Chloramphenicol Proficiency Tests on a Global Scale – Unforeseen Consequences

Mark Sykes

Introduction - Contributors

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What is Proficiency Testing?

If I ask you to do an analysis, how do I know you're giving me the correct answer?

Validated method?

Internal QC?

Accreditation? (ISO 17025)

Bias against external reference

Operation of PT

Test material preparation
- Incurred (dosing study)
- Spiked

Homogeneity test
10 x 2 random samples
LC-MS/MS
ISO 17025 method

Analysis, reporting by participants

Data assessment (z-score)

PT Report

Reporting requirements

Report result corrected for recovery

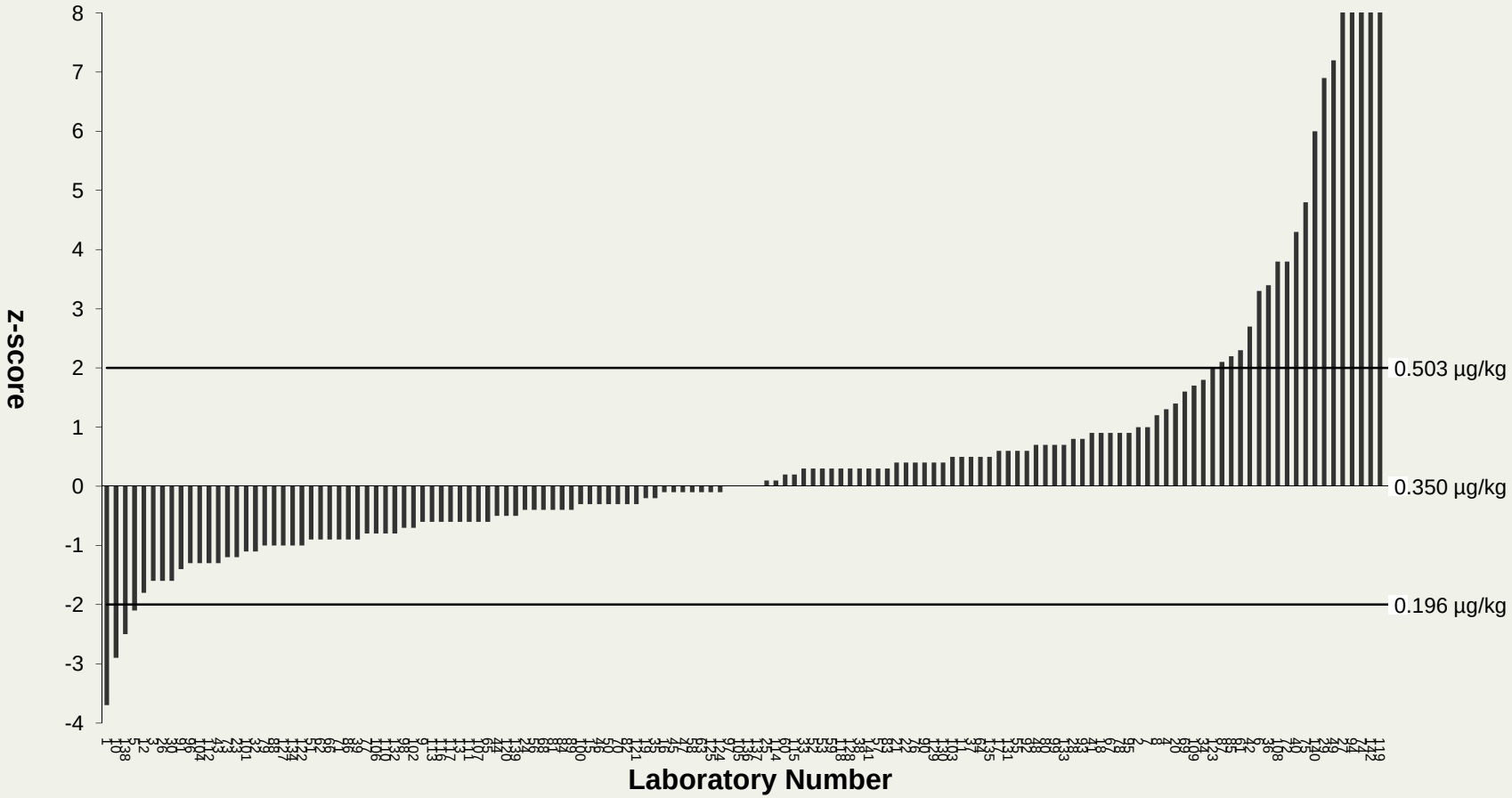
Report method of recovery correction
(internal standard, matrix-extracted calibration, recovery spike, standard addition)

