Istituto Zooprofilattico Sperimentale delle Venezie

Proposed statistical analysis to evaluate the qualitative PT of Salmonella serotyping

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Scheme of proficiency testing "AQUA"

- Diagnostic Microbiology
  (Responsable dr. Michela Corrò)
- Food Microbiology
  (Responsable dr. Maria Grimaldi)

Accreditated ISO/IESC 17043:2010

- Parassitology shellfishes
  (Reference laboratory for Fish health - Responsable dr. Giuseppe Arcangeli)
- Bacteriology and virology of water organisms
  (Reference laboratory for Fish health - Responsable dr. Amedeo Manfrin)
- Virology and Serology for avian flu and Newcastle disease
  (Reference laboratory for avian flu and Newcastle desesae - Responsable dr. Calogero Terregino)
- Serology and molecular biology for bovine and suine diagnostic
  (Responsable dr. Stefano Nardelli)

- **Isolation and *Salmonella* serotyping**
  (Reference laboratory for Salmonella – Responsable dr. Antonia Ricci)

Isolation and *Salmonella* serotyping PT

- **Isolation**:

Positive/Negative sample for *Salmonella* spp.

- ***Salmonella* serotyping**:

  - Identification of somatic antigens
  - Identification of flagellar antigens

Serotype →

S. Typhimurium
S. Bredney
S. Virchow
S. Thompson
…….

2500

Salmonella serotyping PT

- 20 *Salmonella* strains are analysed from each participant
- Routine method is used to analyse the samples

- Results trasmission by e-mail

Standard method is **NOW** available (2014/07/15)
(ISO/TR 6579-3: Guide for serotyping of *Salmonella* spp.)

TECHNICAL REPORT ISO/TR 6579-3

First edition 2014-07-15

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —

Part 3:
Guidelines for serotyping of *Salmonella* spp.

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche, le dénombrement et la sérotypie des Salmonella — Partie 3: Lignes directrices pour la sérotypie des Salmonella spp.

Results for each laboratory

Strain	Somatic antigen	Flagellar antigens	Serotype
1	3,{10}{15}{15,34}	y:1,5	S. Orion
2	6,7,14	k:1,5	*S.* Thompson
3	6,7,14	r :1,2	*S.* Virchow
4	1,4,[5],12	e,h : 1,2	*S.* Typhimurium
5	1,9,12	g,m : -	*S.* Enteritidis
6	1,9,12	g,m : -	*S.* Enteritidis
.			
.			
20	1,4,[5],12	e,h : 1,2	*S.* Typhimurium

Salmonella serotyping PT evaluation

- **Performance of each participant**: Agreement between observed and expected serotypes

- **Performance of overall PT:** Agreement among all answers of all participants

Cohen's K statistics

Cohen's K statistics

K for 2 or more Raters on a 2 or multiple -Level Measurement Scale

- **2 Raters**: results of 2 operators or laboratories, expected vs observed results, results of 2 different methods
- **More Raters**: results of more operators or laboratories, more observed results of one laboratory, results of more different methods

- **2-Level Measurement Scale**: +/-, true/false, presence/absence
- **Multiple-Level Measurement Scale:** an arbitrary number *q* (greater than 2) of nominal or ordinal response categories
  - **Ordinal**: absent, mild, moderate, severe; low, medium, hight;
  - **Nominal**: different pathologies, diagnosis, categories

K in *Salmonella* serotyping PT

K for 2 Raters on a nominal Multiple-Level Measurement Scale: individual evaluation

- **2 Raters:** observed vs expected results for each laboratory
- **Multiple-Level Measurement Scale:** nominal response categories as serotype for an overall of 20 strains

K for *p* Raters on a nominal Multiple-Level Measurement Scale: overall evaluation

- ***p* Raters**: *p* laboratories
- **Multiple-Level Measurement Scale:** nominal response categories as serotype for an overall of 20 strains

Cohen's K statistics

Which is the difference between the simple overall percentage of agreement and the K statistics?

