THE “FEAR FACTOR” IN MICROBIOLGICAL AUTOMATED TEST METHODS

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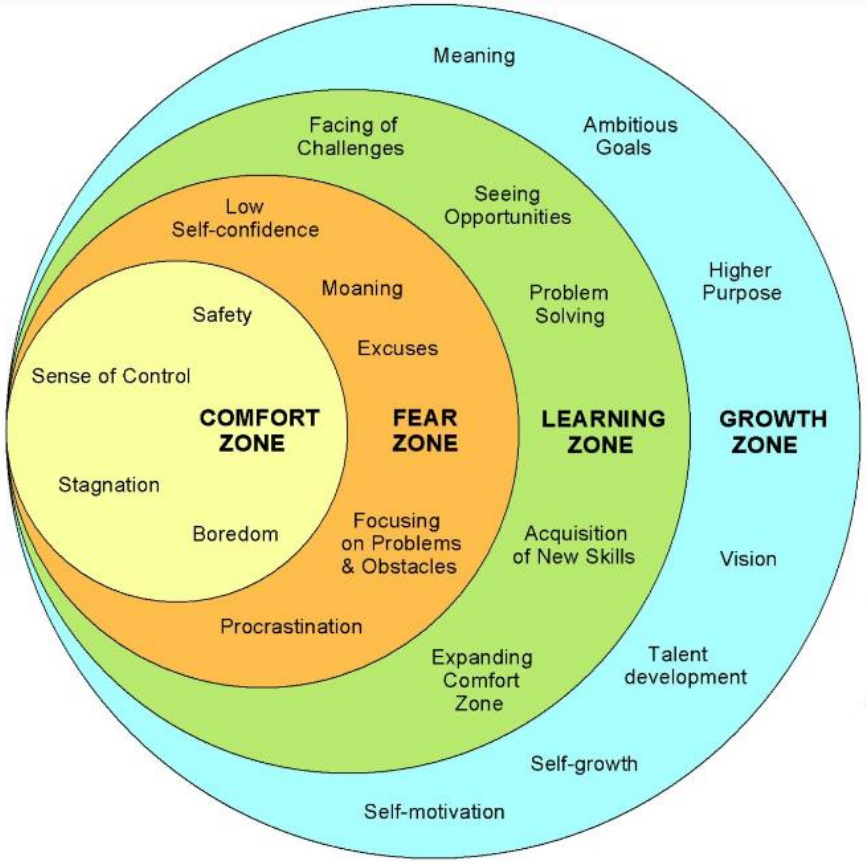
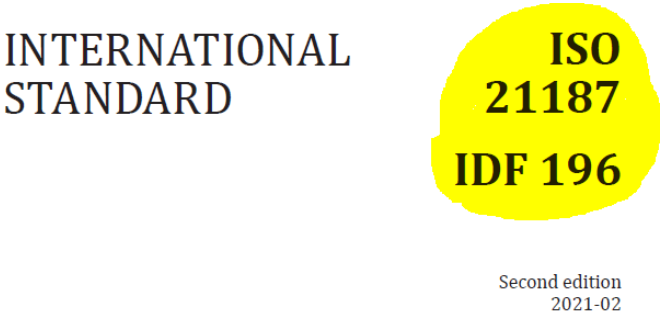

Meaning
Facing of Challenges
Ambitious Goals
Low Self-confidence
Seeing Opportunities
Higher Purpose
Moaning
Problem Solving
Safety
Excuses
Sense of Control
COMFORT ZONE
FEAR ZONE
LEARNING ZONE
GROWTH ZONE
Stagnation
Boredom
Focusing on Problems & Obstacles
Acquisition of New Skills
Vision
Procrastination
Expanding Comfort Zone
Talent development
Self-growth
Self-motivation


Annoyed
Panic
Stressed
Find Purpose
Lack Self-Confidence
Anticipating
Conquer Objectives
Exhilarated
Easy
Set new goals
Tired
Lifeless
Unchallenged
COMFORT ZONE
GROWTH ZONE
STRESS ZONE
Feels Good: Comfy & Safe
Uncomfortable: Requires Focused Attention & Effort
Difficult: Overwhelmed & Unable to Learn
Stable
Secure
Low Risk – Low Reward
Affected by Opinions
Challenged
Willing to Learn
Alive
Frustrated
Stretch
Expectant
Excited
Find Excuses
Disinclined
Fearful
Fed Up
Anxious
Tense
Exhausted

