# PRODUCTION OF REFERENCE MATERIALS AND HOMOGENEITY AND STABILITY TESTING

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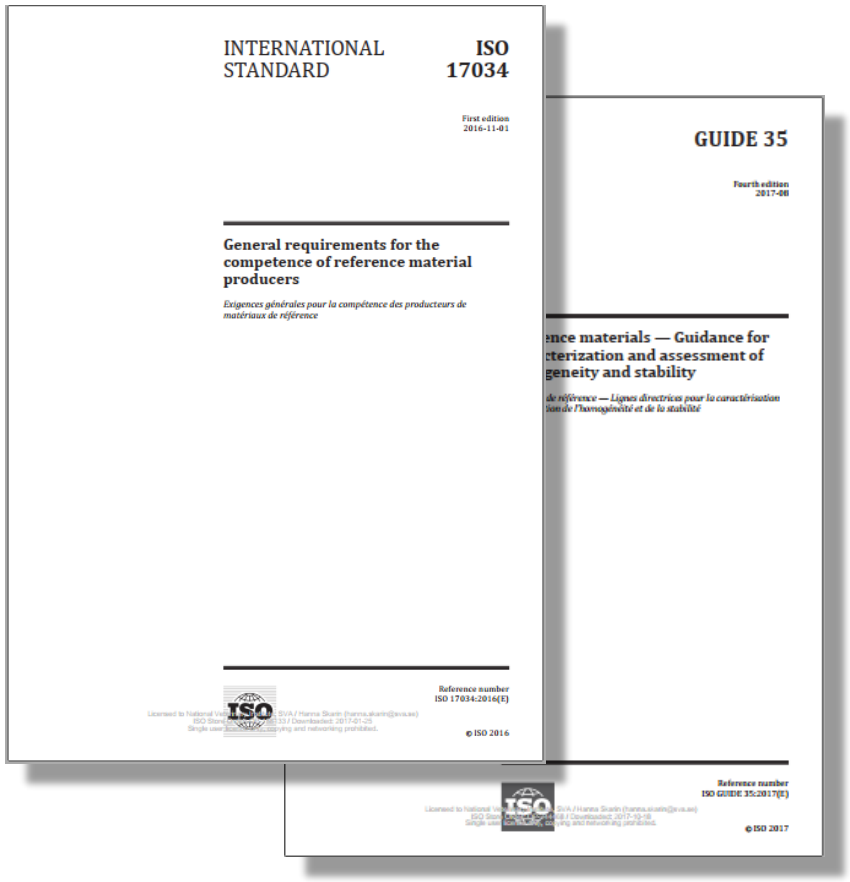
EURL-*Campylobacter Training Course 9* October 2019

# *REQUIREMENTS AND GUIDANCE FOR PRODUCTION OF REFERENCE MATERIALS*

There is an International Standard and three ISO Guides that support production and certification of reference materials.

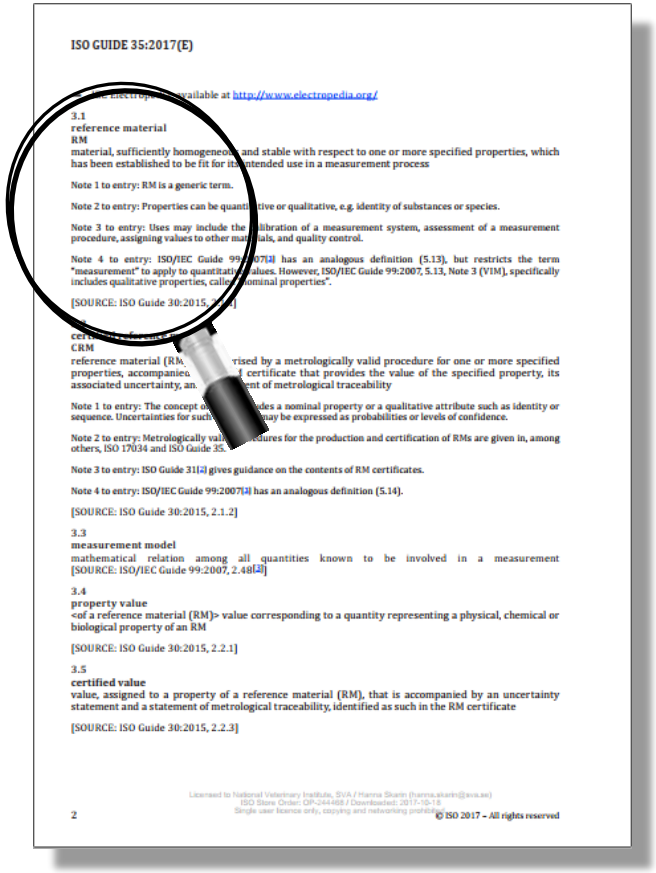
- ISO 17034 - general requirements
- ISO Guide 35 - guidance on technical issues, assessment of homogeneity and stability etc.
- ISO Guide 31 - contents of certificates for certified RMs
- ISO Guide 30 - terms and definitions