The K statistics **adjusts** the overall percentage of agreement for the chance agreement

Example 1: % of agreement

Two doctors analyse 100 x-ray to classify them as pathological or normal:

- **Doctor 1**: he **KNOWS** to destinguish between pathological and normal,

he analyses the x- ray and decides: 4 pathological, 96 normal

- **Doctor 2**: he **DOESN'T** know to destinguish between pathological and normal, he analyses the x- ray and decides: 100 normal

Doctor 1		Doctor 2: Pat.	Doctor 2: Norm.	
	Pat.	0	0	0
	Norm.	4	96	100
		4	96	100

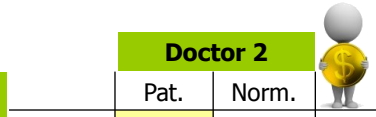
Observed agreement:
(0+96)/100=0.96 ⇔ 96%

Is it correct to say that the agreement is equal to 96%?

Example 2: % of agreement

Two doctors analyse 100 x-ray to classify them as pathological or normal:

- **Both the doctors (1 e 2)** flip a coin and decide: 50% pathological and 50% normal **in this way:**



Doctor 1 \ Doctor 2	Pat.	Norm.	
Pat.	25	25	50
Norm.	25	25	50
	50	50	100

Observed agreement :

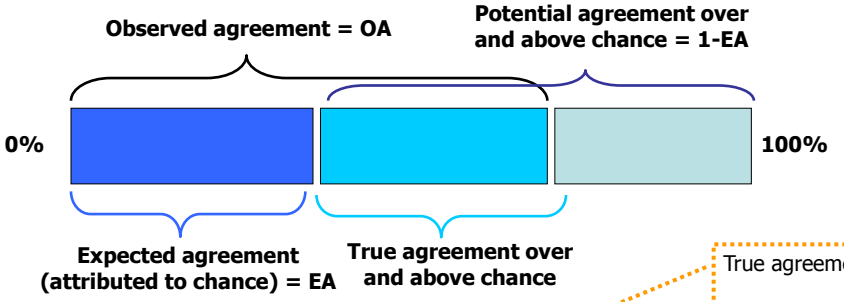
(25+25)/100=0.50 ⇔ 50%

Is it correct to say that the agreement is equal to 50%?

NO

A part of agreement depends on chance → **Remove the percentage of agreement attributed to chance**

Cohen's K



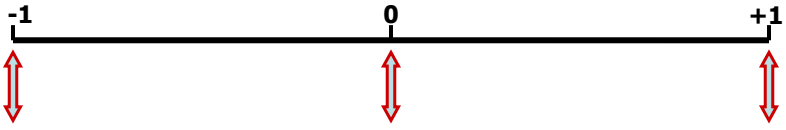
True agreement

Possible maximum agreement NOT chance

$$K = \frac{(OA - EA)}{(1 - EA)}$$

K= indicates the proportion of potential agreement, effectivelly achieved, excluding the chance

Cohen's K interpretation



Maximum disagreement

Observed agreement equal to chance agreement

Maximum agreement

Qualitative interpretation of K according to Landis and Koch scale

< 0	no agreement
0-0.20	slight agreement
0.21-0.40	fair agreement
0.41-0.60	moderate agreement
0.61-0.80	substantial agreement
0.81-1.0	almost perfect agreement.

Cohen's K calculation

1. **OA**, proportion of **observed** concordant results

$$OA = \frac{25+25}{100} = \frac{50}{100} = 0.5$$

2. **EA**, proportion of **expected** concordant results

$$EA = \left(\frac{50 \cdot 50}{100} + \frac{50 \cdot 50}{100} \right) / 100 = 0.5$$

		Doctor 2 result +	Doctor 2 Result -	
Doctor 1	result +	a_{11} 25	a_{12} 25	$a_{1.}$ 50
	result -	a_{21} 25	a_{22} 25	$1_{2.}$ 50
		$a_{.1}$ 50	$a_{.2}$ 50	$a_{..}$ 100