LITERATURE

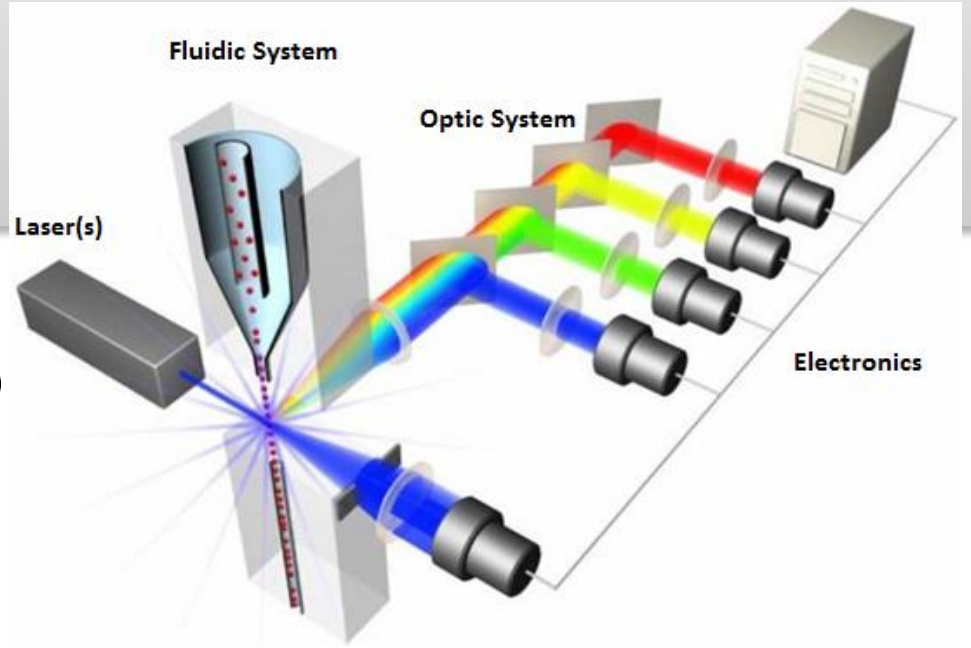
Bulletin 511 - Guidance on the application of conversion equations for determination of microbiological quality of raw milk

ISO 21187/IDF 196 – Milk – Quantitative determination of microbiological quality - Guidance for establishing and verifying a conversion relationship between results of an alternative method and anchor method results



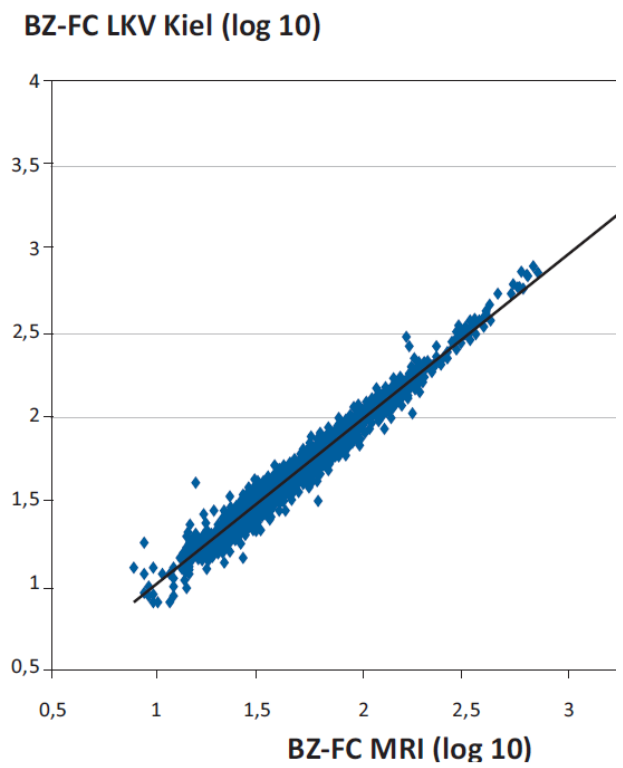
- Historic methods – SPC ➡ CFU
- Today mostly automated Flow Cytometry ➡ TBC
- Different methods – NOT equivalent
- Conversion Factor ?? – TBC into CFU

