# Proficiency testing in Food Microbiology "AQUA" according to ISO/IEC 17043:2010 and ISO/TS 22117:2010

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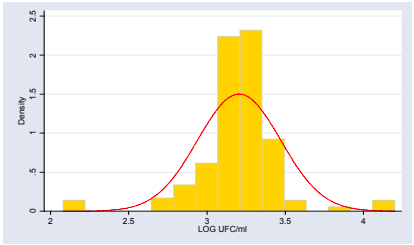
*Eurachem: Istanbul, 4 October 2011*

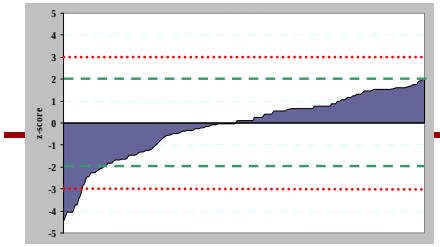
## The PT “AQUA”

## Homogeneity and stability

## The data check




## The statistical analysis




## The comparison of results

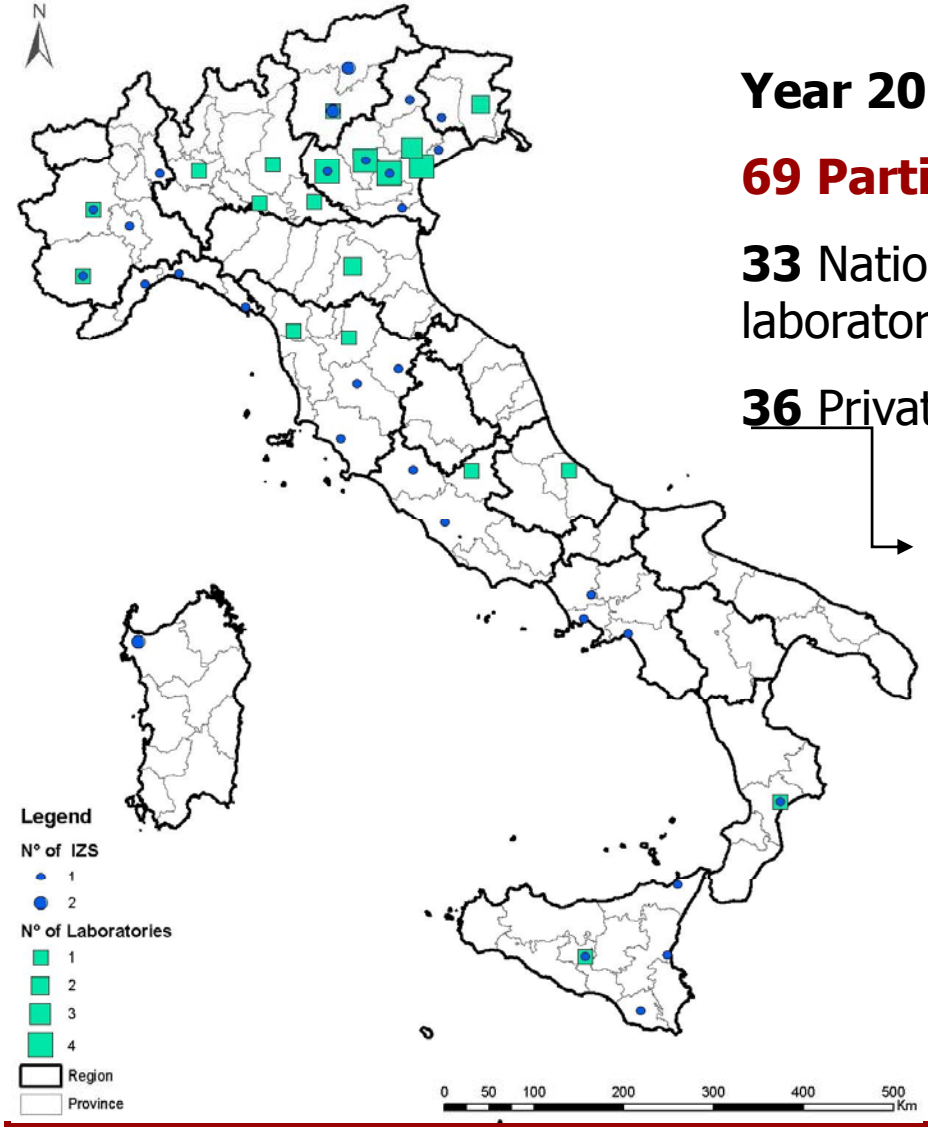
# Scheme of proficiency testing AQUA

ISO/IEC 17043:2010
Conformity assessment- **General requirements** for proficiency testing