Conclusion:Agreement due to chance

3. $$K = \frac{(OA - EA)}{(1 - EA)} = \frac{0.5 - 0.5}{1 - 0.5} = 0 \neq 0.5$$

Generalization: Performance of each participant

Observed	Expected: result +	Expected: Result -	
result +	a_{11}	a_{12}	$\mathbf{a}_{1.}$
result -	a_{21}	a_{22}	$\mathbf{a}_{2.}$
	$\mathbf{a}_{.1}$	$\mathbf{a}_{.2}$	$\mathbf{a}_{..}$

K for 2 raters on a multiple -Level Measurement Scale

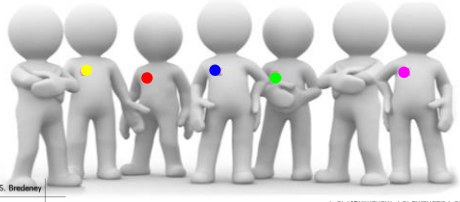
$$K = \frac{AO - AA}{1 - AA} = \frac{\sum_{i=1}^{J}(a_{..} \cdot a_{ii} - a_{i.} \cdot a_{.i})}{a_{..}^2 - \sum_{i=1}^{J}(a_{i.} \cdot a_{.i})}$$

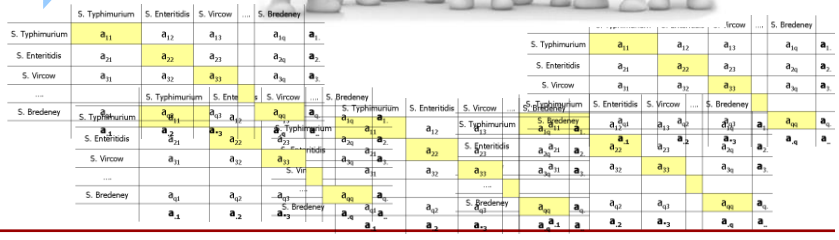
Observed results	Expected results: S. Typhimurium	S. Enteritidis	S. Vircow	....	S. Bredeney	
S. Typhimurium	a_{11}	a_{12}	a_{13}		a_{1q}	$\mathbf{a}_{1.}$
S. Enteritidis	a_{21}	a_{22}	a_{23}		a_{2q}	$\mathbf{a}_{2.}$
S. Vircow	a_{31}	a_{32}	a_{33}		a_{3q}	$\mathbf{a}_{3.}$
....						
S. Bredeney	a_{q1}	a_{q2}	a_{q3}		a_{qq}	$\mathbf{a}_{q.}$
	$\mathbf{a}_{.1}$	$\mathbf{a}_{.2}$	$\mathbf{a}_{.3}$		$\mathbf{a}_{.q}$	$\mathbf{a}_{..}$

Generalization: Performance of overall PT

Observed	Expected: result +	Expected: Result -	
result +	a_{11}	a_{12}	$\mathbf{a}_{1.}$
result -	a_{21}	a_{22}	$\mathbf{a}_{2.}$
	$\mathbf{a}_{.1}$	$\mathbf{a}_{.2}$	$\mathbf{a}_{..}$

K for MORE raters on a multiple -Level Measurement Scale





K statistic, significance and CI

K statistic

$$K = \frac{AO - AA}{1 - AA}$$

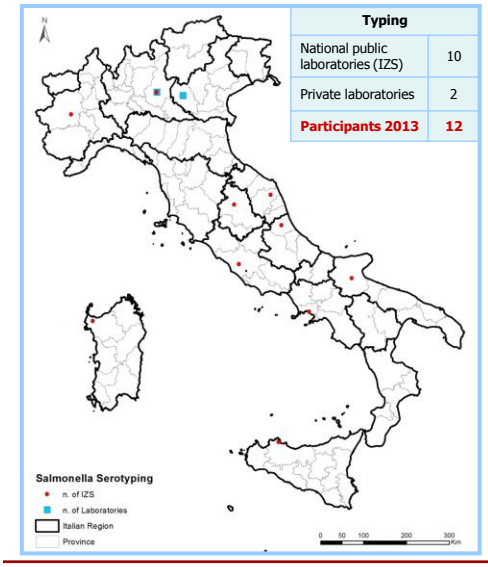
Significance (p-value)

