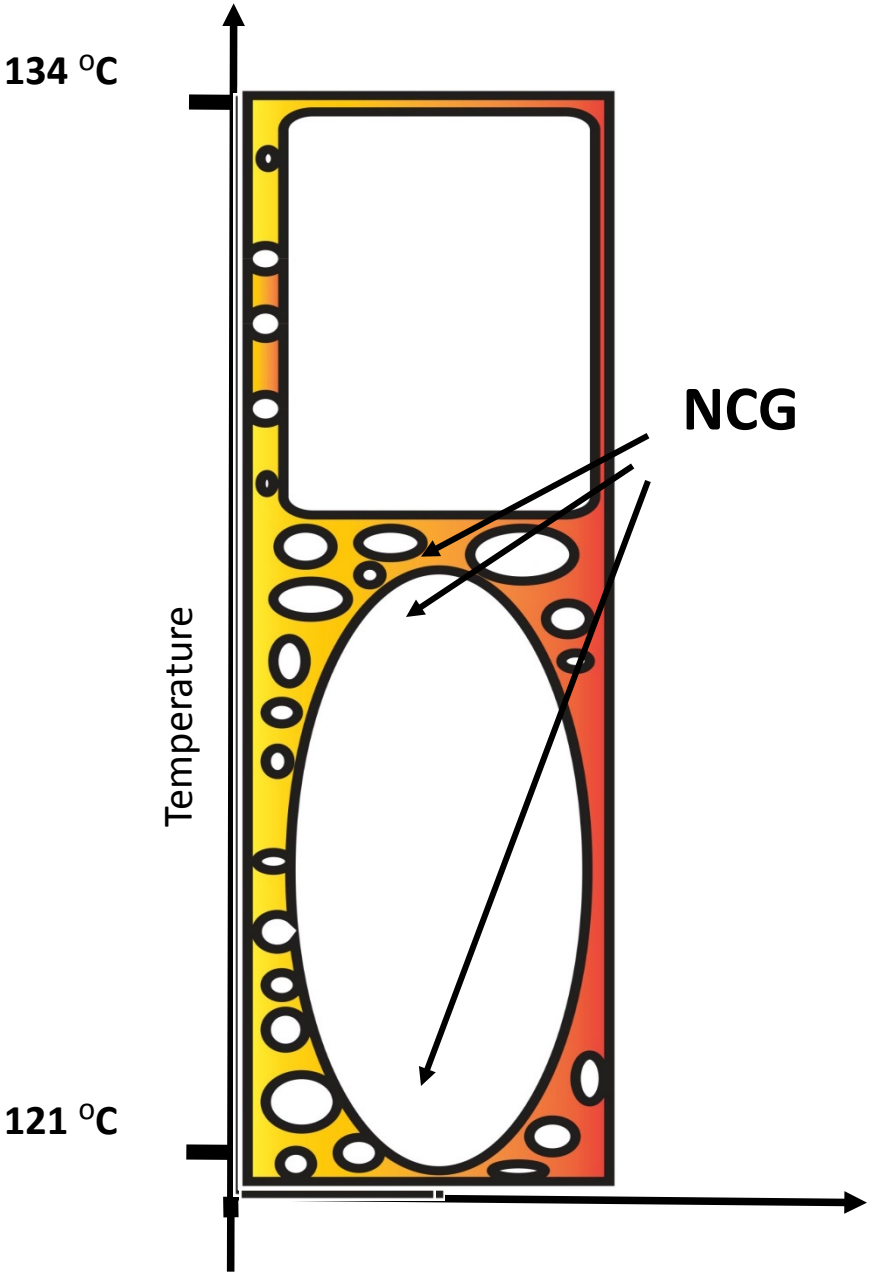


Figure 1: Spore log reduction demonstrating sterility assurance level
Known product bioburden population limit less than 10^3
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
6 logs of 1.0 minute D value will equate to 12 logs of the 0.5 minute bioburden value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

Question 2:



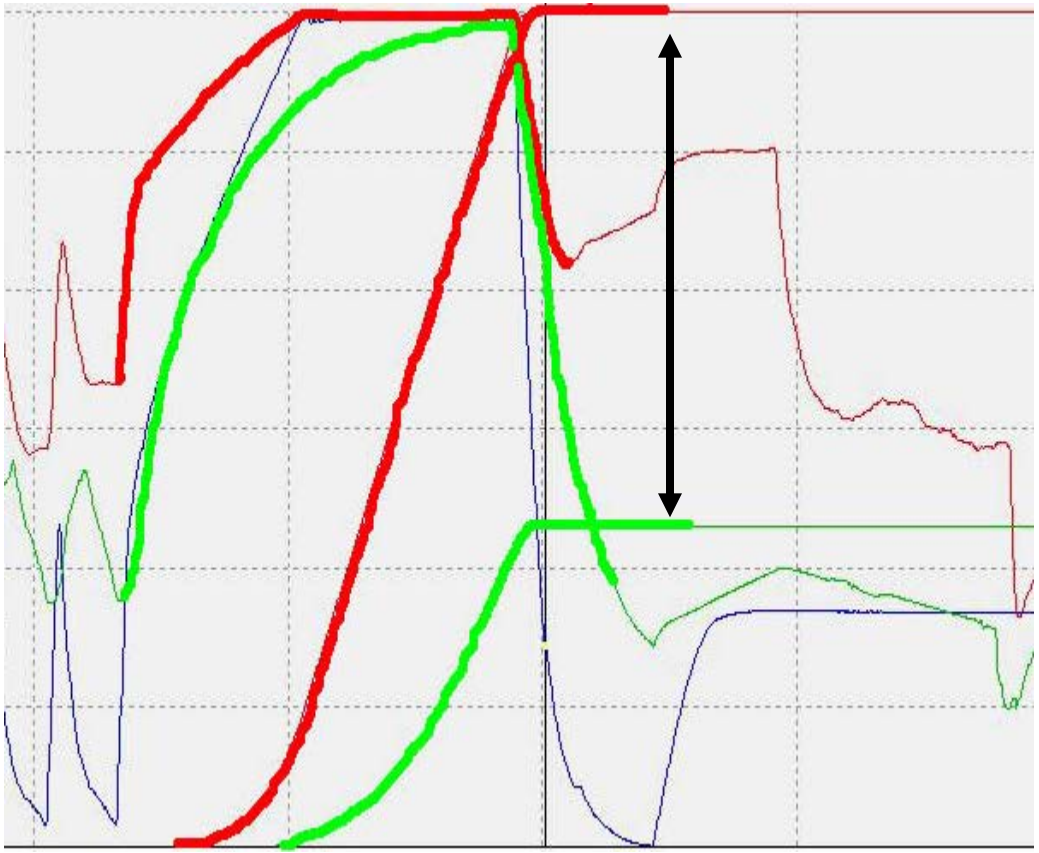
If we prolong sterilization cycles to be sure to achieve SAL 10^{-6}

Do we increase our mistakes with it ???

