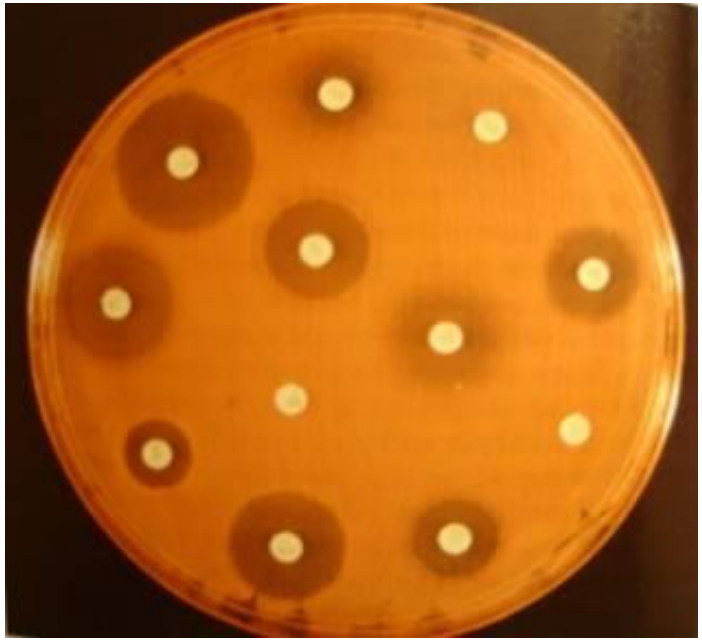
Antimicrobial Susceptibility Testing (AST) : establishing clinical breakpoint CLSI vs. VETCAST/EUCAST

Pierre-Louis Toutain,
Ecole Nationale Vétérinaire
National veterinary School of Toulouse, France
Wuhan 12/10/2015

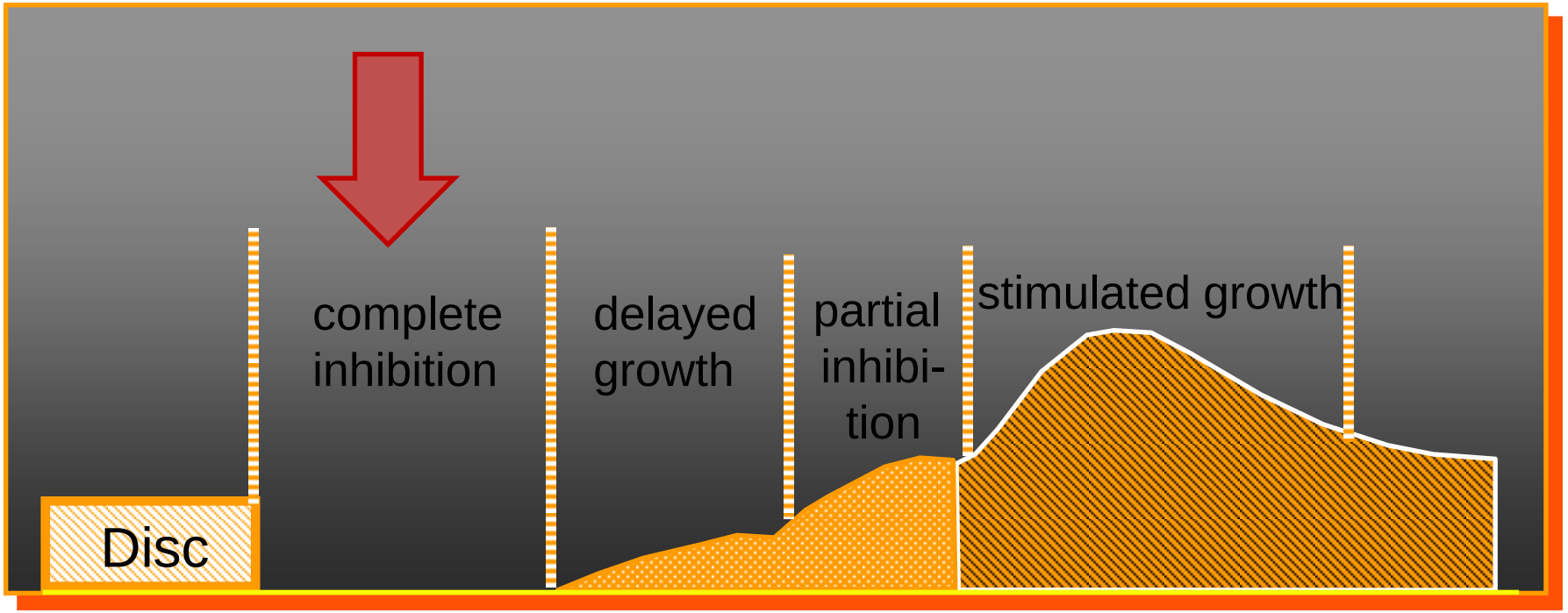
Purpose of AST
(**A**ntimicrobial **S**usceptibility **T**esting)

- To guide the clinical selection of an effective therapeutic intervention for the treatment of microbial infections based upon the results of *in vitro* susceptibility tests (AST) (the word antibiogram is also use).

The disc technic



Growth around the disc



Large diameter of inhibition=susceptible=S
Small diameterof inhibition=resistant=R

Clinically susceptible(s): EUCAST Definitions

- A micro-organism is defined as susceptible by a level of activity associated with a high likelihood of therapeutic success

How to decide R and S?