BACKGROUND



INTRODUCTION

- Hygienic measures of raw milk
- Milk grading schemes
- Incentives or penalties
- Microbiological quality one of key parameters
- Also legal requirements (limit in cfu)
- FC – Industry standard today
- 2 Manufactures
- Challenge to transform from alternative method to anchor method



METHODS APPLIED FOR TBC

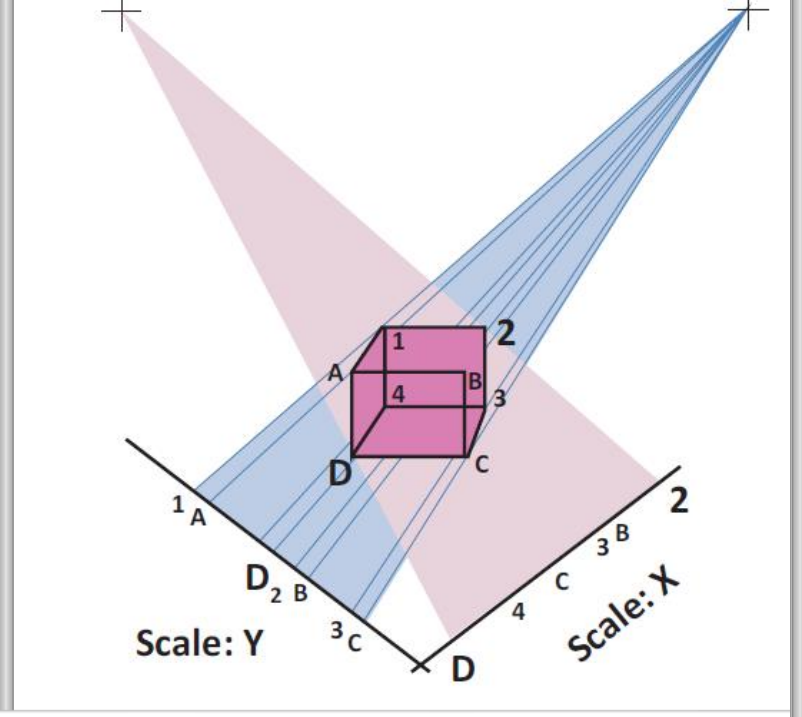
TBC Routine testing: FC, SPC or Aerobic Plate count (am or rm)

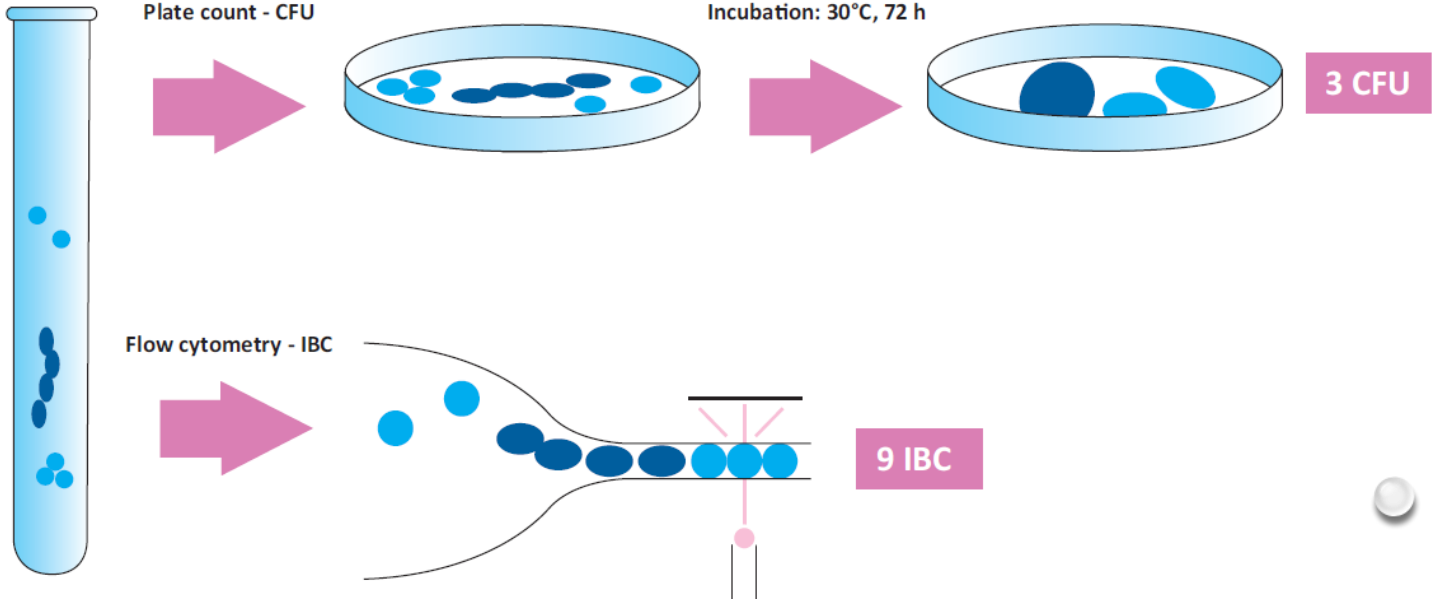
Plate count Methods:

- Several versions (Petrifilm)
- 30 •C for 72 h or 35 •C for 48 h
- Standardized growth medium
- ✚ Simple & Low cost
- ▬ Poor precision, repeatability & reproducibility, one temperature

Flow cytometry

- Interference components
- Fluorescent marker
- Stain DNA & RNA
- Detector measure light pulses
- IBC
- Perform better than SPC methods





CONVERSION INTERLINKING 2 METHODS

- Different measurands
- Plate count rather anchor method
- CFU vs IBC
- Calibration not possible for Microbial analysis
- Conversion developed
- Conversion relationship influenced by:
  - Milk flora growth phase
  - Milk storage time
  - Preservation
  - Animal species
  - High Fat & Protein
  - Seasonal variations
  - Variations in production system
  - Type of bacteria
  - SSC
  - SPC method itself

THE CONVERSION

ISO 21187:2021/IDF 196:2021

HUGE NUMBER OF DATA

COMPLICATED DATA INTERPRETATION

NUMBER OF HIGH LEVEL CALCULATIONS

Number of test samples for linear regression

A.1 For comparison of an estimated and a hypothetical regression coefficient, the test formula for a two-tailed test with probability $(1 - \alpha)$ is shown by Formula (A.1):

$$t = \left| b_{yx} - \beta_{yx} \right| / s_{byx} \quad \text{(A.1)}$$

where

t is $t_{(n-1;1-\alpha/2)}$ which is the numerical value of the Student's t-distribution at its $(1 - \alpha)$ probability level;

b_{yx} is the estimated regression coefficient;

β_{yx} is the hypothetical regression coefficient;

s_{byx} is $s_{yx} / s_x (n-1)^{0,5}$;

n is the number of data pairs.

Replacing $\left| b_{yx} - \beta_{yx} \right|$ by d results in Formula (A.2):

$$t = d / \left[s_{yx} / s_x (n-1)^{0,5} \right] \quad \text{(A.2)}$$

This can be rewritten as Formula (A.3):

$$n = t^2 (s_{yx}^2 / s_x^2) / d^2 + 1 \quad \text{(A.3)}$$

Since s_{yx}^2 can be approximated by $s_y^2 - b_{yx}^2 \cdot s_x^2$ with higher values of n, Formula (A.3) may also be written as Formula (A.4):

$$n = t^2 (s_y^2 / s_x^2 - b_{yx}^2) / d^2 + 1 \quad \text{(A.4)}$$

where

s_y is the standard deviation of the y-values;

s_x is the standard deviation of the x-values.

A.3 An alternative way has been suggested (see Reference [7]), introducing the relative error, δ, of the estimate for the slope of the regression, as shown by Formula (A.5):

$$\delta = \left| b_{yx} - \beta_{yx} \right| / \beta_{yx} \quad \text{(A.5)}$$

Formula (A.1) is transformed to Formula (A.6):

$$t = \left| b_{yx} - \beta_{yx} \right| / \left[s_y^2 (1 - r^2) / (n-1) s_x^2 \right]^{0,5} \quad \text{(A.6)}$$

where r is equal to $b_{yx} \left(s_x / s_y \right)$.