ISO/TS 22117:2010
Specific requirements for **proficiency testing** in Microbiology of food

- Diagnostic Microbiology
  *(Responsable dr. Michela Corrò)*
- Isolation, Identification and Typing of Salmonella
  *(Reference laboratory for Salmonella – Responsable dr. Antonia Ricci)*
- Bacteriology and virology of water organisms
  *(Reference laboratory for Fish health - Responsable dr. Amedeo Manfrin)*
- Parassitology shellfishes
  *(Reference laboratory for Fish health - Responsable dr. Giuseppe Arcangeli)*
- Virology and Serology for avian flu and Newcastle disease
  *(Reference laboratory for avian flu and Newcastle desesae - Responsable dr. Calogero Terregino)*
- Food Microbiology
  *(Responsable dr. Maria Grimaldi)*
- Serology and molecular biology for bovine and suine diagnostic
  *(Responsable dr. Stefano Nardelli)*

# PT in food microbiology




**Year 2011**

**69 Participants**

**33** National public laboratories (IZS)

**36** Private laboratories

one or more results per partecipant (repeatability)

**Team**

Proficiency testing responsable

*Dr. Maria Grimaldi*

Technician responsable

*Dr.ssa Romina Trevisan*

Statistician responsable

*Dr.ssa Marzia Mancin*

# PT in food microbiology

**SHELLFISH Matrix**

**MEAT Matrix**

**MILK Matrix**

# PT in food microbiology

**SHELLFISH Matrix**

**Enumeration of:**
*Escherichia coli* MPN

**Detection of:**
*Salmonella* spp.

**MEAT Matrix**

**Enumeration of:**
*Bacillus cereus*

**Detection of :**
*Escherichia coli O:157*

# PT in food microbiology

**MILK Matrix**

**Enumeration of:**

- Mesophilic bacteria
- Coagulase positive Staphylococci
- Coliform bacteria
- *Escherichia coli*
- Enterobacter spp.
- *Listeria monocytogenes* ufc
- *Listeria monocytogenes* MPN
- Solfite reducing *Clostridium*
- *Clostridium perfringens*
- *Campylobacter* spp.

**Detection of:**

- *Salmonella* spp.
- *Listeria monocytogenes*
- *Cronobacter sakazakii*
- *Campylobacter* spp.
- Staphylococcal enterotoxins

# Example: *Enterobacter* spp.

- **Homogeneity**

  A batch is homogeneus if each sample has the same characteristics

  For counts>40: The international harmonized protocol for the proficiency testing of analytical chemistry laboratories (IUPAC technical report, 2006)

  For counts<40:ISO/TS 22117, Annex B: T1-T2 test

- **Stability**

  A batch is stable if it preserves its own microbiologic characteristics within limited values during the time of analysis (first and third day)

  The international harmonized protocol for the proficiency testing of analytical chemistry laboratories (IUPAC technical report, 2006)

# Observations

**Scope**: the batch has to be homogeneus and stable with a standard deviation target $\sigma_p$=0.25 "fitness for purpose" (from previous PT)

- **IF** the batch **is not homogeneus** for $\sigma_p$=0.25

**DISCARD OF BATCH AND PRODUCTION OF A NEW BATCH**

- **IF** the batch is homogeneus for $\sigma_p$=0.25
- **IF** the batch **is not stable** for $\sigma_p$=0.25

**IN THE CALCULATION OF Z-SCORE THE STANDARD DEVIATION OF THE STABILITY IS TAKEN INTO ACCOUNT**

# Procedures of our PT

INFORMATION about :
- Conservation and Treatment of samples
- Analysis to do and suggestions about procedures and legislative requirements to follow
- Registration of results