Glossary

- **Breakpoint (BP) (clinical)**
  - A numerical value for S or I or R
  - A decision (*risk management*) taking into account CO values plus other considerations (harmonization…)
- **Cutoffs (CO)**
  - Numerical values to decide of the BP
    - Epidemiological or microbiological CO (CO_{WT})
    - PK/PD CO (CO_{PD})
    - Clinical CO (CO_{CL})
  - CO are computed (*risk assessment*)

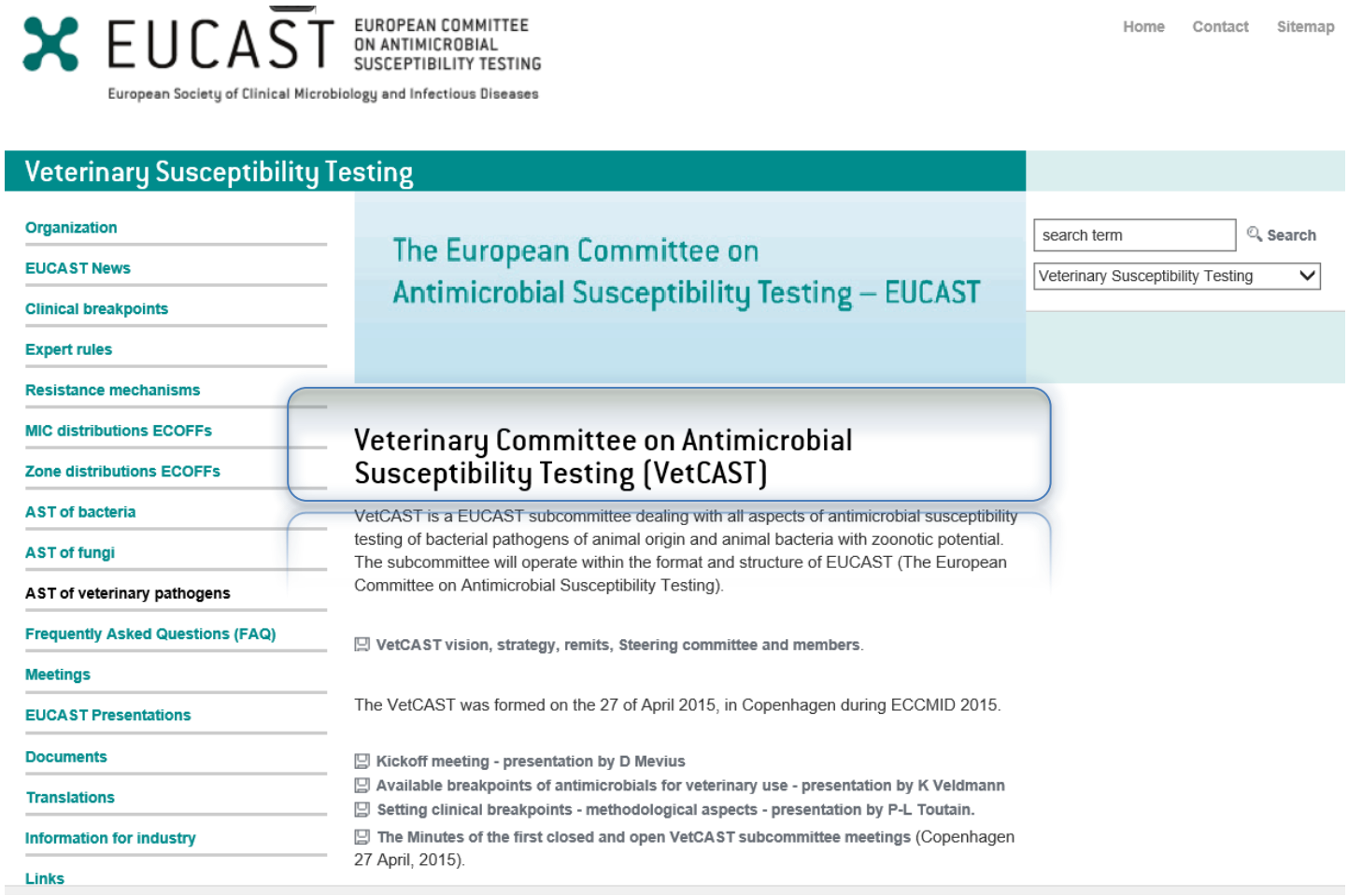
Two international organizations

EUCAST
(see their website)

Remits of EUCAST

1. To harmonise clinical breakpoints for existing antimicrobial agents in Europe,
2. To determine clinical breakpoints for new agents,
3. To set epidemiological (microbiological) breakpoints (cut-off),
4. To revise breakpoints as required,
5. To harmonise methodology for antimicrobial susceptibility testing,
6. To develop a website with MIC and zone diameter distributions of antimicrobial agents for a wide range of organisms
7. To liaise with European governmental agencies and European networks involved with antimicrobial resistance and resistance surveillance.

VETCAST, a subcommittee of EUCAST



What is VETCAST?

- VETCAST is a EUCAST subcommittee
  - It deals with all aspects of antimicrobial susceptibility testing (AST) of bacterial pathogens of animal origin and animal bacteria with zoonotic potential.
  - The subcommittee will operate within the format and structure of EUCAST (The European Committee on Antimicrobial Susceptibility Testing).