Report CCβ (or LOQ)

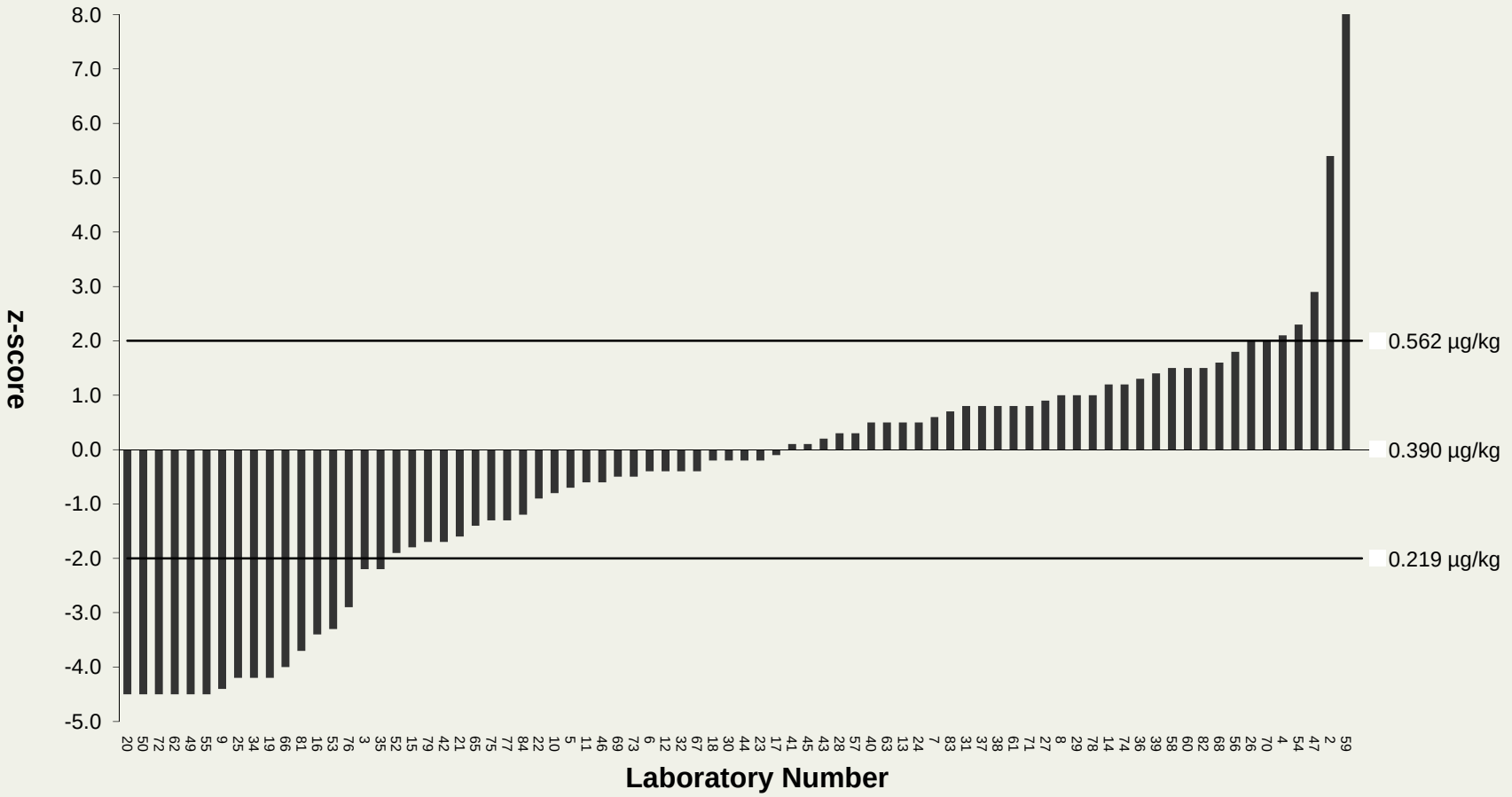
Report analytical method (optional)