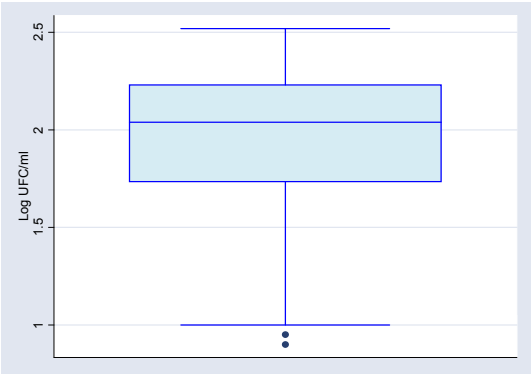
Routine method

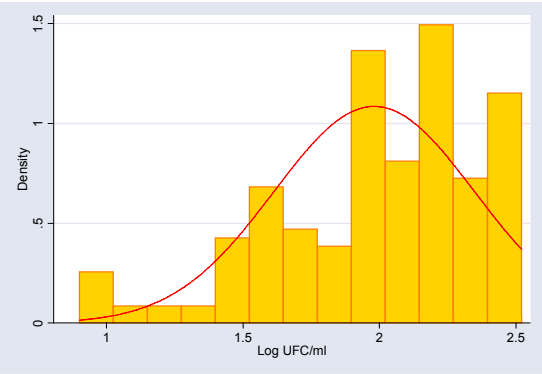
Counts >10

# Example: *Enterobacter* spp.

- Check of data (incorrect results) and equivalence of methods
- Z- score: provided that the data distribution is ROUGHLY symmetrical and unimodal
- Other methods according to the number of participants: 0.5log, percentile, MAD

38 laboratories;
194 (188) observations



# Example: *Enterobacter* spp.

- Descriptive statistic of data for NOT equivalent method

| variable | N | min | max | mean | p50 | sd | cv |
|---|---|---|---|---|---|---|---|
| log_data | 6 | 9.047821 | 9.21034 | 9.174705 | 9.21034 | .0654588 | .0071347 |

**Too few data: NO Z-score calculation for this method**

- Descriptive statistic of data for equivalent methods

| variable | N | min | max | mean | p50 | sd | cv |
|---|---|---|---|---|---|---|---|
| log_data | 188 | .9 | 2.52 | 1.9805 | 2.04 | .3676 | .1856 |

- Z-score calculation

# Example: *Enterobacter* spp.

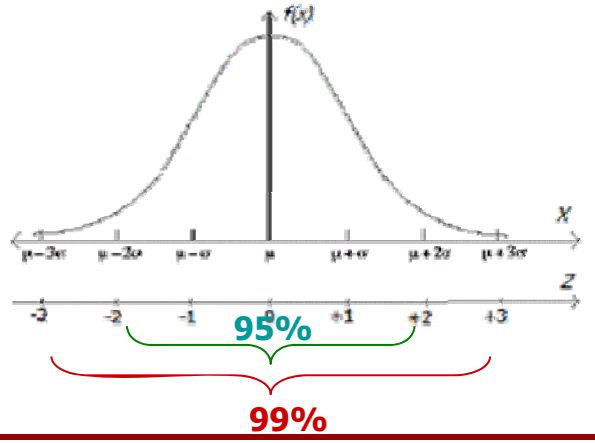
$$Z - score = \frac{(X_i - \overline{X})}{\sigma_t}$$

$X_i$ = observed value

$\overline{X}$ = assigned value like "**consensus value from participants**" obtained by the algoritm A of Annex C, ISO 13528

$\sigma_t$ = 0.25, "**fitness for purpose**" about the ISO 13528

| | |
|---|---|
| -2 ≤ z-score ≤ +2 | Acceptable results |
| -3 < z-score < -2 e 2 < z-score < 3 | Questionable results |
| z- score ≤ -3 e z- score ≥+3 | Not acceptable results |