# *DEFINITION*

Reference material (RM) is defined in ISO 17034 and Guide 35 as

”material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process”



ISO GUIDE 35:2017(E)

available at http://www.electropedia.org/

3.1
**reference material**
**RM**
material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: RM is a generic term.

Note 2 to entry: Properties can be quantitative or qualitative, e.g. identity of substances or species.

Note 3 to entry: Uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control.

Note 4 to entry: ISO/IEC Guide 99:2007[3] has an analogous definition (5.13), but restricts the term "measurement" to apply to quantitative values. However, ISO/IEC Guide 99:2007, 5.13, Note 3 (VIM), specifically includes qualitative properties, called "nominal properties".

[SOURCE: ISO Guide 30:2015, 2.1.1]

**certified reference material**
**CRM**
reference material (RM), characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

Note 1 to entry: The concept of value includes a nominal property or a qualitative attribute such as identity or sequence. Uncertainties for such values may be expressed as probabilities or levels of confidence.

Note 2 to entry: Metrologically valid procedures for the production and certification of RMs are given in, among others, ISO 17034 and ISO Guide 35.

Note 3 to entry: ISO Guide 31[2] gives guidance on the contents of RM certificates.

Note 4 to entry: ISO/IEC Guide 99:2007[3] has an analogous definition (5.14).

[SOURCE: ISO Guide 30:2015, 2.1.2]

3.3
**measurement model**
mathematical relation among all quantities known to be involved in a measurement
[SOURCE: ISO/IEC Guide 99:2007, 2.48[3]]

3.4
**property value**
<of a reference material (RM)> value corresponding to a quantity representing a physical, chemical or biological property of an RM

[SOURCE: ISO Guide 30:2015, 2.2.1]

3.5
**certified value**
value, assigned to a property of a reference material (RM), that is accompanied by an uncertainty statement and a statement of metrological traceability, identified as such in the RM certificate

[SOURCE: ISO Guide 30:2015, 2.2.3]

## *FOLLOWING REQUIREMENTS MUST BE FULFILLED FOR RMS*

- representative for its intended use

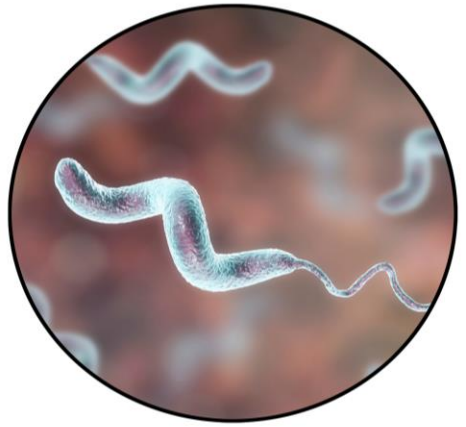
  representative = resemble routine samples as much as possible

- homogeneity specified within defined limits

- stability specified within limits over a specified period of time

# *FOR CAMPYLOBACTER THERE ARE TWO PRODUCERS OF QUANTITATIVE MICROBIOLOGICAL RMS*

- National Food Agency (Sweden) - Freeze-dried material
- Biosisto (The Netherlands) - Cryo cultures