The 2 main objectives of VetCAST

1. To determine antimicrobial breakpoints specific to the veterinary field;
   - Currently, absence of animal species-specific breakpoints
2. To harmonize veterinary antimicrobial susceptibility testing in the European Union (EU);

VETCAST for EMA

- To provide advice on the type and quality of the MIC, pharmacokinetic (PK) and clinical data needed for setting clinical breakpoints
- To define clinical MIC breakpoints for new veterinary antimicrobial agents
- To revise breakpoints for generic drugs
- To advice on the bacterial spectrum of veterinary antimicrobial agents

CLSI/VAST
(NCCLS)

CLSI: VAST

- The CLSI Veterinary Antimicrobial Susceptibility Testing (VAST) committee is in charge in defining clinical breakpoints for new and generic veterinary antimicrobial agents.

CLSI ‘s VAST

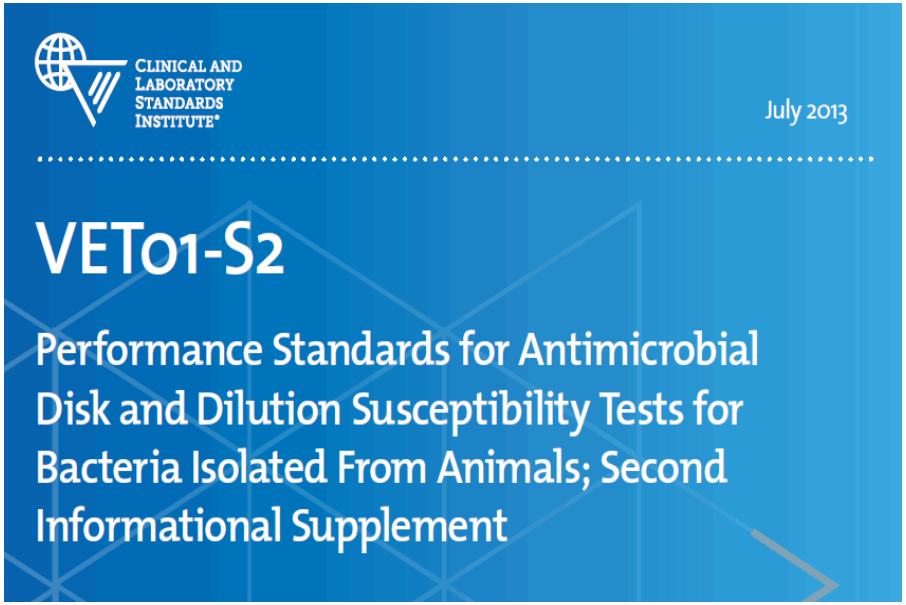
About CLSI’s Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST)

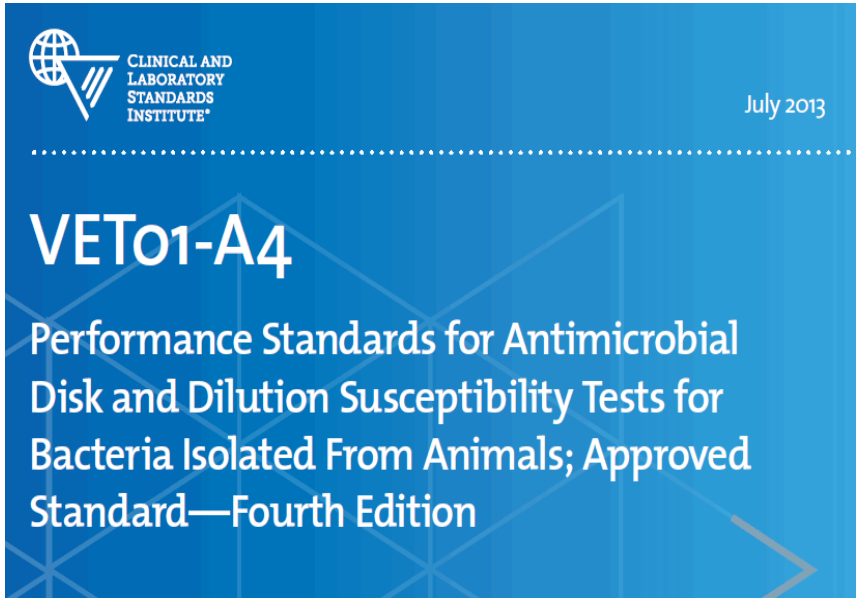
Volunteers for the Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) collaborate to develop standards and guidelines that **promote accurate antimicrobial susceptibility testing and appropriate reporting.**

CLSI/VAST: members

- Individuals from the following types of areas join together to pool knowledge, expertise, and viewpoints:
  - Veterinary microbiology laboratories
  - Government agencies (FDA)
  - Veterinary care providers and educators
  - Pharmaceutical and diagnostic microbiology laboratories

CLSI released many documents explaining how they proceed





Add to your basket (not free of charge as for EUCAST)

CLSI/VAST approved breakpoints for label applications in food animals

Table 1: CLSI/VAST approved breakpoints for label applications in food animals.