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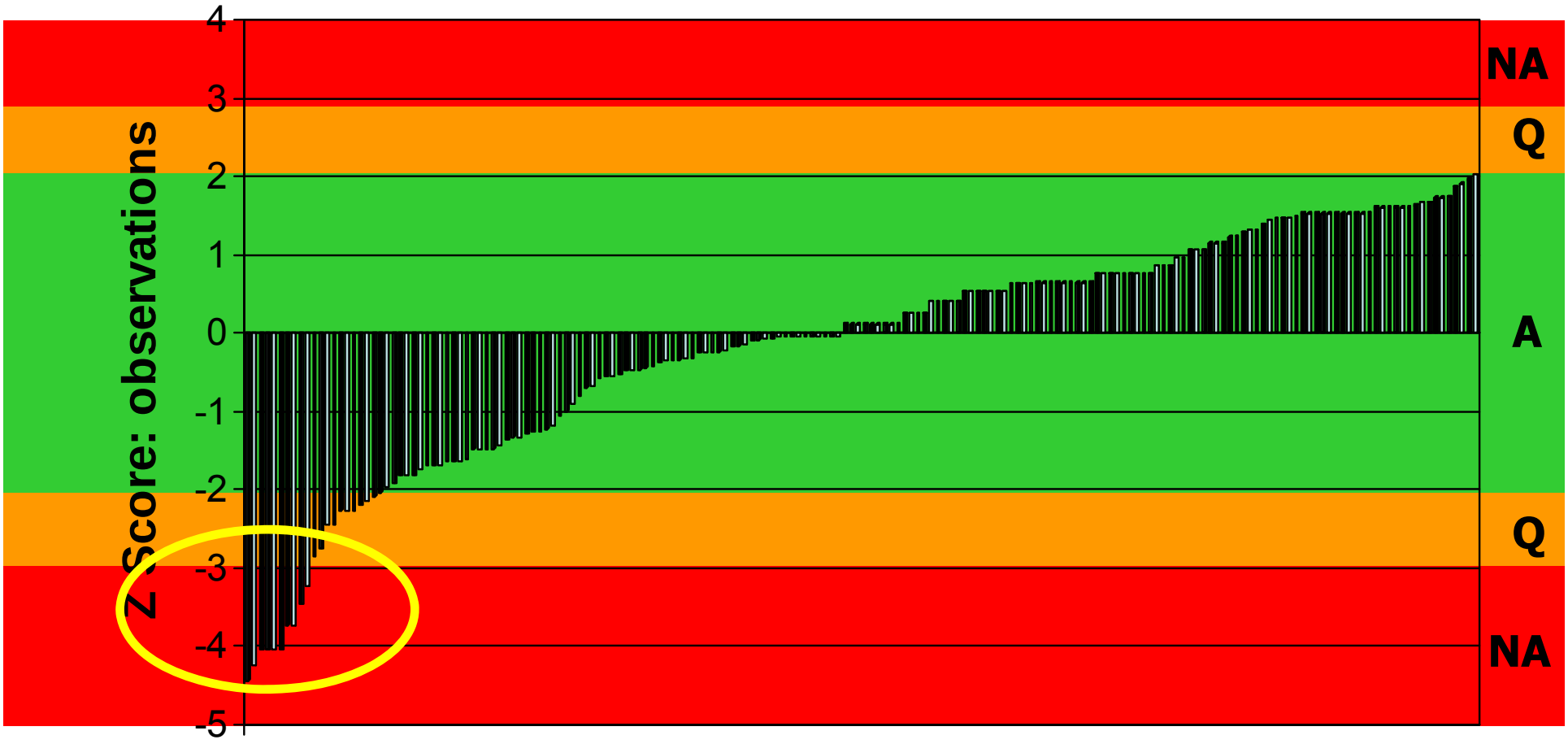