Excessive condensate

- The heavier our sterilization packs are, the more condensate we are generating at heating up
- If this condensate is trapped into sterilization pack it does not gain temperature as fast as surfaces that are not in the condensate

Effect of excessive condensate on sterilization efficacy



Difference in F value

Condensate (green)

Without condensate

(red)

Up to:

-60%

…at short cycles

Materials and methods

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

Spore production

(Writz-Conklin staining)

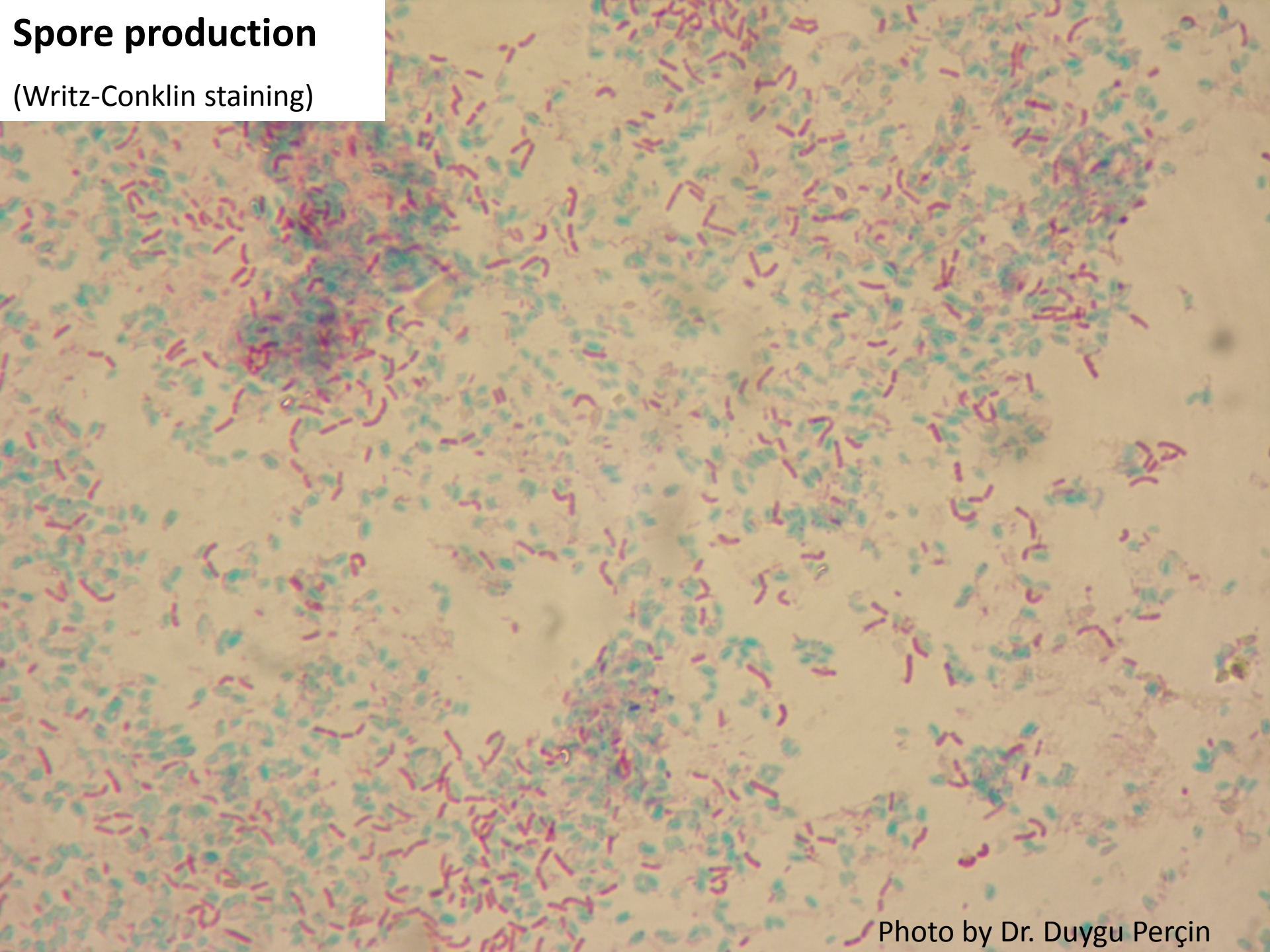
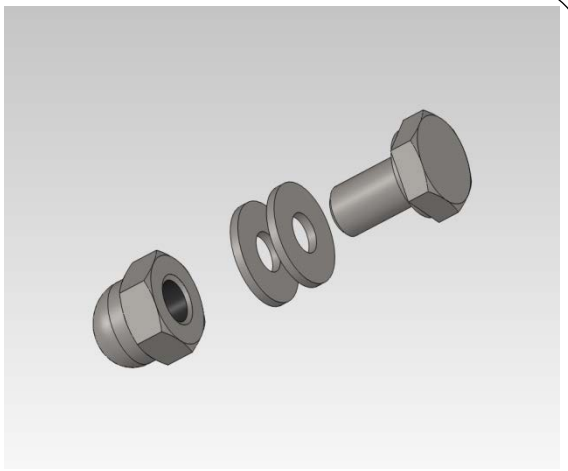


