



**BacTrac Calibration,  
Standardisation and Validation**

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# MICROBIAL ENUMERATION

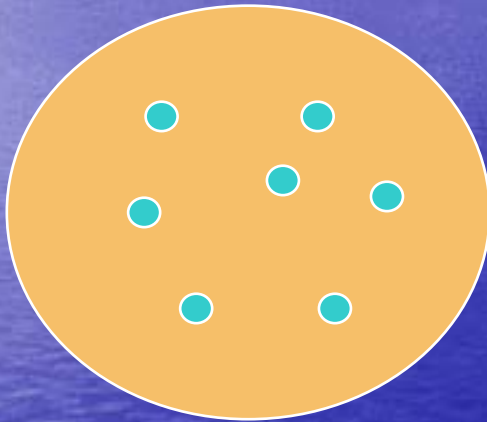
## PLATE

- Gives a number of so called „colony forming units = CFU“

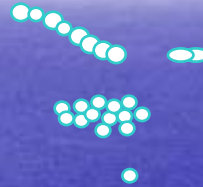
## BacTrac

- Gives a detection time depending of the activity and number of microbial cells present in a sample
- NOTE: Short detection = high number (activity)

# The Mystery of CFU Determination



TVC = 7 CFU



TVC = 71 CFU

# BacTrac Calibration – Method. differences

## PLATE

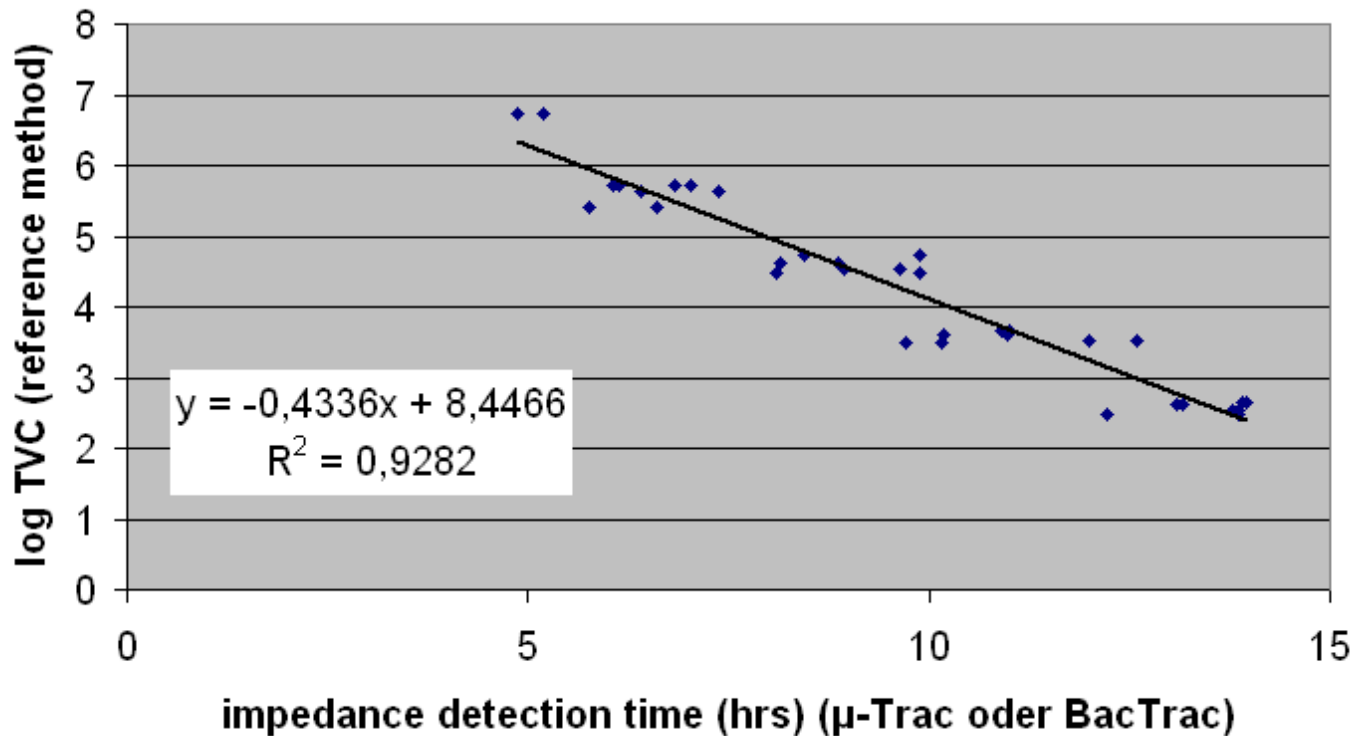
- Definition of „CFU“ – not always originating from a single cell
- Result calculated using a statistical approach in a countable range
- Semi solid substrate

## BacTrac

- Different metabolic activities of different microbes
- Direct result without dilution error
- Liquid substrates

# Relationship BacTrac - Plate

linear regression



Syx = 0,35  
r = -0,9634  
y = log CFU

# BacTrac Calibration - Fundamentals

- identical starting material (same homogenate)
- standardised reference method
- suitable range of contamination (max  $10^9$  - 10)
- adequate distribution over a 4 - 5 log range
- precise volumina
- **contamination flora and metabolic situation of the contamination flora**
- geographical variations of the flora

# Flora and metabolic situation

## Example 1

### Raw milk

- gram. neg germs are dominating
- liquid

### Past. milk

- Selection for gram pos. Thermotolerant germs
- liquid

☞ Different flora = no combined calibration !