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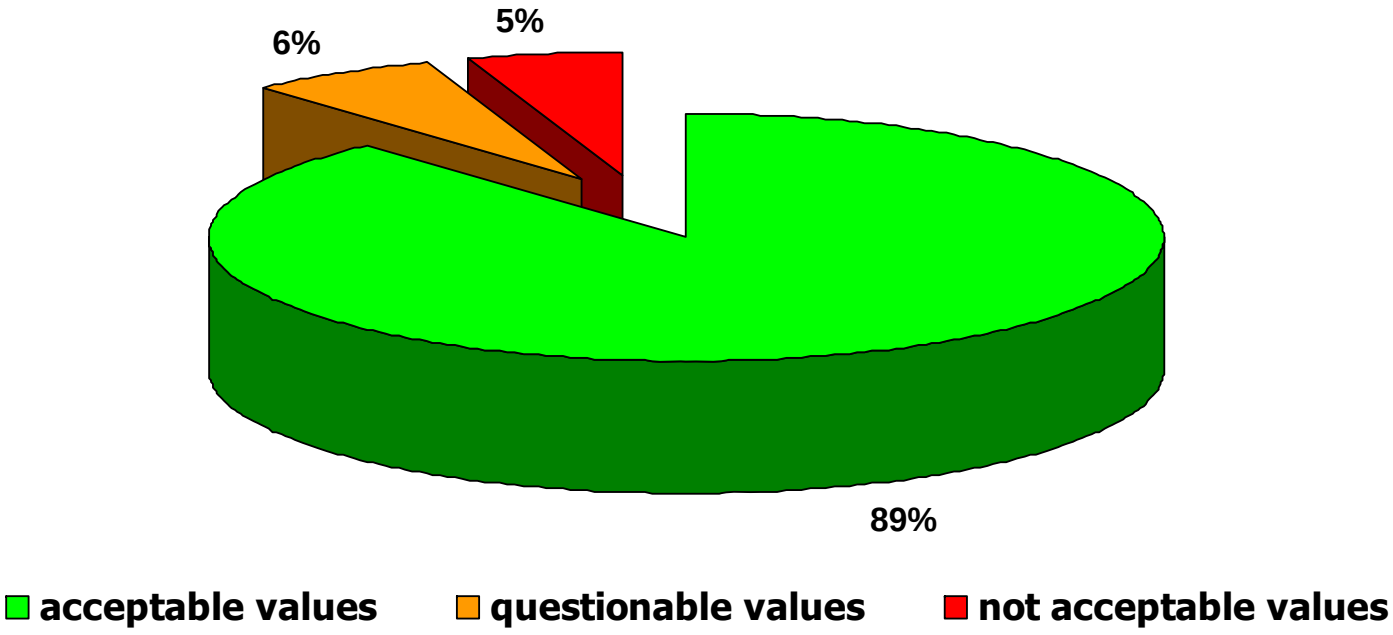