Commission Decision 2002/657/EC

Typical results, PT 02230



Atypical results, PT 02256



Trend analysis

ELISA kits vs LC-MS/MS

ELISA labs	Result	Int std or rec	CCB ug/kg
1	<0.2	N	0.2
9	0.015	0	0
19	0.033	61.36%	
33	<0.30	Y95.8%	0.3
37	0.46		
48	<0.2	N	
49	0.004		
51	<LOQ		0.3
62	0.003	Y 108	0.03
80	<0.006	95.0	

AV	0.390
sp	0.086
z -2	0.219
z +2	0.562

Why ELISA kits affected?

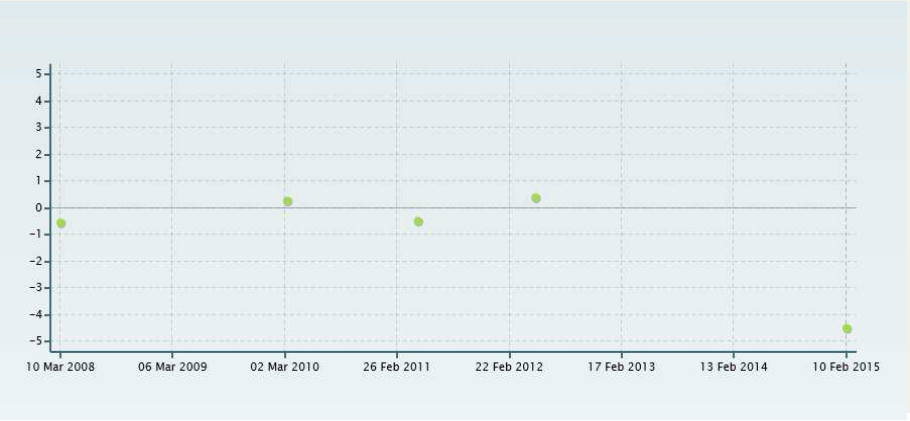
Type of prawn?
Pacific White Shrimp (*Litopenaeus vannamei*)

Spiking protocol?
No change, similar concentrations

Homogeneity test flawed?
LC-MS/MS data agrees

Statistical anomaly?

ELISA kits flawed?



R-Biopharm investigation

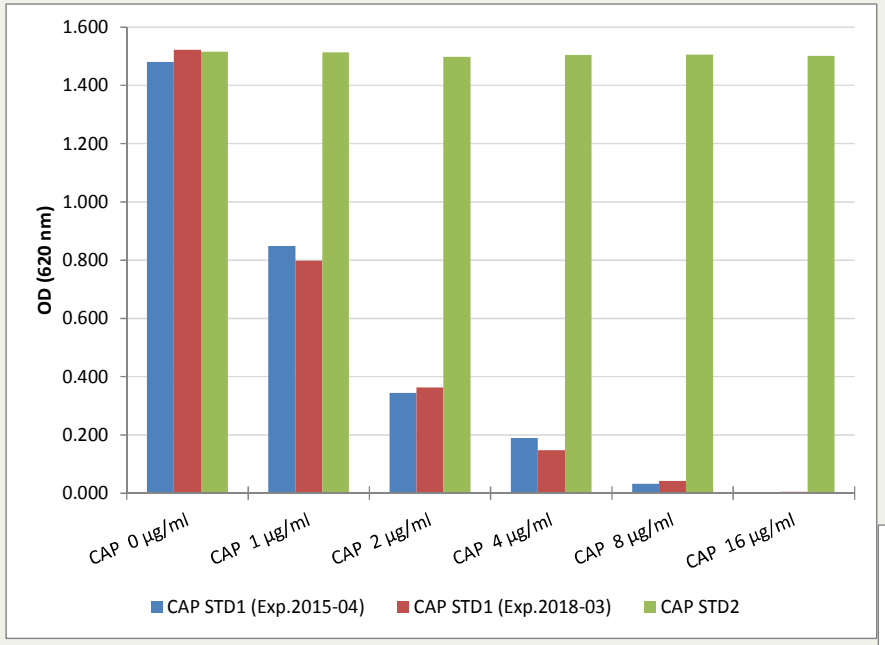
2 CAP standards purchased

STD1 (RIDASCREEN® CAP ELISA kit)