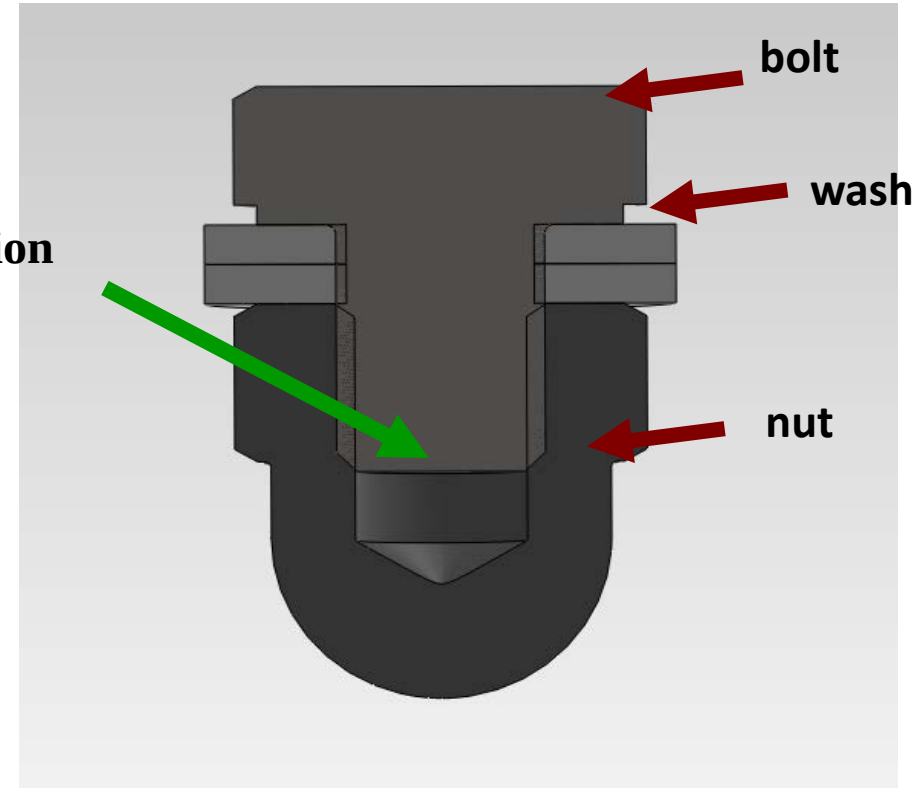
Photo by Dr. Duygu Perçin

Bolted nuts

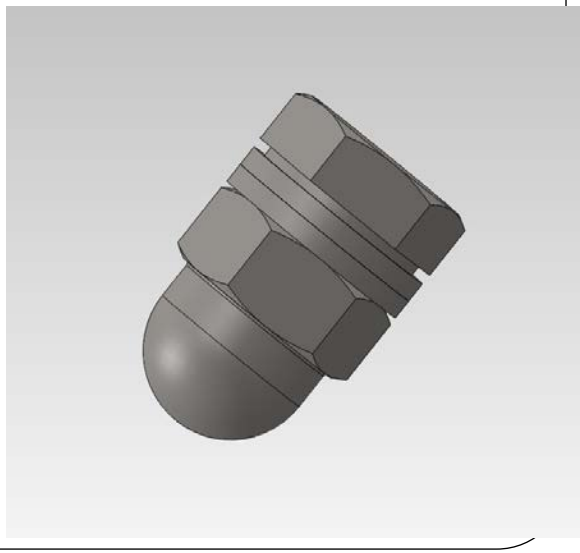
Photo by Peter Kozin

Bolted nuts





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

Transfer into broth and incubation

Turbidity in broths in 72 hours

Photo by Duygu Perçin

Gram staining of turbid broth

Photo by Duygu Perçin

STEP 1: Results of bolted nuts 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	+

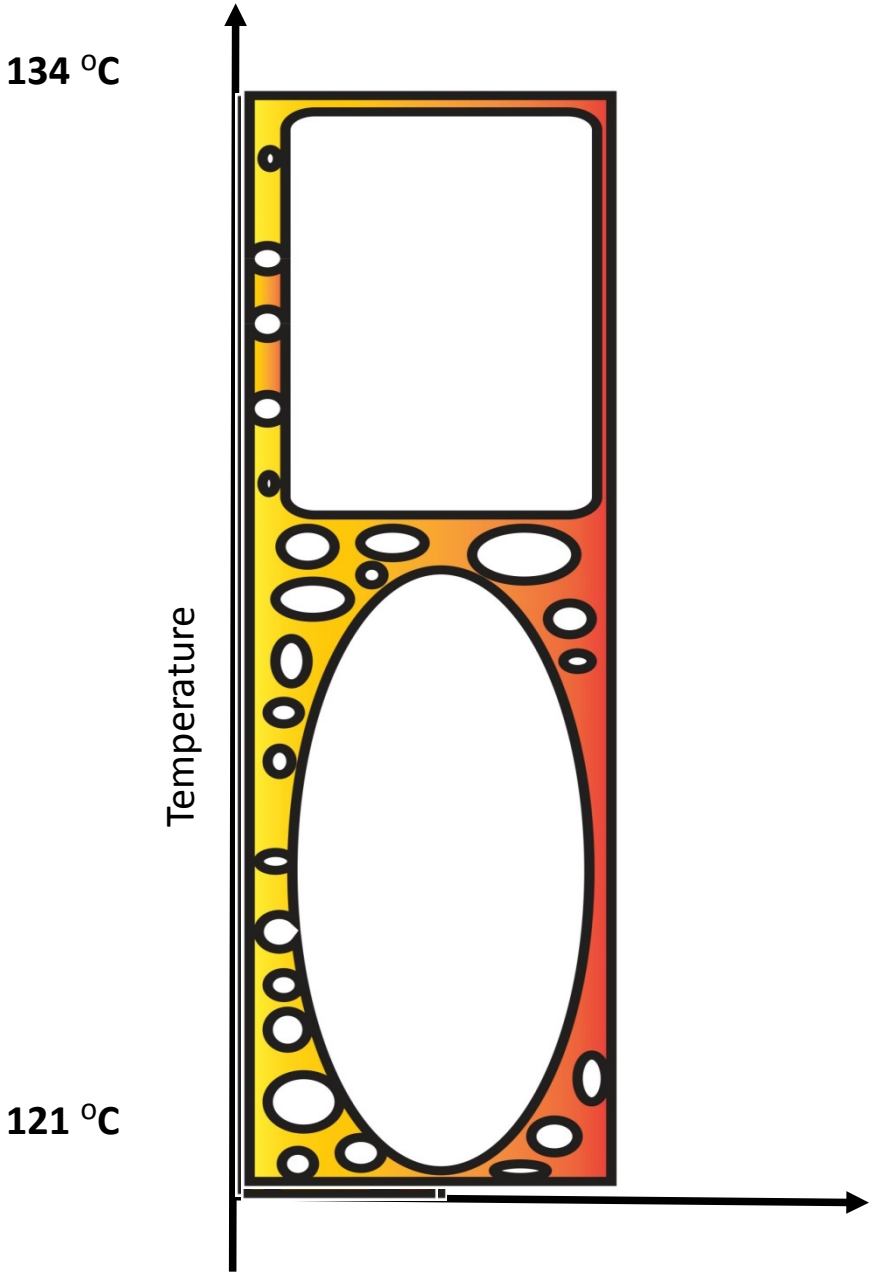
STEP 2: Results from bolted nuts with less load and metal plates ($2cm^2$)

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 bolted nuts 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