# Flora and metabolic situation

## Example 2

### Milk powder

- selection for gram pos. Thermotolerant germs
- dry low  $a_w$ -value

### Past. milk

- selection for gram pos. Thermotolerant germs
- Liquid

☞ Different metabolic situation = no combined calibration !



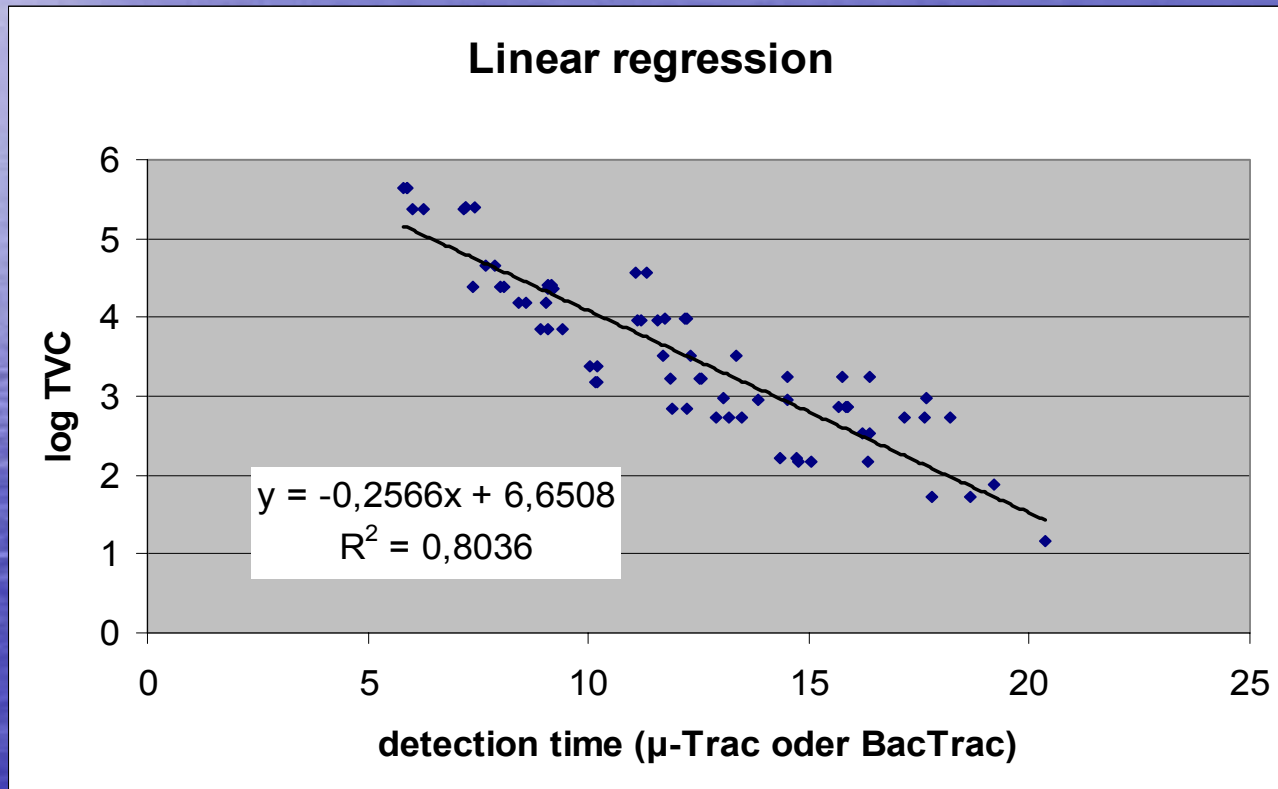
# Calibrations – Evaluation criteria

- Coefficient of correlation  $r$
- Dispersion  $S_{yx}$
- Expected results:

$$r = -0,85 \text{ to } -1.0$$

$$S_{yx} = < 0.5 \text{ (unit = log)}$$

# Calibration Coliforms Soft Cheese



Syx = 0,479

r = -0,8964

# IMPEDANCE and STANDARDISATION (Europe)

## ☞ **DIN and §64 LMBG, ÖNORM**

DIN 10115: basic standard (principles)

DIN 10120: Salmonella detection (horizontal)

DIN 10122: TVC enumeration

## ☞ **AFNOR**

NF V08-105: basic standard (principles)

NF V08-106: E.coli in sea food

# Validation – legal requirements

- ISO 16140 - Validation guidelines food
- EG 2073/2005 – Validation requirements
- Pharm. Eur. 6 – Pharmaceutical applications

# Official third party validations (ISO 16140)

- 👉 Enumeration of E.coli in shellfish (IFREMER, finished)
- 👉 Detection of Enterobacteriaceae (AFNOR, finished)
- 👉 Determination of aerobic mesophilic counts (AFNOR in progress)
- 👉 Detection of Salmonella (AOAC planned)