		Zone Diameter (mm)			Concentrations (µg/ml)			
Antimicrobial	Disease/Pathogen(s)	S	I	R	S	I	R	Extended dilutions
Ceftiofur	**Bovine respiratory disease** - *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* **Swine respiratory disease** - *Actinobacillus pleuropneumoniae, Pasteurella multocida, Salmonella choleraesuis, Streptococcus suis*	≥21	18-21	≤8	≤2	4	≥8	0.5-8
Ceftiofur (intramammary)	**Bovine mastitis** - *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli*	≥21	18-21	≤8	≤2	4	≥8	0.5-8
Enrofloxacin	**Bovine respiratory disease** - *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*	≥21	17-20	≤16	≤0.25	0.5-1	≥2	0.12-2
Florfenicol	**Bovine respiratory disease** - *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* **Swine respiratory disease** – *Actinobacillus pleuropneumoniae, Pasteurella multocida, Streptococcus suis* Type 2.	≥19	15-18	≤14	≤2	4	≥8	0.25-8
Florfenicol	**Swine respiratory disease** – *Salmonella choleraesuis*	---	---	---	≤4	8	≥16	0.25-8
Penicillin/ Novobiocin	**Bovine mastitis** – *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis*	≥18	15-17	≤14	≤1/2	2/4	≥4/8	---
Pirlimycin	**Bovine mastitis** - *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis*	≥13	---	≤12	≤2	---	≥4	---
Spectinomycin sulfate	**Bovine respiratory disease** - *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*	≥14	11-13	≤10	≤32	64	≥128	8-64
Tiamulin	**Swine respiratory disease** - *Actinobacillus pleuropneumoniae*	≥9	---	≤8	≤16	---	≥32	4-32
Tilmicosin	**Bovine respiratory disease** - *Mannheimia haemolytica*	≥14	11-13	≤10	≤8	16	≥32	4-32
Tilmicosin	**Swine respiratory disease** - *Pasteurella multocida, Actinobacillus pleuropneumoniae*	≥11	---	≤10	≤16		≥32	4-32