# *LYOPHILIZATION / FREEZE-DRYING*

# *A CLOSER LOOK AT THE FREEZE-DRY PROCESS*

- **Freezing** - formation of ice crystals
- **Primary drying - ** removal of ice crystals
  Sublimation (at -35°C)
- **Secondary drying -** removal of unfrozen water
  Desorption (at 25°C)

## *FREEZE-DRYING CHALLENGES*

- Anaerobic and micro-aerophilic organisms, such as *Campylobacter* spp., are more difficult to freeze-dry due to their sensitivity to oxygen

- Gram negative bacteria show lower freeze-drying survival than gram positive

- A succesful freeze-drying process doesn’t guarantee a long shelf-life

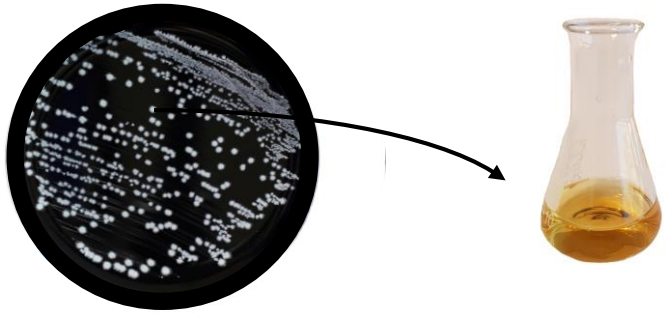
Temperature and humidity are important factors that affect the storage stability.

## *FREEZE-DRYING PROCEDURE*

- Selection and identification of the test strain
- Decide the batch size
- Culture
- Formulation
- Filling vials
- Partial stoppering
- Load freeze-dryer
- Freeze-drying
- Cap/seal

# CULTURE

Enrichment in 10 ml BHI

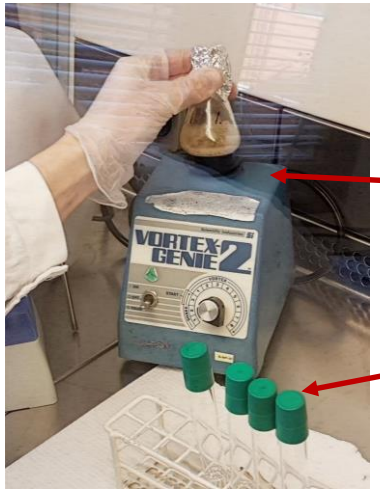


Inoculate a small part of a colony rather than a whole colony.

Incubation time differ for different strains and species

| | |
|---|---|
| • C. jejuni | 18h |
| • C. coli | 18h |
| • C. lari | 24h |
| • C. hyointestinalis | 50h |
| • C. lanienae | 48h |
| • C. upsaliensis | 48h |
| • C. helveticus | 22h |