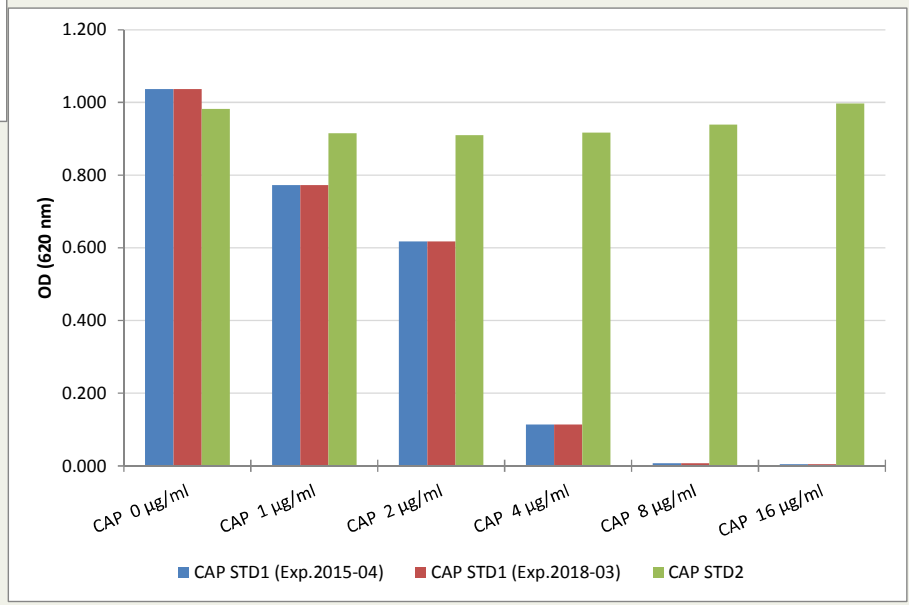
STD2 same lot number as PT02256

Sample	Sample treatment	Result [µg/kg]
FAPAS Sample 02256	Spiked with CAP STD1 0.1 µg/kg	0.095
Buffer	Blank	< LOD
Buffer	Spiked with CAP STD2 0.1 µg/kg	< LOD
Buffer	Spiked with CAP STD2 0.5 µg/kg	< LOD
Buffer	Spiked with CAP STD2 1.0 µg/kg	< LOD
Buffer	Spiked with CAP STD1 0.1 µg/kg	0.084
Buffer	Spiked with CAP STD1 0.5 µg/kg	0.54

Microbial inhibition (R-Biopharm)

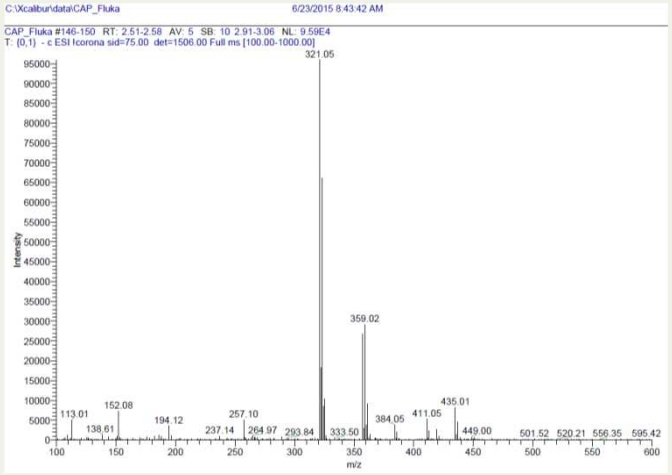


Response of *E. coli*



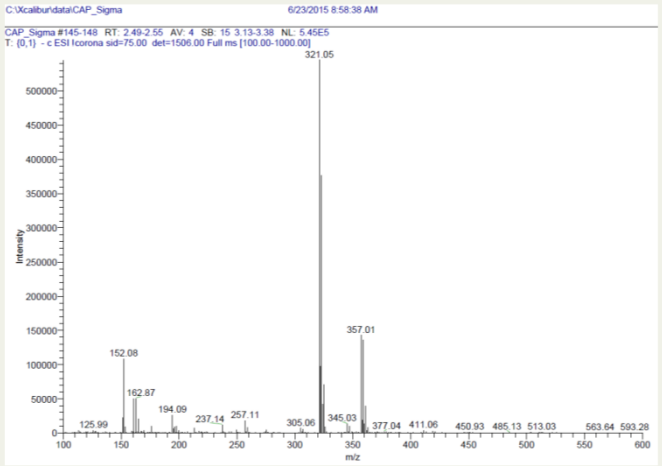
Response of *S. aureus*

Mass spectrometry analysis (R-Biopharm)