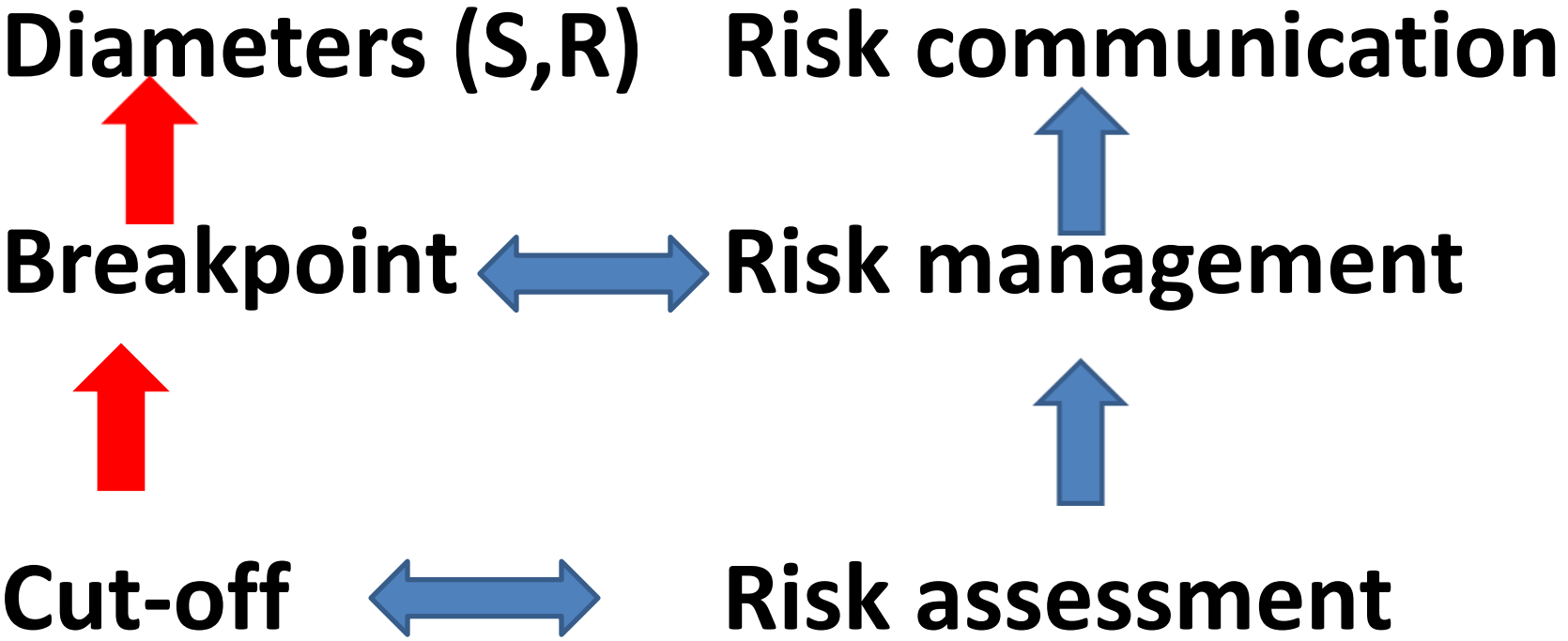
A given animal species

A given disease

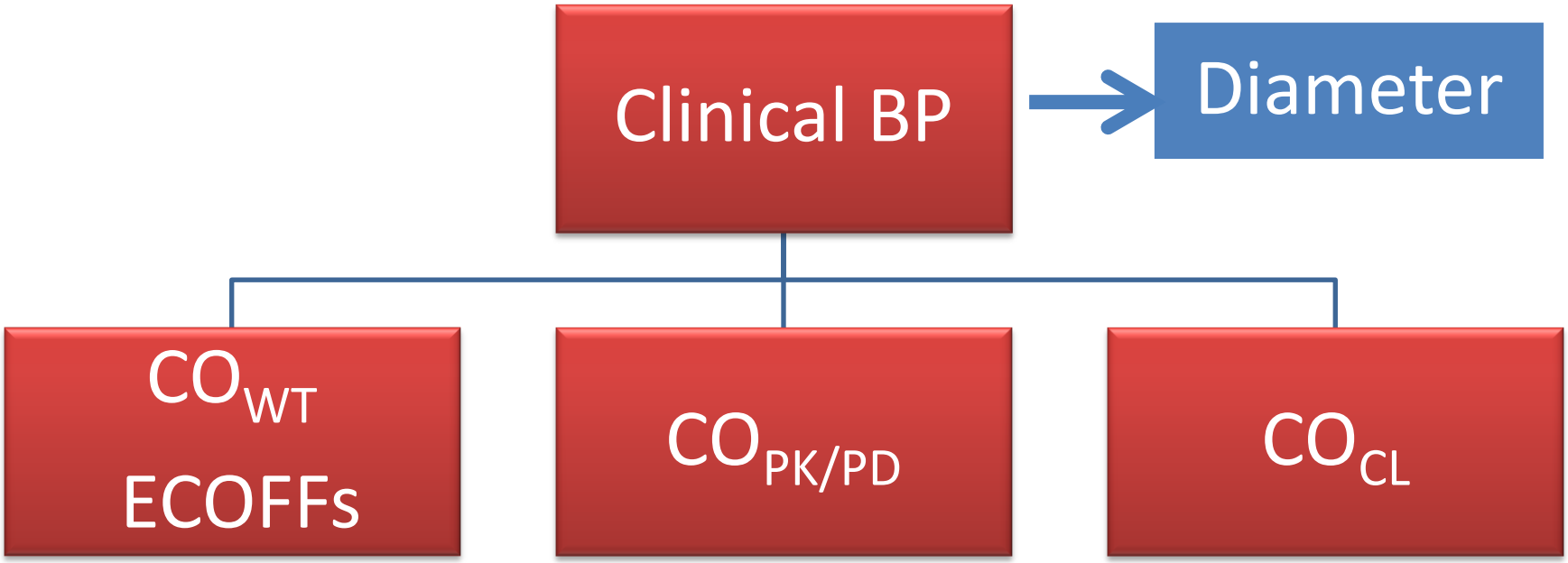
2 given pathogens

Setting clinical breakpoint
Methodological aspects

Glossary: risk analysis



Development of a (clinical) Breakpoint (BP)



Epidemiological cut-off values

(CO_{WT} , ECOFFs)

Epidemiological cut-off values (ECOFFs)

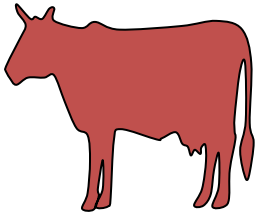
- ECOFFs (or microbiological breakpoints) are related to the distribution of MICs of wild type organisms lacking acquired or mutational resistance to the antimicrobial agent in question.
- The ECOFF is essentially the upper MIC value of the wild type distribution.

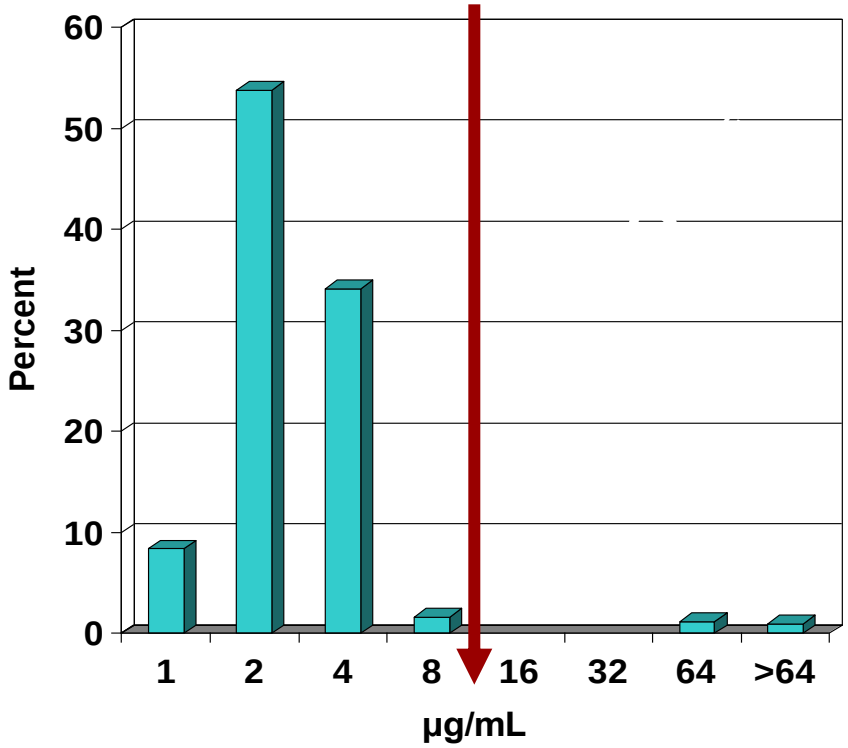
ECOFFs: SOP

- ECOFFs are defined for relevant species according to procedures detailed in in the introduction to the MIC and zone diameter distributions website and in EUCAST SOP 7.0 (in preparation).