# FORMULATION




1

Culture

Peptone saline water

Vortex and dilution

2


3

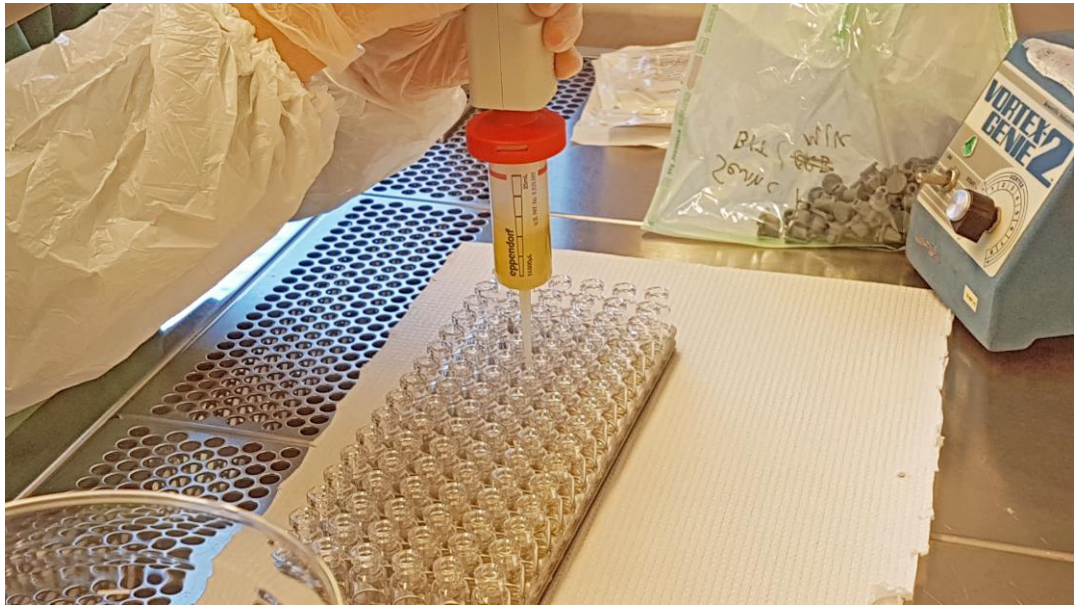
Protective agents

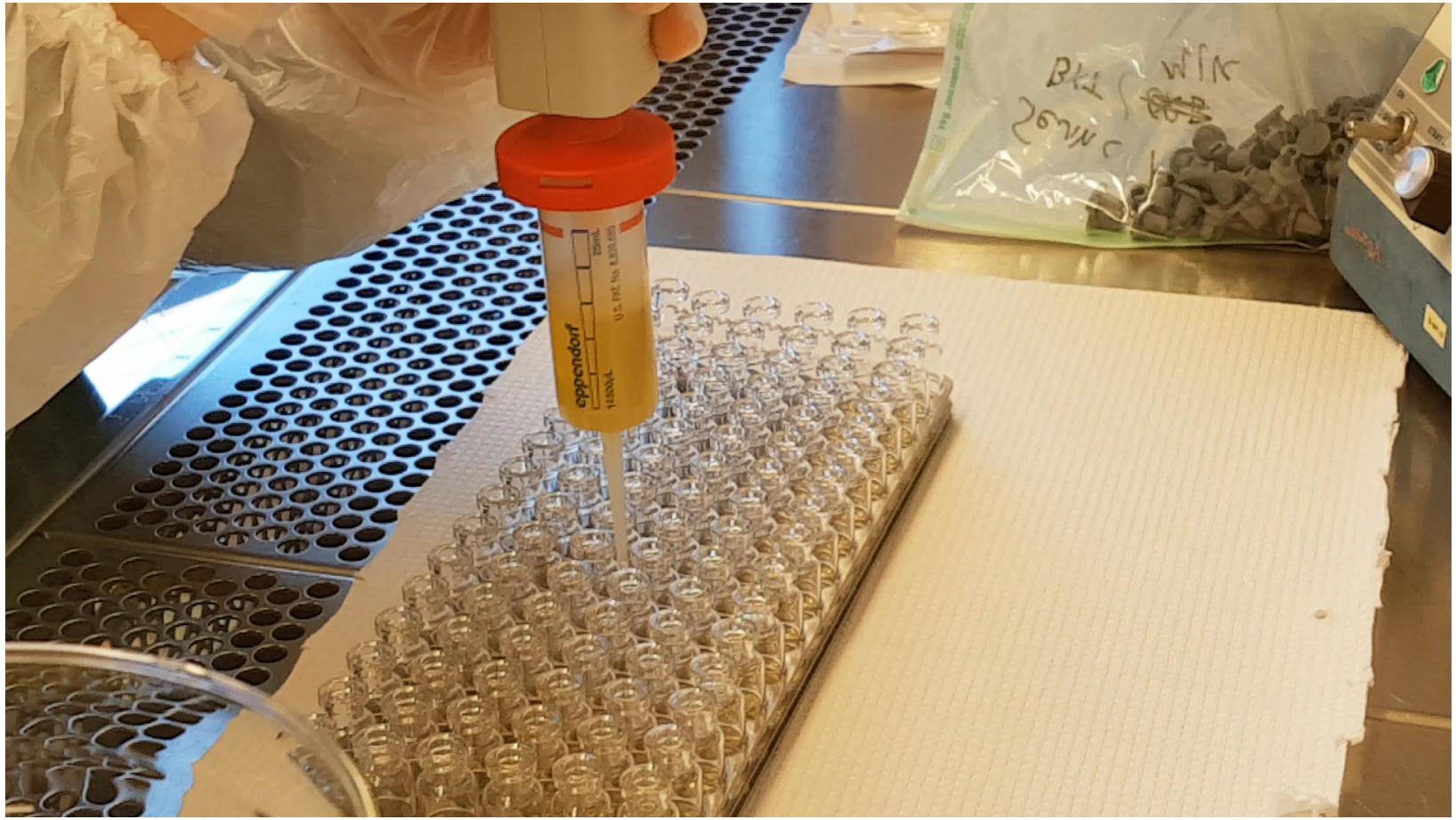
**SPG:** saccharose phosphate glutamate, and peptone