Extending Formula (A.6) results in Formula (A.7):

$$t = \left(\left| b_{yx} - \beta_{yx} \right| / \beta_{yx} \right) / \left\{ \left[s_y^2 (1 - r^2) / (n-1) s_x^2 \right]^{0,5} / \beta_{yx} \right\} \quad \text{(A.7)}$$

On introducing Formula (A.5), Formula (A.7) becomes Formula (A.8):

$$t = \delta / \left\langle \left\{ \left[s_y^2 (1 - r^2) / (n-1) s_x^2 \right]^{0,5} \cdot s_x \right\} / r \cdot s_y \right\rangle \quad \text{(A.8)}$$

or Formula (A.9):

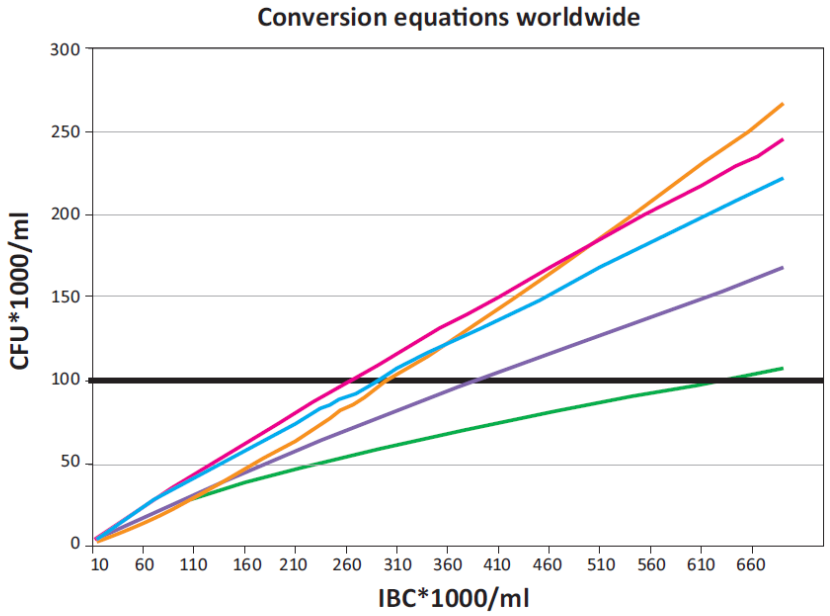
$$t = \delta / \left[(1 - r^2) / (n-1) r^2 \right]^{0,5} \quad \text{(A.9)}$$

Resolving n results in Formula (A.10):

$$n = \left[t^2 (1 - r^2) / (\delta^2 \cdot r^2) \right] + 1 \quad \text{(A.10)}$$

CONVERSION FACTOR TODAY

- DIFFICULT TO STANDARDIZE
- COSTLY TO DEVELOP & MAINTAIN
- SOME DIFFERENCES IN THE EQUATIONS



IBC/ml	CFU/ml Applying the Danish conversion	CFU/ml Applying conversion factors from other regions
25 000	6 000	3–11 000
50 000	12 000	7–22 000
100 000	24 000	13–41 000
200 000	46 000	27–78 000
450 000	101 000	69–181 000
750 000	165 000	104–263 000

Figure 7. Conversion equations from some European countries. The example shows that routine results ranging from 260 000 to 650 000 IBC/mL correspond to 100 000 Estimated CFU/mL (black line), depending on the applied conversion equation. Source: C Baumgartner, MPR Bayern, DE.

3 SOLUTIONS POSSIBLE

1. Conversion with lab specific equations
2. National or global conversion
3. Avoid the conversion

	Laboratory specific conversion	Harmonized national conversion	No conversion
Compliance with most legal limits	+++	+++	+
Compliance with present farmer settlement models	+++	+++	+
Legal disputes over converted or non-converted results Legal disputes over microbiology of perishable products are generally complicated, due to bacterial growth and timing	+	+	+++ Estimated CFUs may be very different from actual CFUs, see Figure 4
Good tool for improving and maintenance of on-farm hygiene	++	++	++
Results independent of analyzing laboratory	+	+++	+++
Simplicity and cost of development and maintenance	+	++	+++
Known by stakeholders	+++	+++	+

THANK YOU