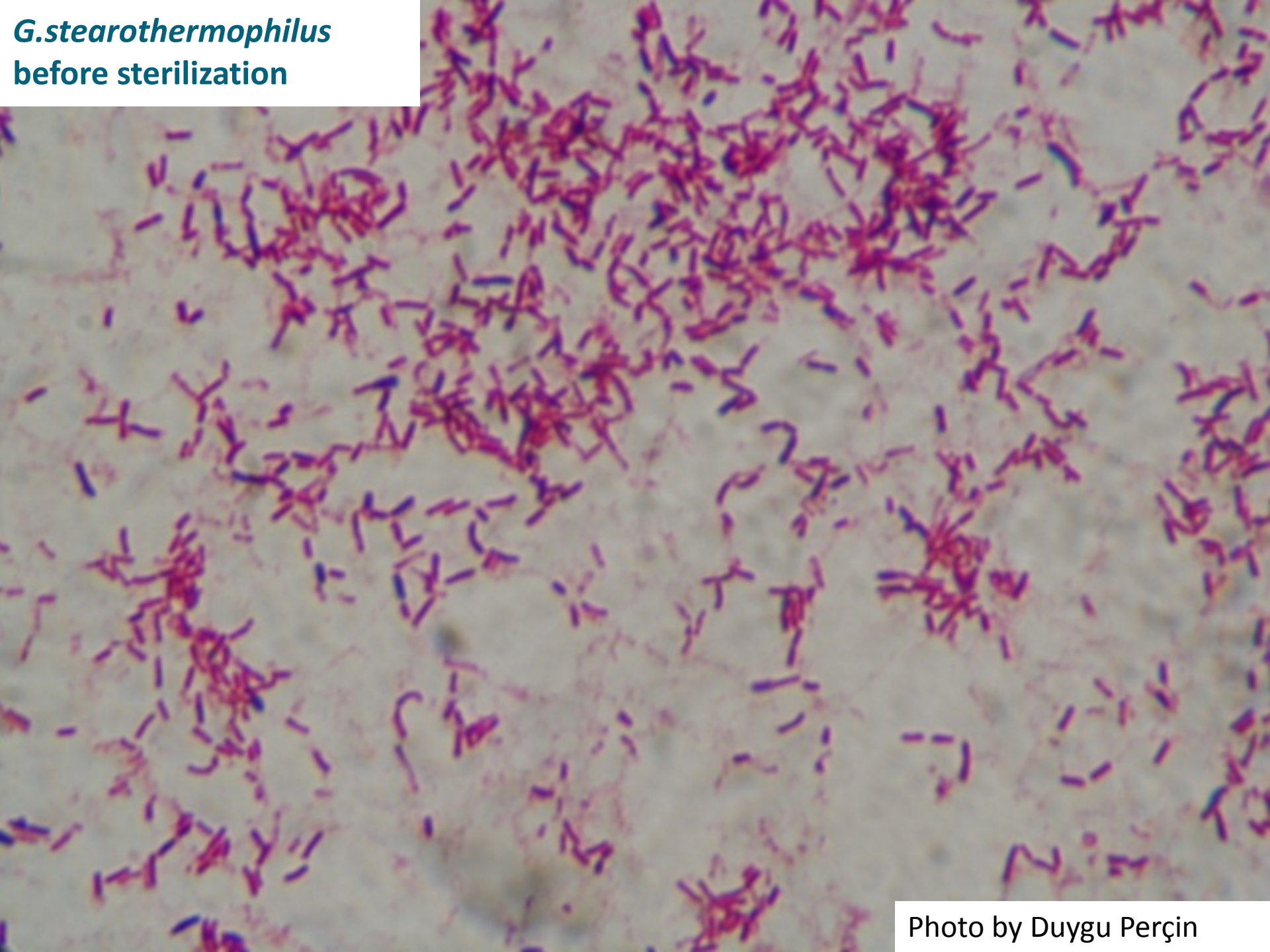
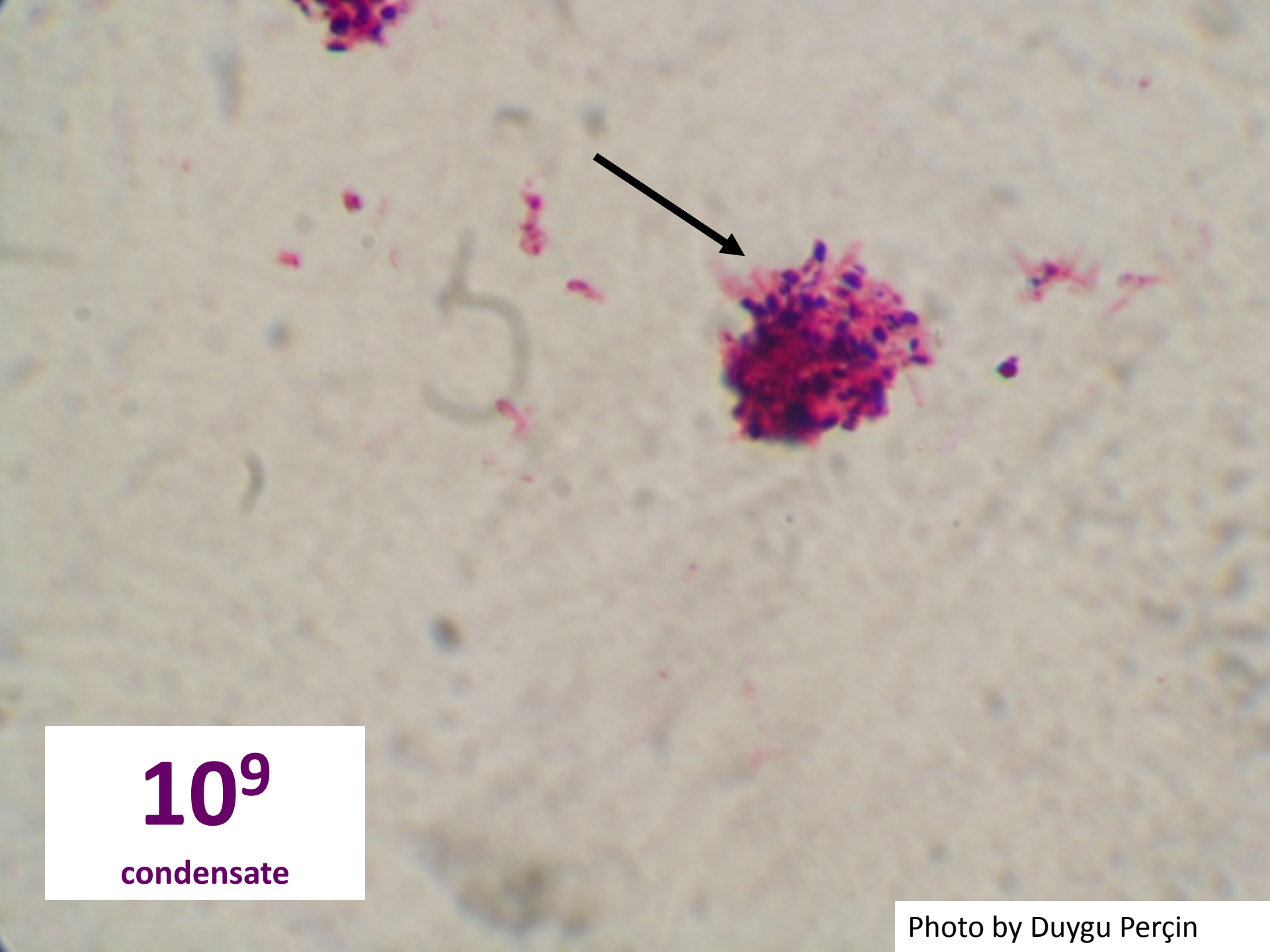