**ISB:** horse serum, inositol, nutrient broth


culture + SPG to ISB

# FILLING VIALS




eppendorf

# PARTIAL STOPPERING

Partially stoppered vial

# *LOAD FREEZE-DRYER AND FREEZE-DRY*

# *CAP/SEAL*

# THE END PRODUCT

Dried cake →

Uniform colour and texture of the cake after freeze-drying

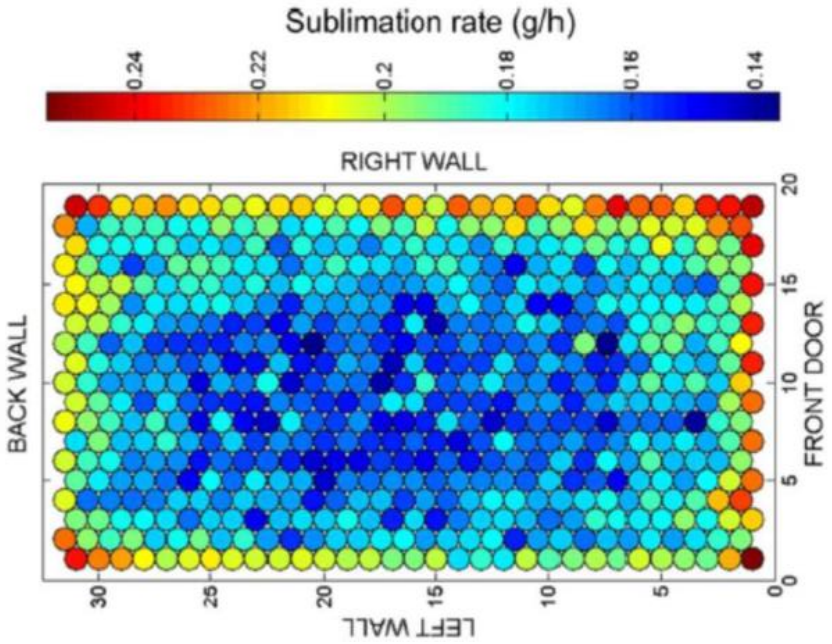
# *LIMITATIONS OF BATCH FREEZE-DRYING*

Variability in sublimation rate

Uneven heat transfer

↓

Vial-to-vial product variability




Kauppinen et al.

## *HOMOGENEITY ASSESSMENT*

An experimental homogeneity study is provided in ISO Guide 35

> ”experimental homogeneity tests require measurements of a representative number of randomly chosen units. The units can be chosen for example by random selection, stratified random selection or systematic selection from a random start point.”

# HOMOGENEITY STUDY FOR QUANTITATIVE PROPERTIES

$N_{prod}$ = the total number of units produced

$N_{min}$ = recommended minimum number of units

$$N_{min} = \max\left(10, \sqrt[3]{N_{prod}}\right)$$

Example 1: You prepare 3 000 vials of a RM and intend to undertake a homogeneity study.
The cube root of 3000 is 14,4. This study requires 15 vials for the homogeneity study.