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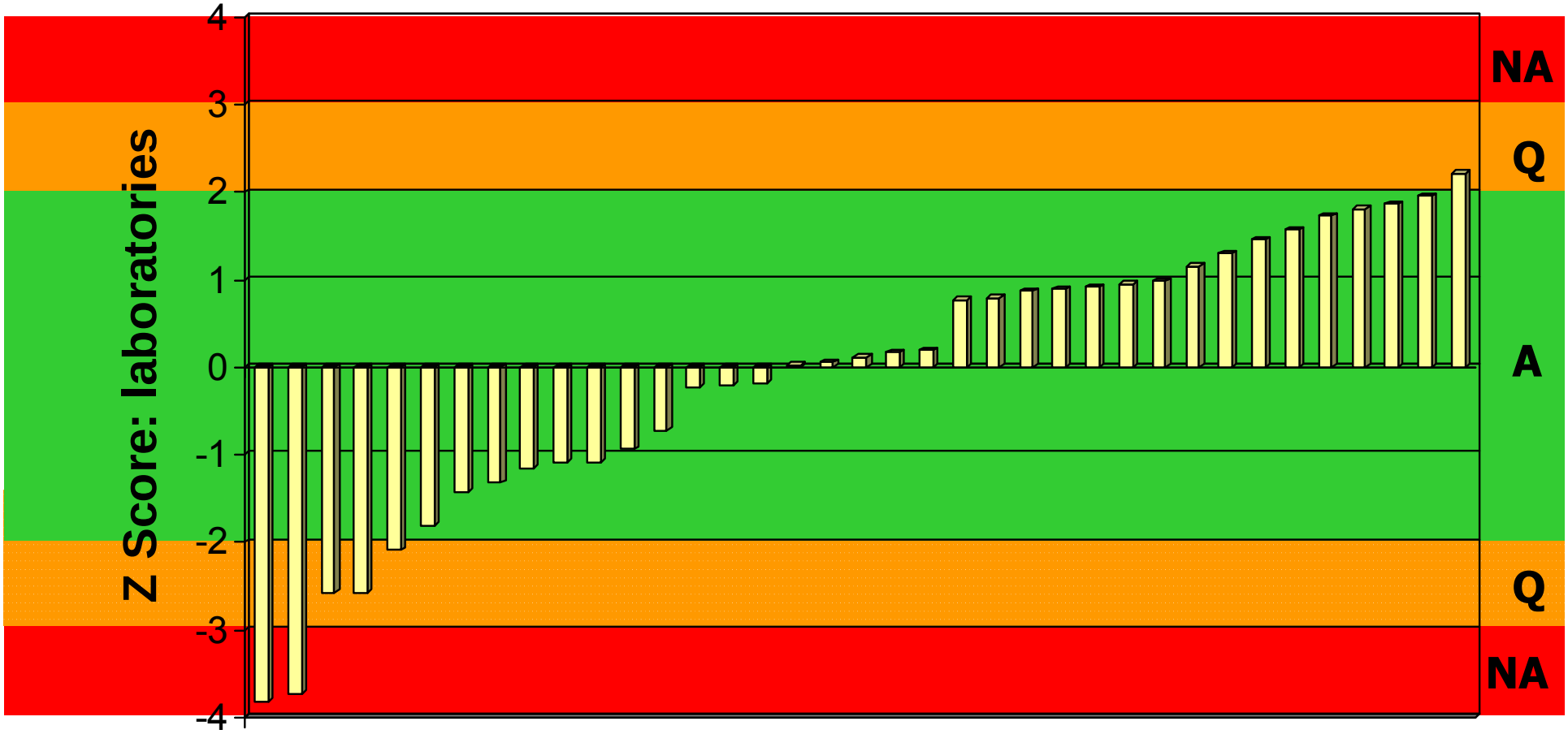
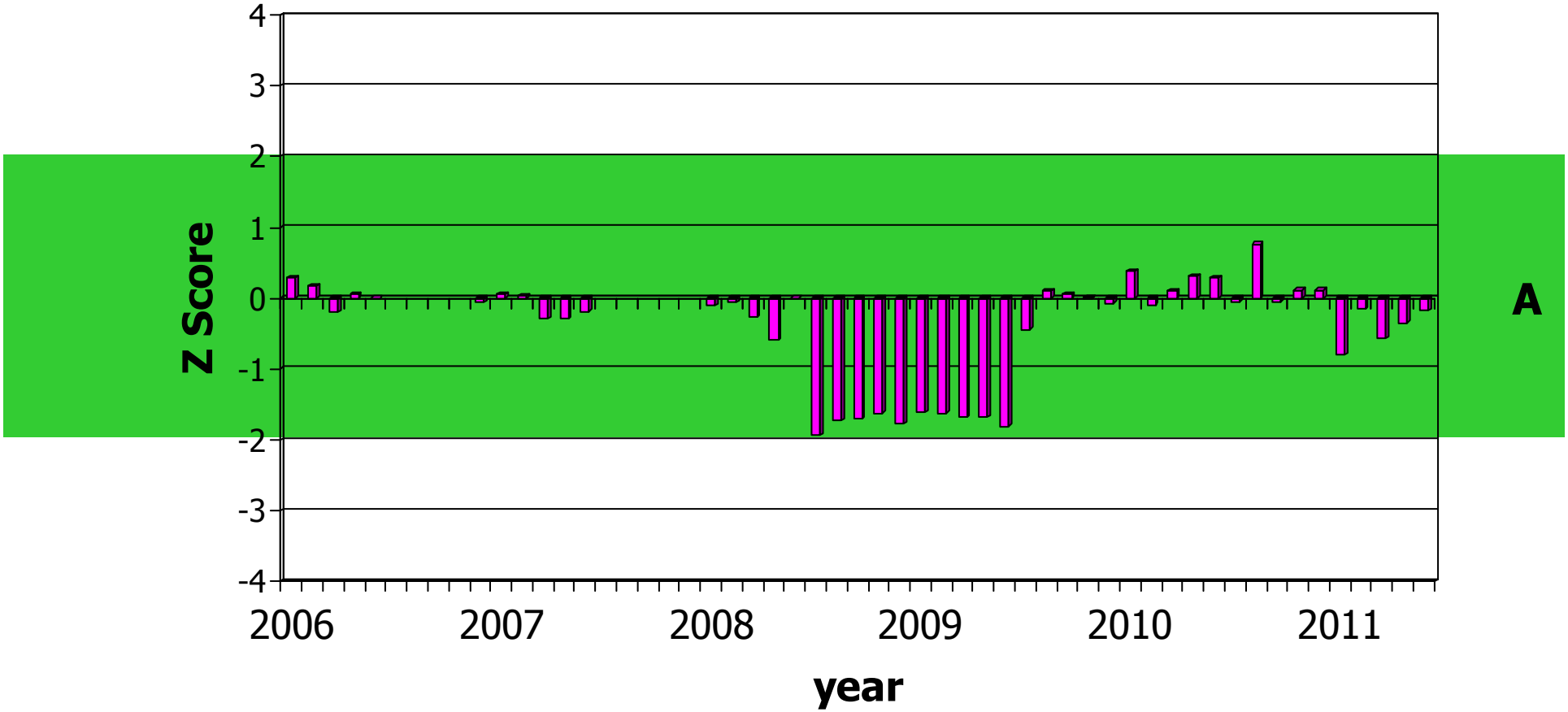