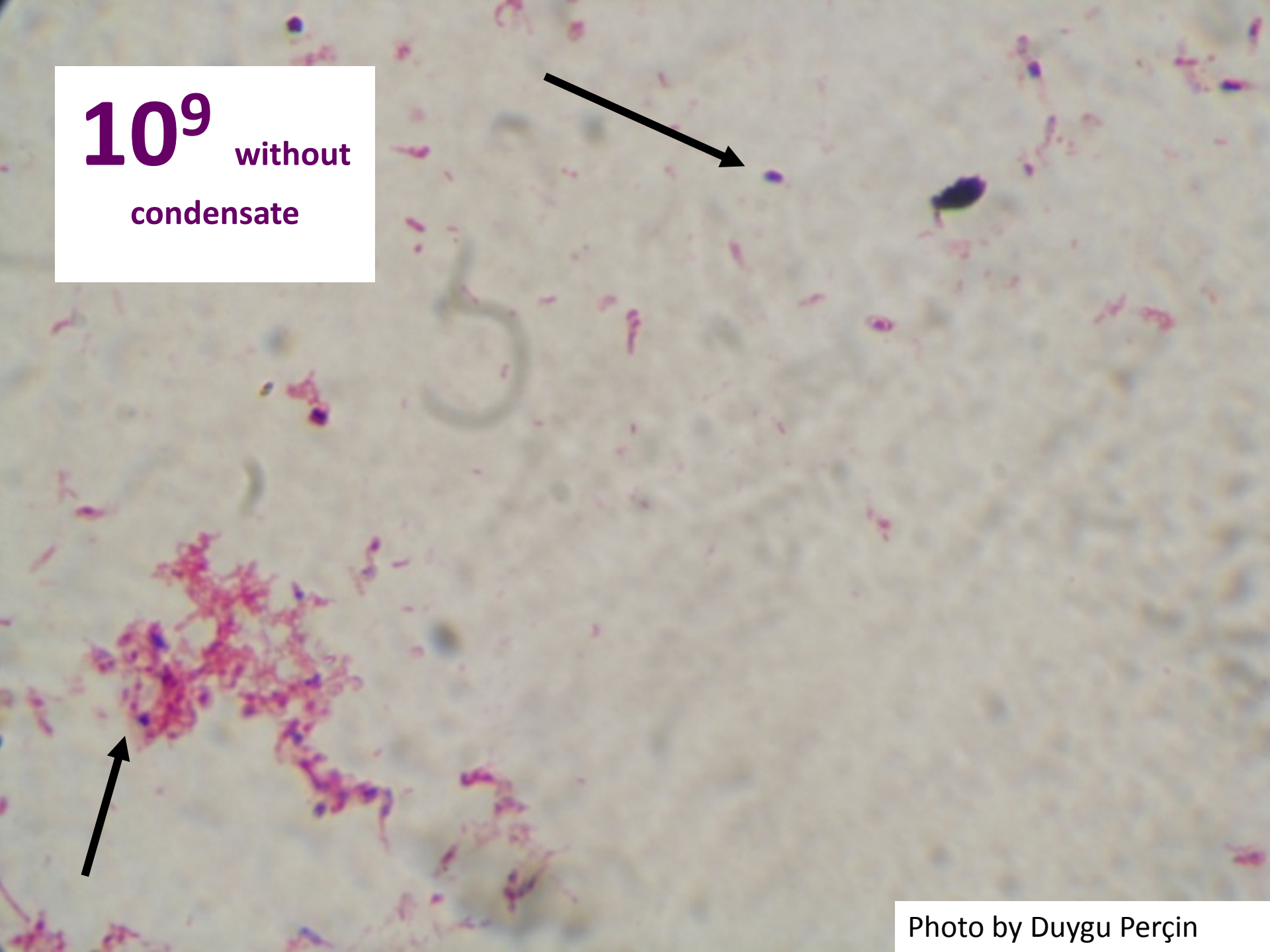
Photo by Duygu Perçin

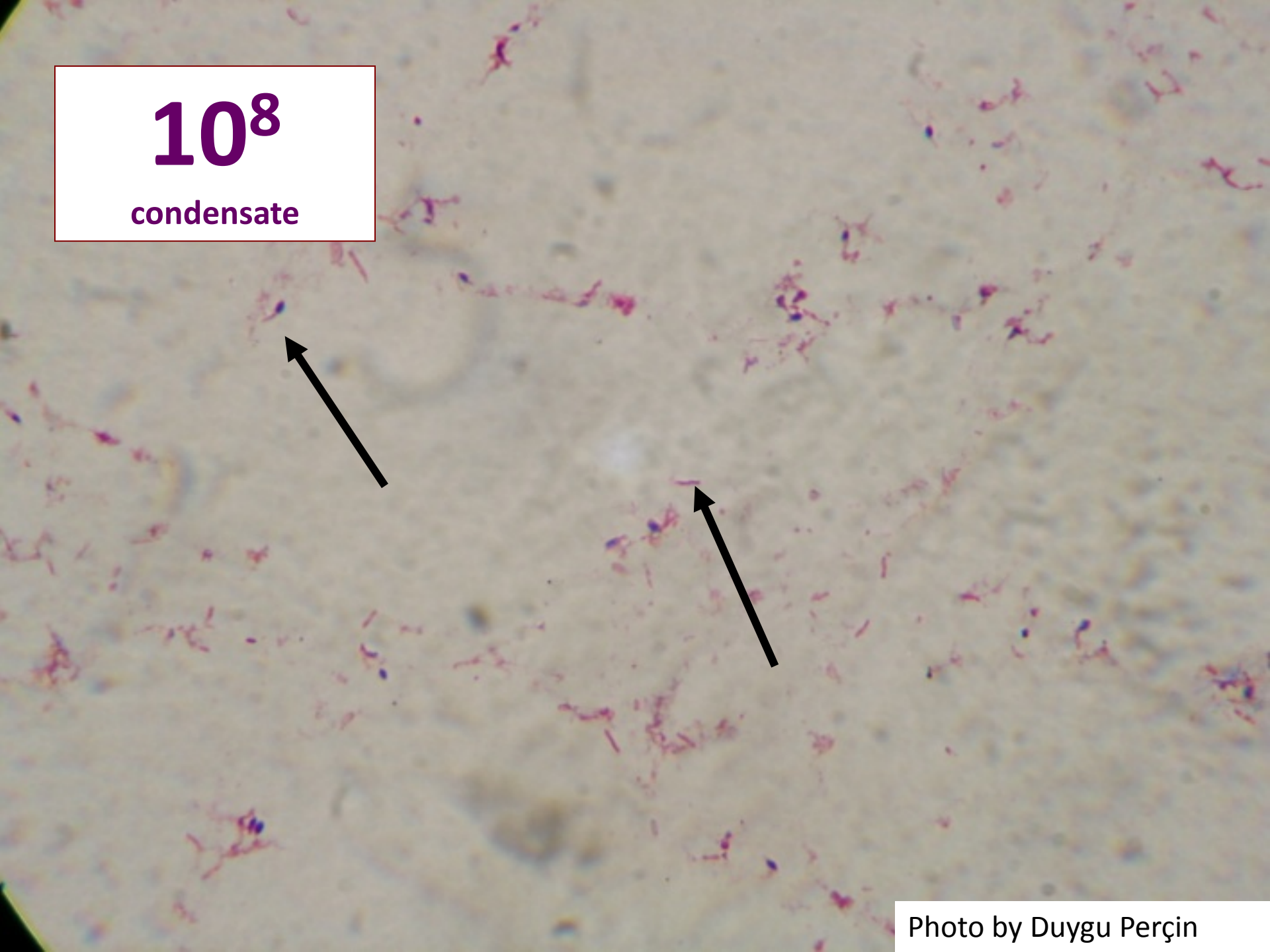


10^9

condensate