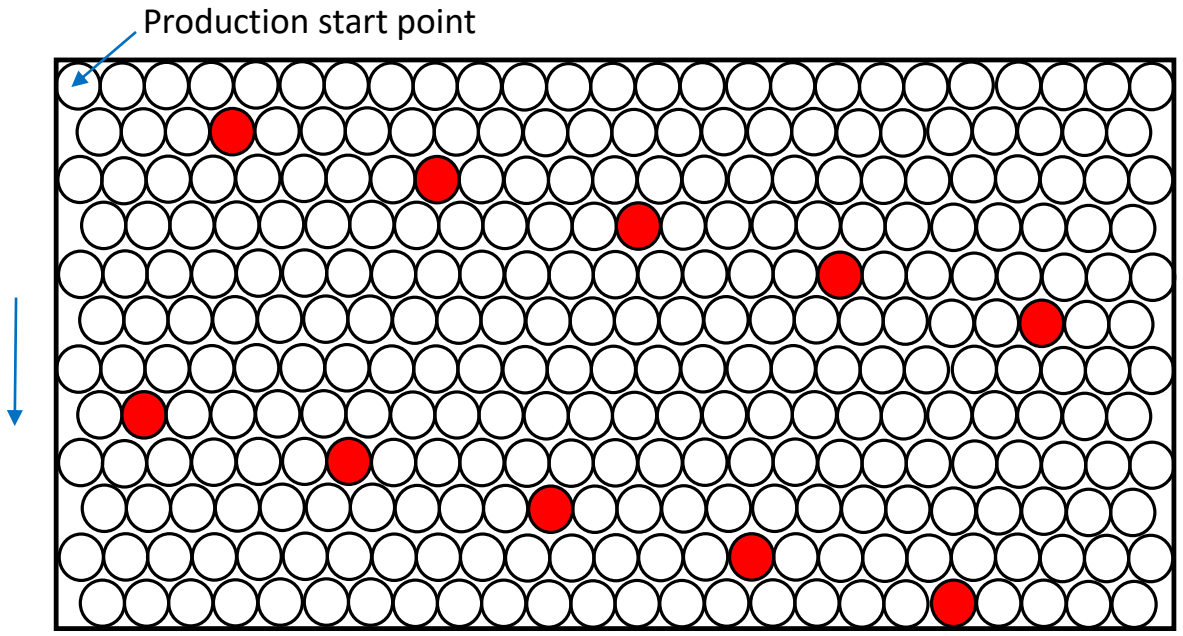
Example 2. You prepare 500 vials of a RM and intend to undertake a homogeneity study.
The cube root of 500 is 7,9. This study requires 10 vials for the homogeneity study.

## *HOMOGENEITY STUDY ON SMALL BATCHES*

| | |
|---|---|
| $N_{prod}$ < 100 units | homogeneity should be assessed on the larger of three units or 10% of the batch size |