The epidemiological cutoff: ECOFF/COWT

The case of unimodal distribution

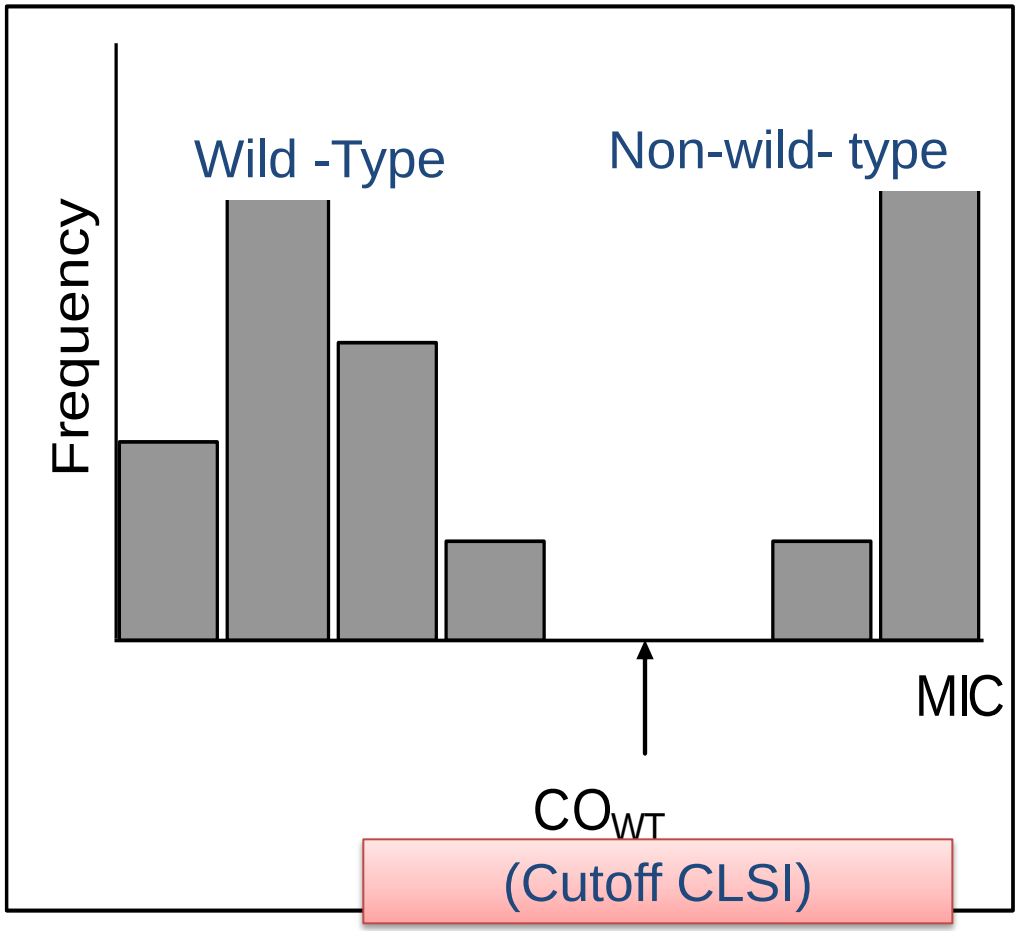




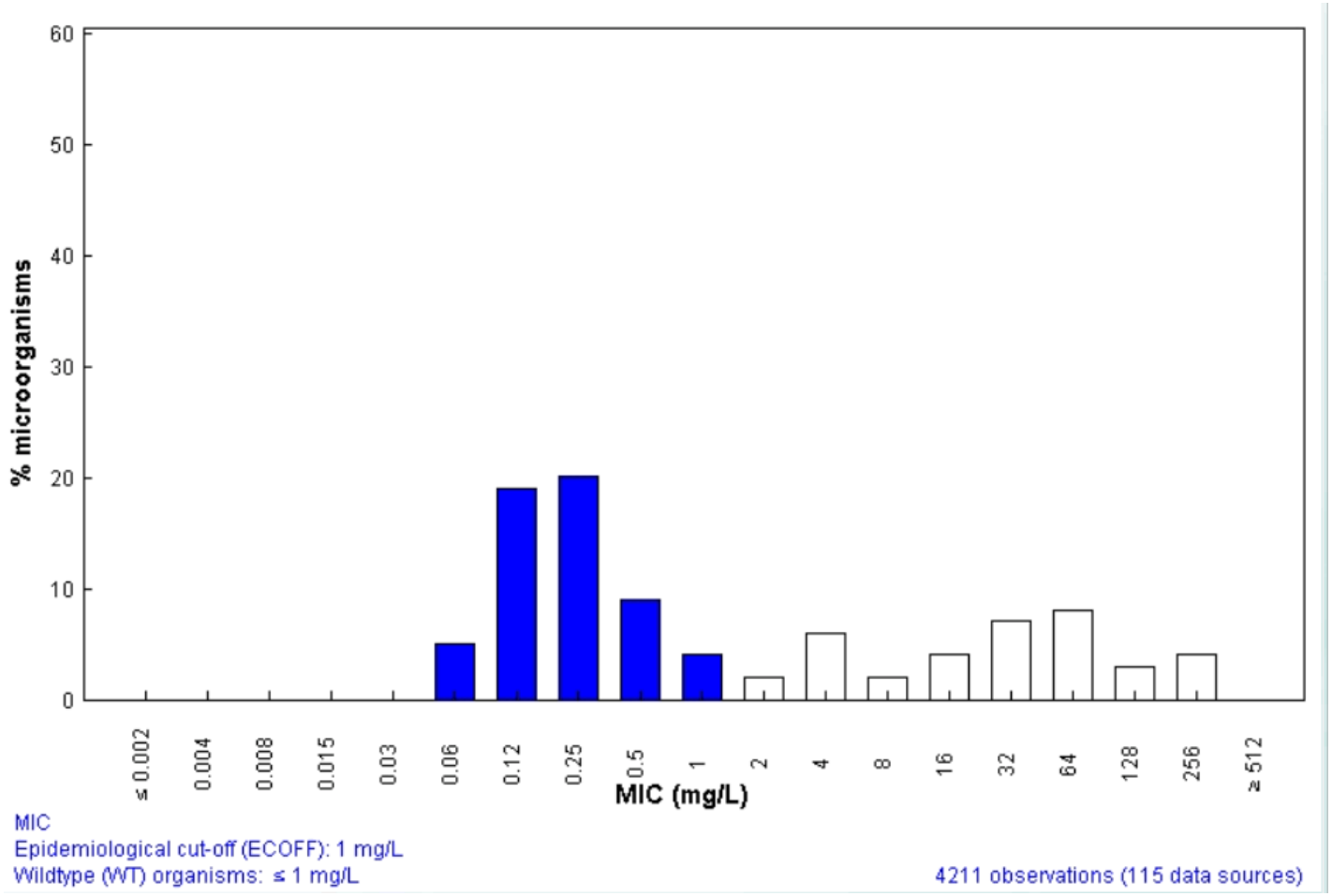
e.g., MIC distributions for tulathromycin with 728 strains of *M. haemolytica* isolated from bovine respiratory disease. Note the "wild-type" population with MIC values ≤8 µg/ml.