# Example: Trend of *Enterobacter* spp.




The results obtained by our institute in the last 6 PTs for *Enterobacter* spp

# THANK YOU FOR YOUR ATTENTION

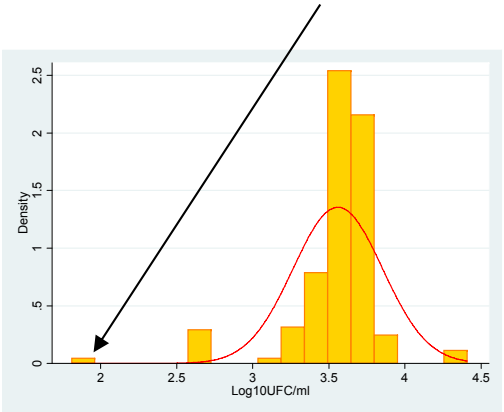
# ISO/TS 22117:2010

## Other methods of performance evaluation

- **± 0.5log10 rule: high number of participants**

  the result is good, in internal quality control procedures for microbiology laboratories, if the counts are within ± 0.5log10. Statistically the 95% CIs around a mean count are generally not more ± 0.5log10

- **Percentiles: ≥50 participants and outlying counts**
  - p10<x<p90: Score 2
  - p5<x<p10 or p90<x<p95: Score 1
  - x<p5 or x>p95 Score 0