$$z = \frac{\hat{K}}{s.e_0.(\hat{K})} \approx N(0,1)$$

Confidence interval

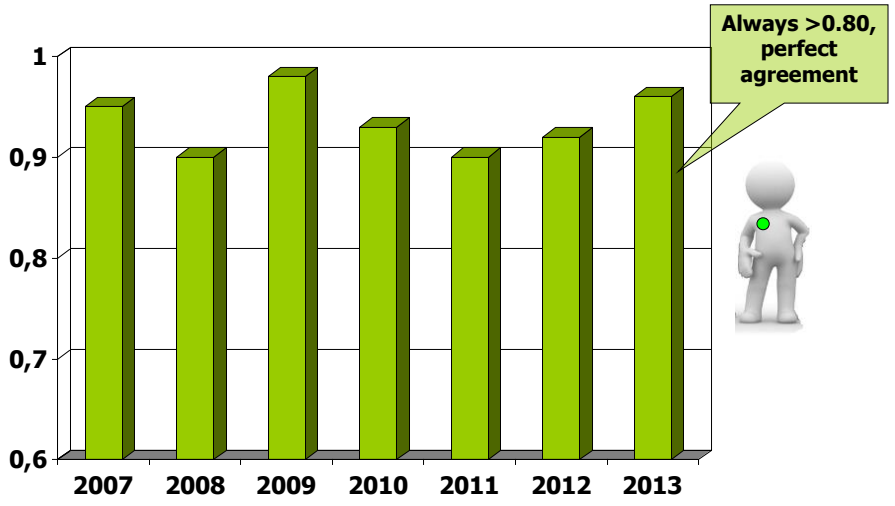
$$\hat{K} - z_{1-\alpha/2} \cdot s.e.(\hat{K}) \leq K \leq \hat{K} + z_{1-\alpha/2} \cdot s.e.(\hat{K})$$

Performance evaluation of *Salmonella* serotyping PT: 2013

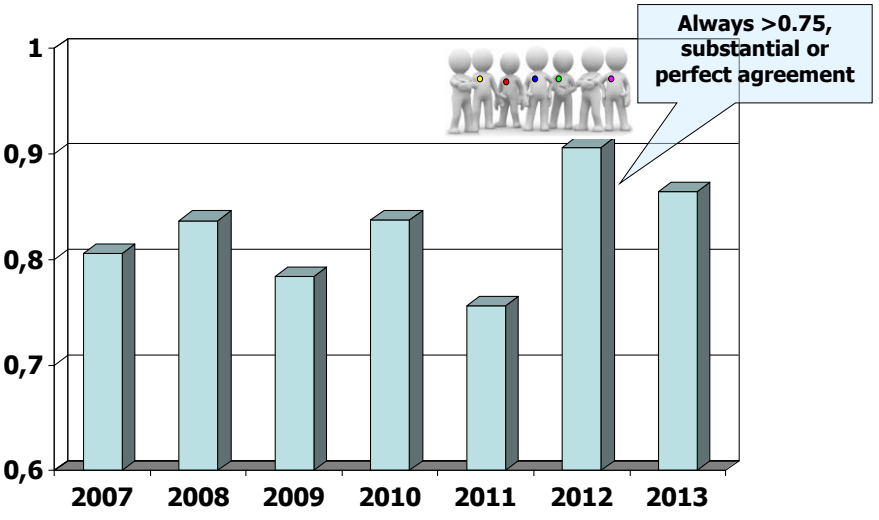


	K	p-value
LAB 1	0.89	0.00
LAB 2	0.83	0.00
LAB 3	1.00	0.00
LAB 4	0.94	0.00
LAB 5	1.00	0.00
LAB 6	0.78	0.00
LAB 7	1.00	0.00
LAB 8	0.89	0.00
LAB 9	0.94	0.00
LAB 10	1.00	0.00
LAB 11	0.89	0.00
LAB 12	1.00	0.00
Overall	0.86	0.00

Performance for each laboratory over time



Performance of PT over time



THANK YOU FOR YOUR ATTENTION