The epidemiological cutoff: ECOFF/CO_{WT}

Bimodal distribution



Ciprofloxacin & Acinetobacter baumannii



Statistical method for ECOFF/CO_{WT}

ORIGINAL ARTICLE

10.1111/j.1469-0691.2006.01377.x

Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values

J. Turnidge[1], *G. Kahlmeter*[2] *and G. Kronvall*[3]

RangeFinder and ECOFFinder

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2010, p. 4445–4452 Vol. 48, No. 12
0095-1137/10/$12.00 doi:10.1128/JCM.01101-10

Normalized Resistance Interpretation as a Tool for Establishing Epidemiological MIC Susceptibility Breakpoints[▽]

Göran Kronvall*

Department of Microbiology and Tumor Biology–MTC, Clinical Microbiology, Karolinska Institutet, Karolinska University Hospital Solna, Stockholm, Sweden

Why no to split a wild population

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2009, p. 1628–1629 Vol. 53, No. 4
0066-4804/09/$08.00+0 doi:10.1128/AAC.01624-08

Breakpoints for Susceptibility Testing Should Not Divide Wild-Type Distributions of Important Target Species▽

Maiken Cavling Arendrup,[1]* Gunnar Kahlmeter,[2] Juan Luis Rodriguez-Tudela,[3] and J. Peter Donnelly[4]

Unit of Mycology and Parasitology, Statens Serum Institut, Copenhagen, Denmark[1]; Department of Clinical Microbiology, Central Hospital, Växjö, Sweden[2]; Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain[3]; and Department of Haematology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands[4]

Received 10 December 2008/Returned for modification 7 January 2009/Accepted 25 January 2009

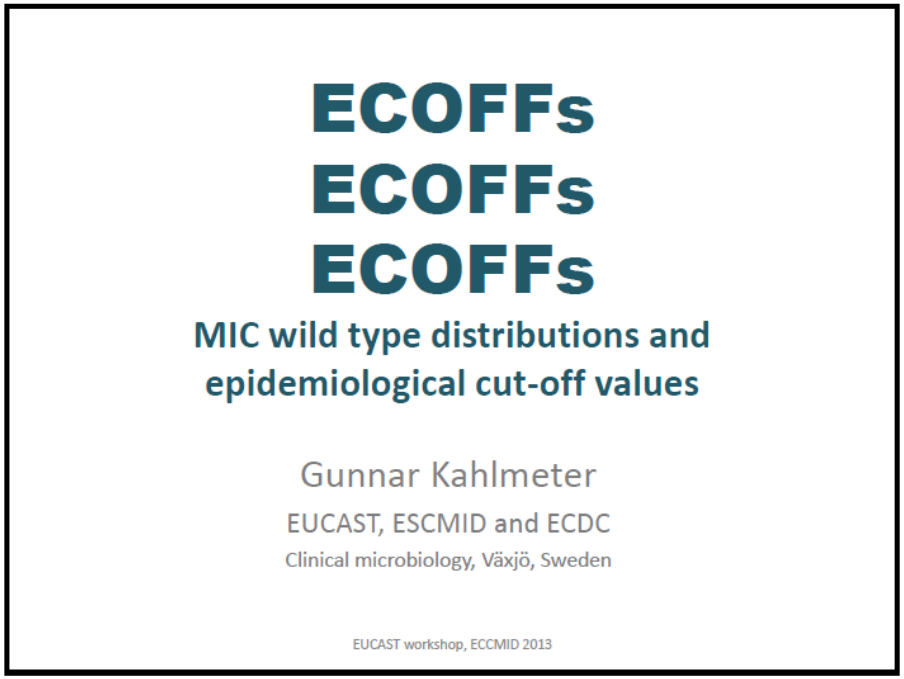
The fluconazole MIC distributions for *Candida glabrata* from testing 34 different clinical isolates and performing 51 tests on a single isolate mirrored each other. Since what is perceived as biological variation in isolates without resistance mechanisms is mainly methodological variation, breakpoints which divide this distribution not only lack a sound biological basis but also result in poor reproducibility of susceptibility characterization. This makes 2, 4, 8, and possibly 16 μg/ml unsuitable breakpoints for *C. glabrata* and fluconazole.