Robust method and indipendent from the distribution

# ISO/TS 22117:2010

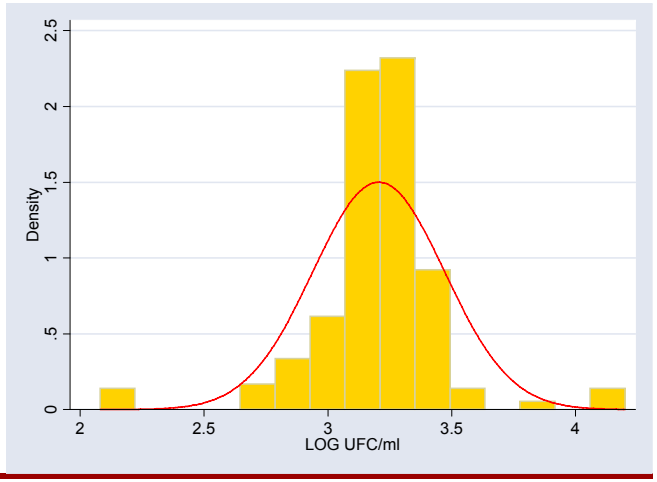
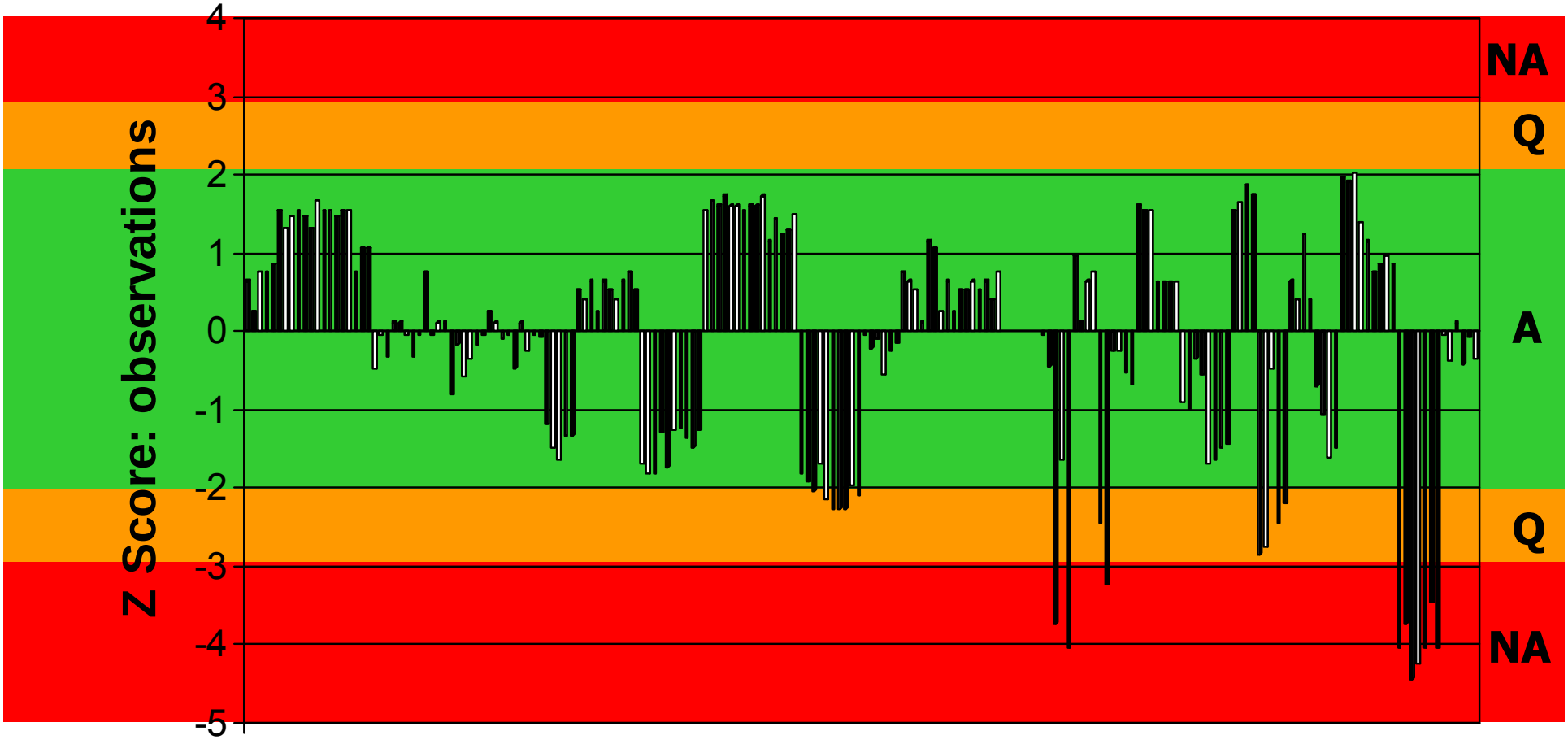
## Other methods of performance evaluation

**Median absolute deviation (MAD) from the data's median: <50 participants and outlying counts for new PT schemes with <100 participants**

- x within ± 2 $\sigma_{MAD}$: Score 2
- x between ± 2 $\sigma_{MAD}$ and ± 2.58 $\sigma_{MAD}$ : Score 1
- x outside ± 2.58 $\sigma_{MAD}$: Score 0

$$MAD = median_i(|X_i - median_j(X_j)|)$$
$$\sigma_{MAD} = K \cdot MAD$$

if K=1.486 and the distribution of data is normal $\sigma_{MAD}$ is consistent estimator for σ



# Example: *Enterobacter* spp.