Photo by Duygu Perçin


10^9 without condensate
Photo by Duygu Perçin


10^8
condensate
Photo by Duygu Perçin

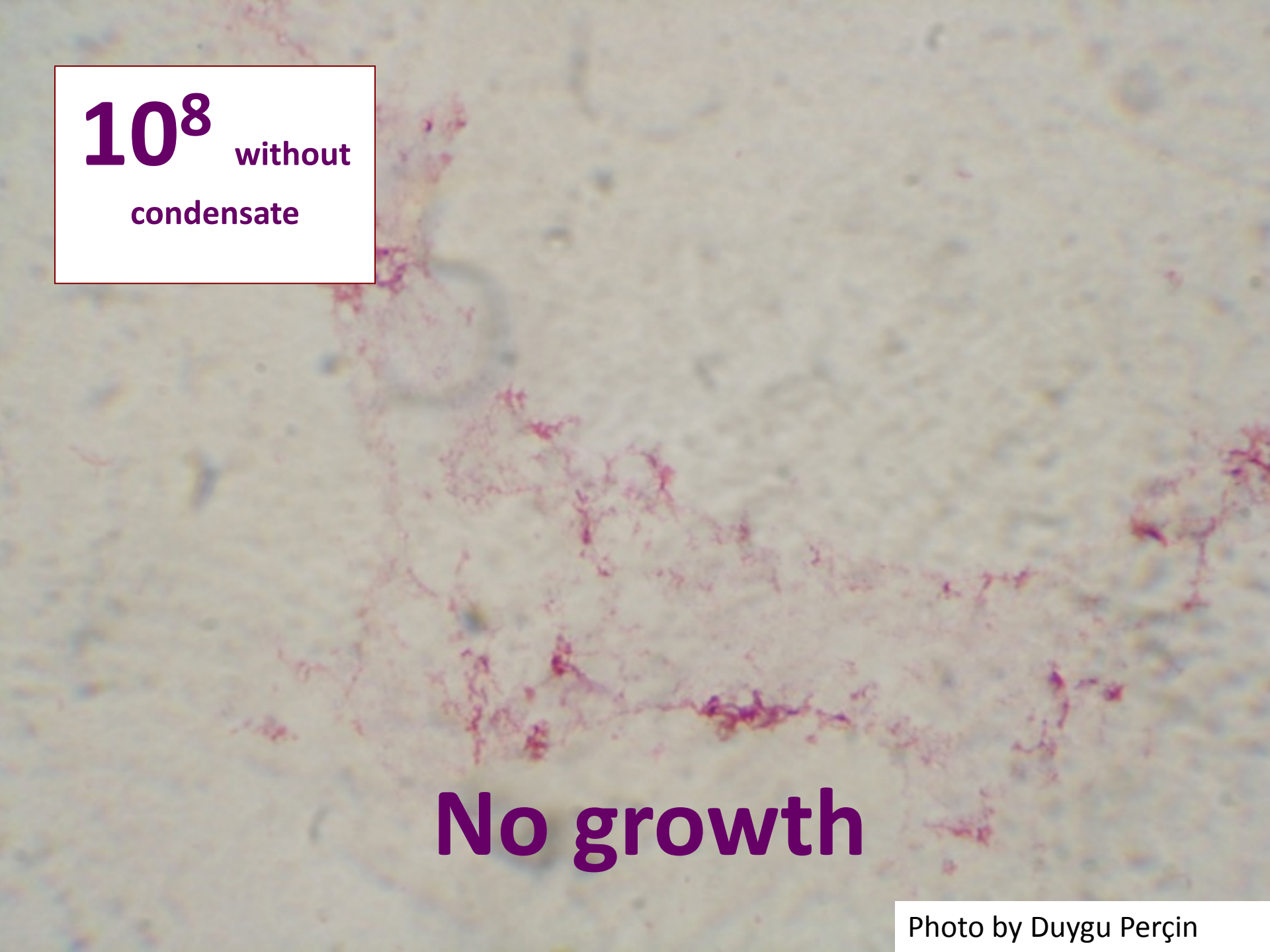
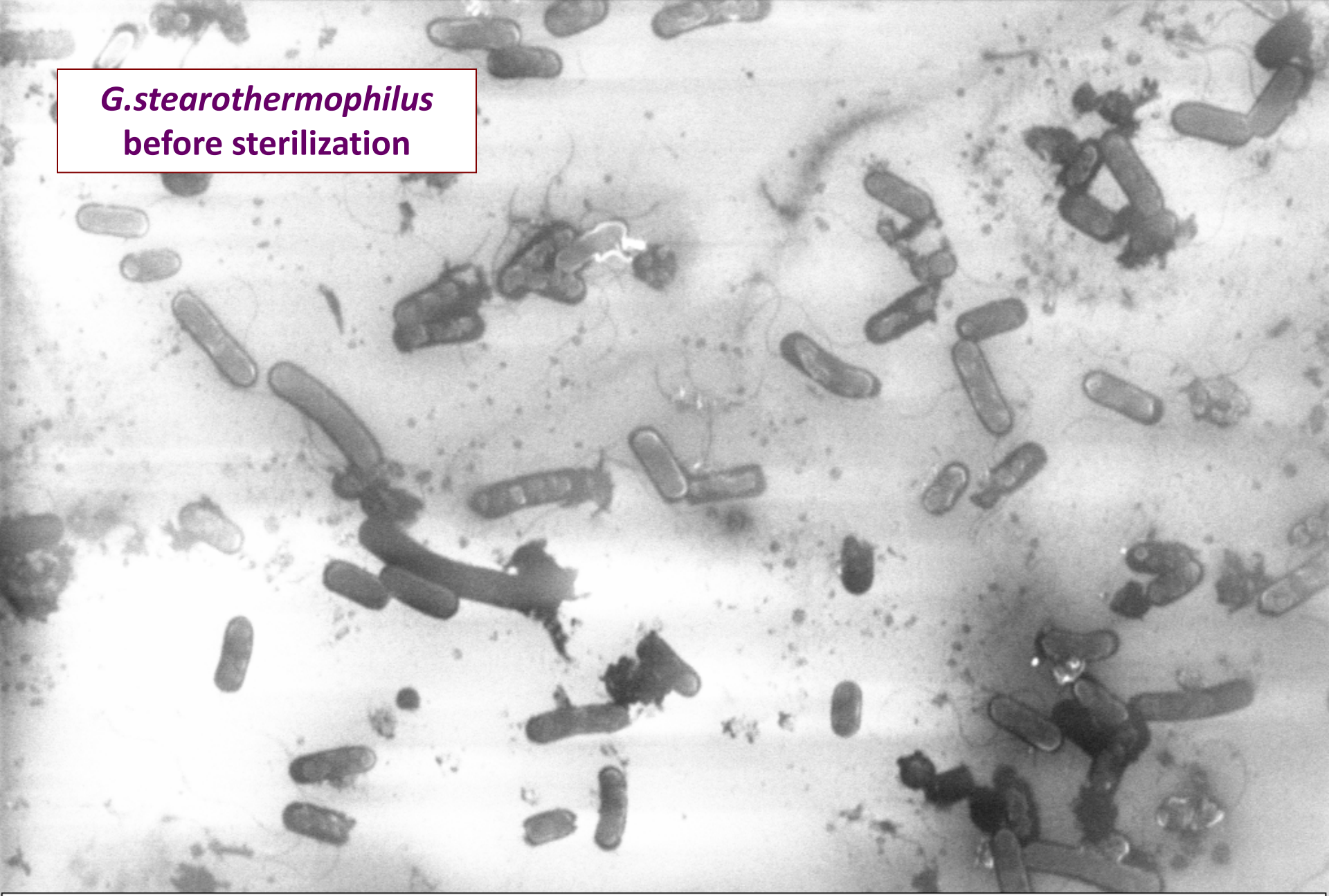
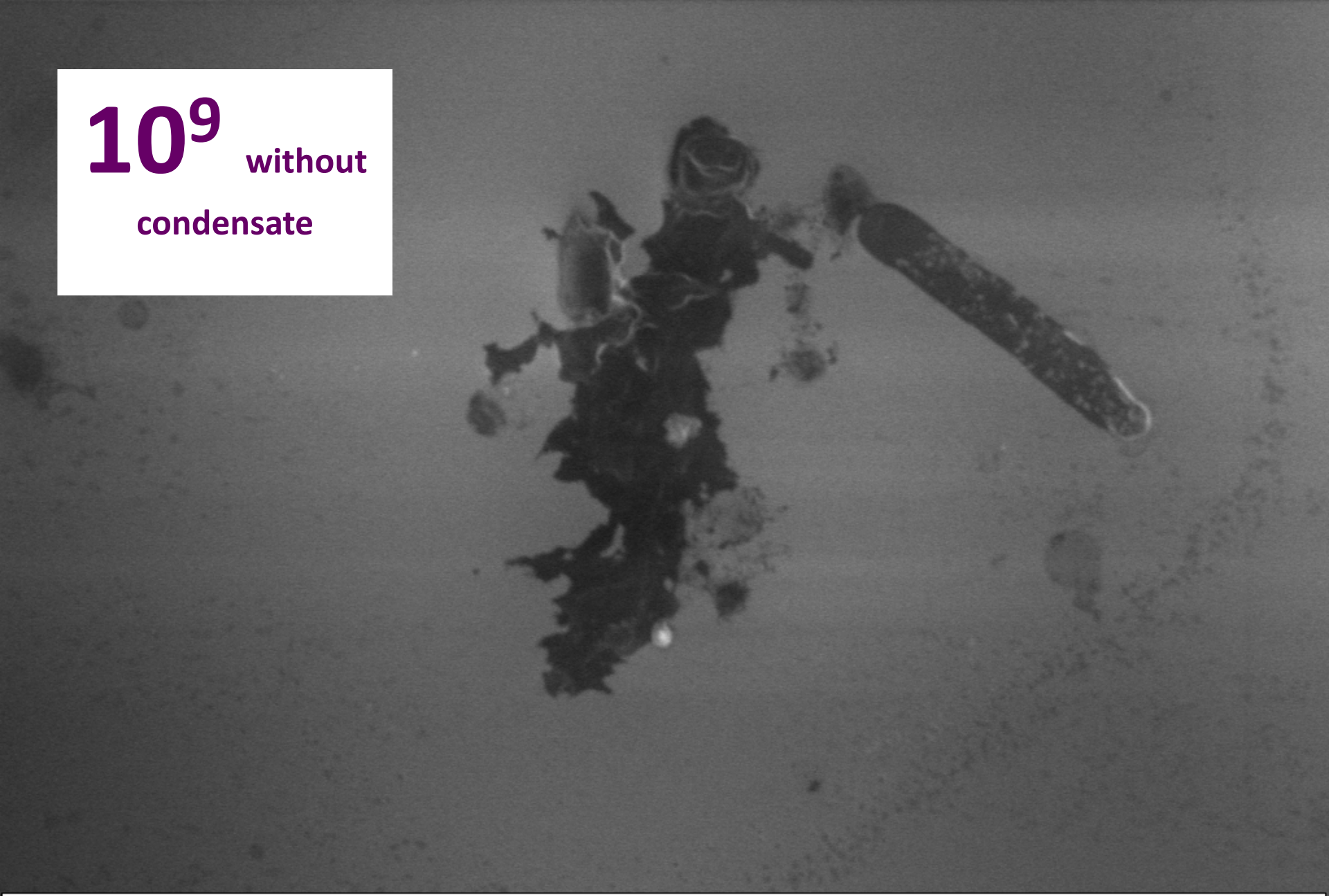