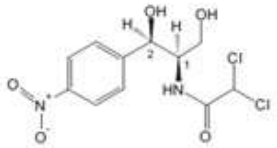
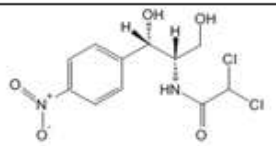
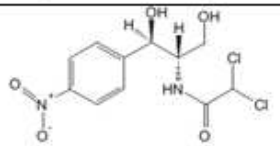
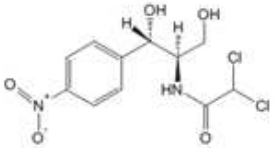
STD2

Supported by NMR analysis

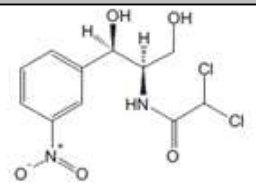
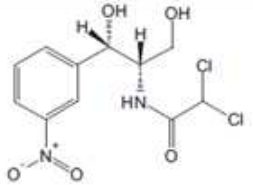
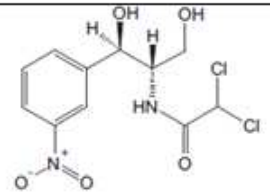
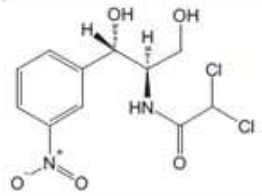


STD1

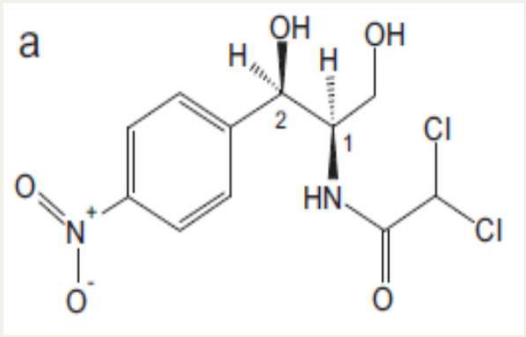
Hypothesis – stereoisomers?

Configuration	Stereoisomer	Structure	Trivial name	Biological activity	Antimicrobial activity
para	RR-p-CAP		chloramphenicol or levomycetin	yes	yes
	SS-p-CAP		dextramycin	yes	no
	RS-p-CAP		synthomycin	yes	no
	SR-p-CAP		synthomycin	yes	no

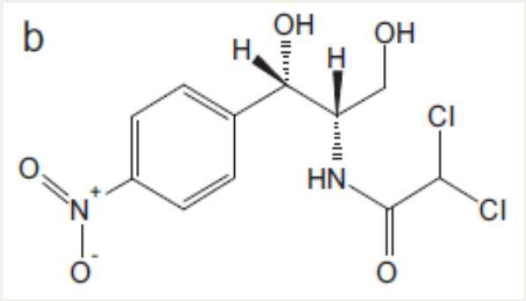
meta-stereoisomers

Configuration	Stereoisomer	Structure	Trivial name	Biological activity	Antimicrobial activity
meta	RR-m-CAP		none	no	no
	SS-m-CAP		none	no	no
	RS-m-CAP		none	no	no
	SR-m-CAP		none	no	no

Chiral analysis (R-Biopharm-RIKILT)



RR-p-CAP Chloramphenicol
CAS 56-75-7
Antimicrobial activity



SS-p-CAP Dextramycin
CAS 56-75-7
No antimicrobial activity

Commission Decision 657/2002 - discuss

Summary

Standard materials labelled as CAP might be different

Most labs use LC-MS/MS

Most labs not using chiral LC

ELISA kits are stereoisomer-specific

How will the vet medicines community address the stereoisomer question?