# SYSTEMATIC SAMPLIG FOR THE HOMOGENEITY STUDY

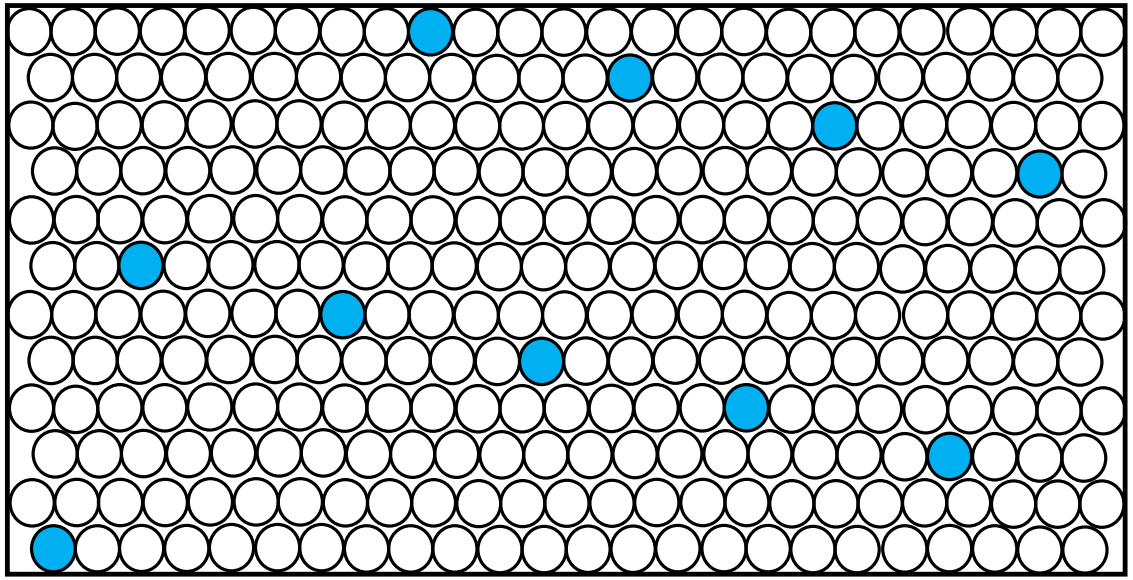
$N_{prod}$ = 294 vials




Important to have an interval between the samples in order to cover the whole filling line.

In this example every 29th vial is selected for the homogeneity study.

# Systematic selection from a random start point



# *STATISTICAL TREATMENT*

Number of randomly chosen units = 10

Homogeneity: s < 0.15 log CFU, max-min < 0.5 log CFU

An example

**C. jejuni (SVA021)**

| Sample | Plate 1 (0 dilution) No of CFU | log CFU/vial |
|---|---|---|
| 1 | 187 | 4,27 |
| 2 | 189,5 | 4,28 |
| 3 | 187,5 | 4,27 |
| 4 | 190 | 4,28 |
| 5 | 188 | 4,27 |
| 6 | 158 | 4,20 |
| 7 | 164,5 | 4,22 |
| 8 | 212 | 4,33 |
| 9 | 194,5 | 4,29 |
| 10 | 233 | 4,37 |

| | CFU/ampoule | log CFU/ampoule |
|---|---|---|
| **Mv** | 190 | 4,28 |
| s | 21 | 0,05 |
| Min | 158 | 4,20 |
| **Max** | 233 | 4,37 |
| Max-Min | 75 | 0,17 |

## *STABILITY MONITORING*

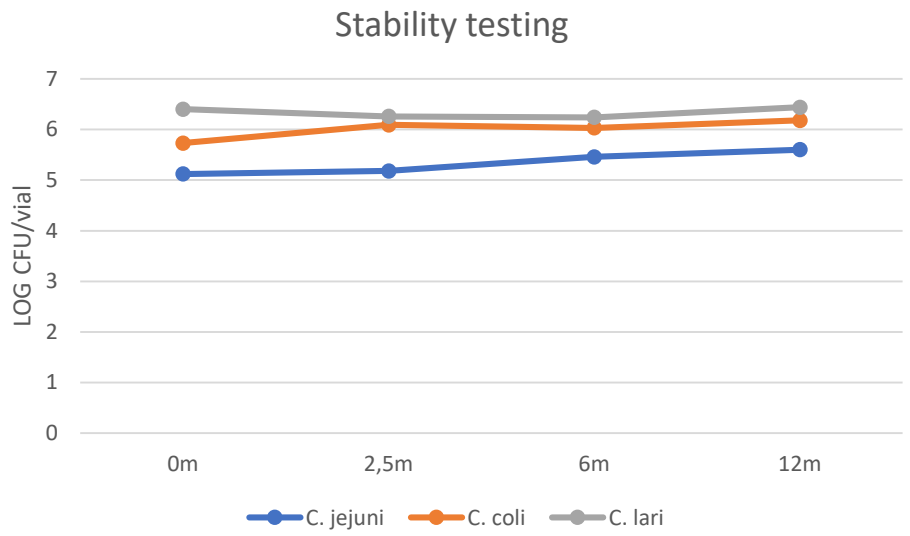
- Most RMs are stored for extended periods

  - necessary to assess the stability during storage conditions

- Nearly all RMs have to be transported to the location of use

  - necessary to assess the stability during transport conditions

# STABILITY MONITORING: C. JEJUNI, C. COLI AND C. LARI



## *TESTING OF PT*

The whole PT is tested

- when designing the PT (pilot tests)
- right before sending the PT (pre-PT)
- when most labs have received their PT (2 days after sending)
- the last date set for running the PT

Thank you for your attention!

Questions?