Photo by Duygu Perçin


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
Nanoteknoloji Araştırma Merkezi
Nanotechnology Research Center


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
Nanoteknoloji Araştırma Merkezi
Nanotechnology Research Center


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
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Nanoteknoloji Araştırma Merkezi
Nanotechnology Research Center


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
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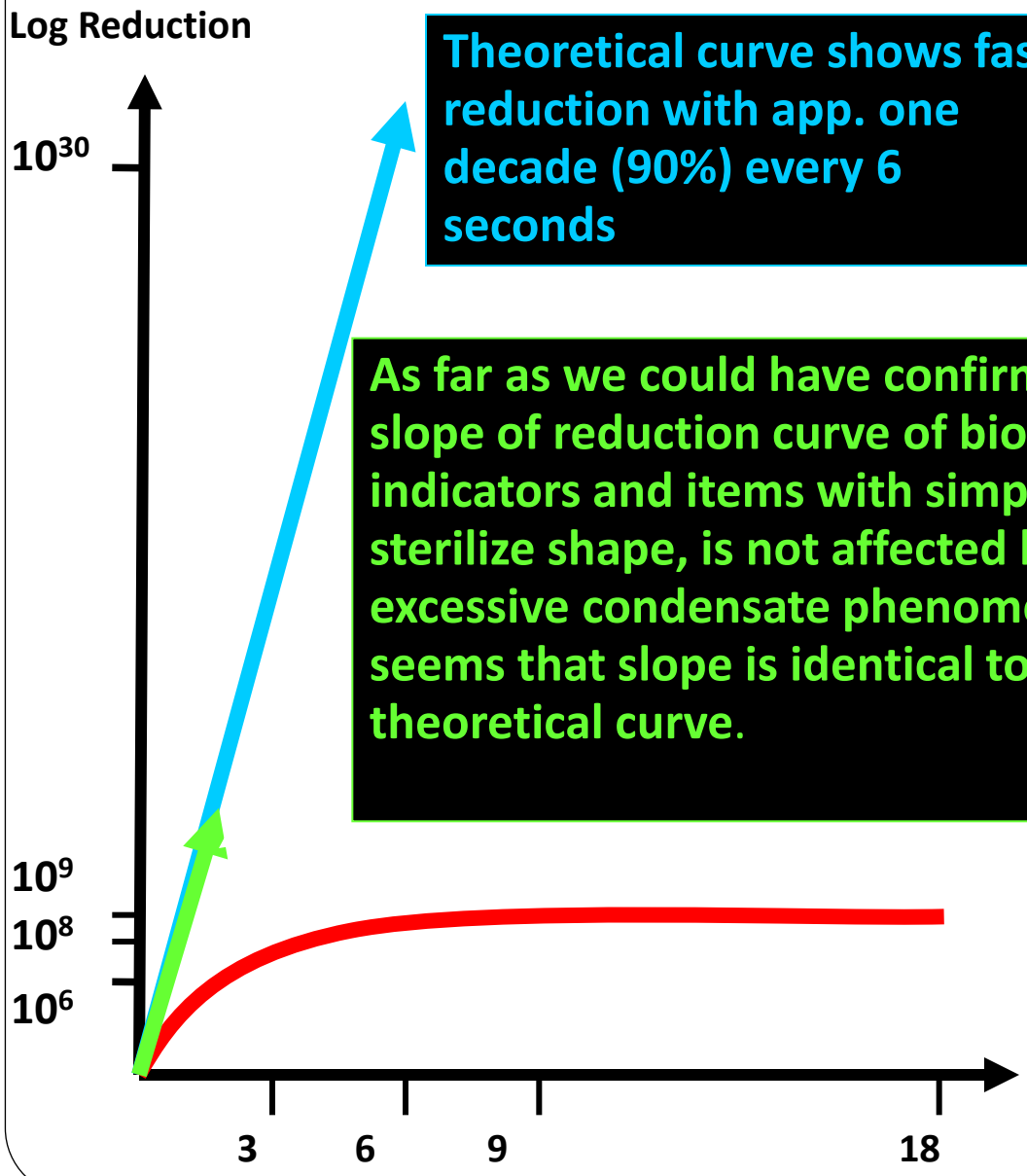
Reduction at 134 °C



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 from the study

- 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

The impact of excessive condensate on the sterility assurance level*

D. Perzin*, P. Kozin†, W. Rendens‡

Fig. 1: The instrument used to produce excessive condensate

Fig. 2: Nuts and screws which were used as test devices

Objective: The aims of this study are to determine the efficacy of the sterilization cycle when excessive condensate occurs and to investigate whether the Sterility Assurance Level (SAL) theory following first-order kinetics is applicable to condensate.

Methods: Nuts and bolts which are similar to the ones used in surgical instruments were used. Sterile nuts were inoculated with differing amounts of Geobacillus stearothermophilus ATCC 7953 spores, bolted and sterilized. The nuts and bolts were put through two cycles one without condensate, the other one in which excessive condensate was produced by a solid metal device weighing 3 kgs. After sterilization, the nuts were unbolted, and incubated in tryptic soy broth at 56 °C.

Results: The F-value was found to be 60 % lower in a cycle with excessive condensate in comparison to a cycle without condensate. In both conditions G. stearothermophilus did not grow on nuts and bolts inoculated with 10^2, 10^4, 10^6 spores, not even in the shortest cycle of 3 min. Of the nuts inoculated with 10^6 spores, only the ones that were exposed to excessive condensate showed growth in the 3 min cycle. The nuts inoculated with 10^6 spores and sterilized in conditions without condensate for 3, 4, and 5 minutes and with condensate in 7, 10, and 18 min cycles showed growth.

Conclusions: Excessive condensation lowers sterilization efficacy. A spore concentration of 10^6 is the tipping point to see this effect. The method of using high spore load can be used for the design qualifications of steam sterilizers.

KEY WORDS
- steam sterilization
- sterility assurance level
- condensate

Introduction

For a 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 1×10^{-6}. This is called the sterility assurance level (SAL). This norm is based on the assumption that the inactivation of microorganisms by physical or chemical means follows first-order kinetics. A SAL of 10^{-6} is the quantitative result which has to be reached through a sterilization process. This norm is not based on scientific findings, but is the result of the application of the rule of approximate values (1–3). The elimination of micro-organisms from a device during a sterilization process is time-dependent, influenced by the intensity of the sterilization process and of the level of the initial microbial contamination. Routine sterilization in Central Sterile Supply Departments (CSSD) always contains a number of uncertainties linked to non-condensable gases, insufficient cleaning and excessive condensate. However, the effects of these uncertainties on the sterilization process cannot be accurately ascertained despite the use of all kinds of indicators to monitor sterilization efficacy (4).

Excessive condensation on heavy surgical instruments during the sterilization cycle is one of the most important and frequently occurring problems in CSSDs. This occurs when trays with heavy instruments form part of the steriliser load. Condensation is necessary to achieve adequate sterilization during the steam sterilization cycle. Con-

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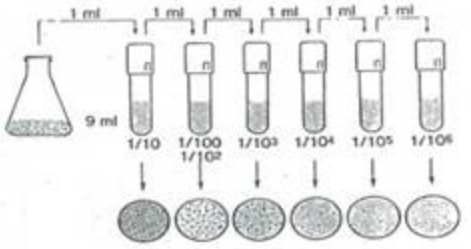
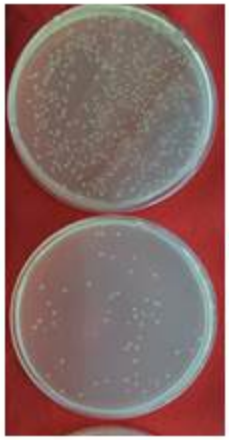
* The data was partly presented at 14th World Congress of Sterilization, 3–5 September 2013, Antalya, Turkey

STERRAD NX (ASP, USA)
28 min standard cycle
1 – 2 – 3 – 4 injections





Results

	Colony counts				
	Before sterilization	After sterilization			
		After 1 injection	After 2 injections	After 3 injections	After 4 injections
Bolted nuts	10^8	$1x10^6$	$1x10^6$	$1,5x10^5$	$2x10^4$
	10^2-10^7	No growth	No growth	No growth	No growth
Plates	10^2-10^8	No growth	No growth	No growth	No growth

In conclusion

- 10^8 spore concentration is a breakpoint for both steam and H_2O_2 gas plasma sterilization methods
- Theoretical mathematical models are not applicable on high inoculum of microorganisms equal to or more than 10^8
- Biological load difference is huge!
  - 10^6 = 1.000.000
  - 10^7 = 10.000.000
  - 10^8 = 100.000.000
  - 10^9 = 1.000.000.000
- SAL concept is questionable…
- Impact of BI (~10^6 spores) to approve SAL concept is even more questionable!

If you have positive BI it means you might be in trouble!

If you have negative BI it doesn't mean that you might be at ease!

It will be of value in sterility assurance only

- if it is used and interpreted correctly,
- if the user takes appropriate action in response to the results

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Thank you!