MIC-data available tetra/doxycycline EUCAST

	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
Lactobacillus reuteri	0	0	0	0	0	0	0	0	0	0	0	1	7	19	26	1	5	31	22	ND
Lactobacillus rhamnosus	0	0	0	0	0	0	0	0	45	75	85	5	3	1	3	5	2	1	0	ND
Lactobacillus sakei	0	0	0	0	0	0	0	0	0	0	4	27	29	16	3	1	0	3	0	ND
Lactococcus lactis	0	0	0	0	0	1	48	23	5	1	0	0	0	0	0	3	2	6	0	ND
Listeria monocytogenes	0	0	0	0	0	2	6	20	76	12	0	0	0	0	0	0	0	0	0	1.0
Mannheimia haemolytica	0	0	0	0	0	0	0	3	29	23	1	1	2	16	58	11	4	0	0	2.0
Moraxella catarrhalis	0	0	0	0	0	2	172	3888	3357	807	256	114	36	20	26	1	1	0	0	2.0
Morganella morganii	0	0	0	0	0	0	0	0	6	40	31	18	5	4	23	15	25	3	0	8.0
Neisseria gonorrhoeae	0	0	0	38	39	112	382	497	932	1172	1314	366	116	370	298	93	37	6	3	ND
Neisseria meningitidis	0	0	0	0	0	0	0	1	27	28	0	0	0	0	0	0	0	0	0	ND
Pasteurella multocida	0	0	0	0	0	3	19	111	54	44	18	9	17	40	13	7	1	0	0	2.0
Propionibacterium acnes	0	0	0	0	5	0	7	25	164	93	5	1	3	0	1	0	0	0	0	ND
Proteus mirabilis	0	0	0	0	0	0	0	0	1	7	4	2	5	81	715	172	28	1	0	128.0
Proteus spp	0	0	0	0	0	0	0	0	0	3	13	3	2	8	4	2	0	15	0	ND
Proteus vulgaris	0	0	0	0	0	0	0	0	1	1	2	7	0	3	4	0	0	0	0	ND
	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	2	4	1	39	172	157	63	0	0	0	0	ND
Raoultella spp	0	0	0	0	0	0	0	0	3	29	9	3	1	0	0	1	3	0	0	ND
Raoultella spp	0	0	0	0	0	0	0	0	1	8	1	1	1	0	9	0	0	0	0	ND
Salmonella spp	0	0	0	0	0	0	0	1	127	1001	5466	814	38	32	338	609	296	0	0	8.0
Serratia liquefaciens	0	0	0	0	0	0	0	0	0	5	11	34	26	4	5	1	0	0	0	ND
Serratia marcescens	0	0	0	0	0	0	0	0	1	1	4	25	12	12	20	0	0	0	0	ND
Serratia spp	0	0	0	0	0	0	0	0	0	0	2	8	82	13	6	13	13	3	0	32.0
Staphylococcus aureus	0	0	0	0	0	0	54	502	765	79	5	1	5	6	32	194	126	110	0	1.0
Staphylococcus aureus MRSA	0	0	0	0	0	0	0	3	345	52	2	0	0	0	4	4	1	0	0	1.0
Staphylococcus aureus MSSA	0	0	0	0	0	0	1	9	515	26	2	1	1	1	7	10	3	3	0	1.0
Staphylococcus coagulase negative	0	0	0	0	0	0	32	130	171	15	78	23	5	7	40	5	13	16	0	1.0
Staphylococcus coagulase negative MRSE	0	0	0	0	0	0	0	23	129	21	178	63	21	4	8	26	23	32	0	1.0
Staphylococcus epidermidis	0	0	0	0	0	0	1	52	98	26	126	33	1	3	9	10	9	8	0	1.0
Staphylococcus epidermidis MSSE	0	0	0	0	0	0	0	16	33	1	20	3	0	1	1	5	4	0	0	1.0
Staphylococcus haemolyticus	0	0	0	0	0	0	1	7	2	16	13	5	7	1	0	4	3	7	0	ND
	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
Staphylococcus saprophyticus	0	0	0	0	0	0	0	2	14	2	0	0	0	0	0	0	0	0	0	ND
Stenotrophomonas maltophilia	0	0	0	0	0	0	0	0	1	2	1	15	63	79	31	1	0	0	0	ND
Streptococcus agalactiae	0	0	0	0	0	0	8	11	33	13	0	1	1	12	93	153	33	0	0	1.0
Streptococcus anginosus	0	0	0	0	0	0	0	20	31	5	9	6	6	2	3	1	1	1	0	ND
Streptococcus group G	0	0	0	0	0	0	0	3	61	33	10	6	18	8	9	29	11	0	0	2.0
Streptococcus oralis	0	0	0	0	0	0	7	43	76	15	3	2	0	8	16	22	4	0	0	2.0
Streptococcus pneumoniae	0	0	0	1	4	173	2934	6909	1878	120	65	75	97	306	939	392	14	6	0	1.0
Streptococcus pyogenes	0	0	0	0	12	200	1018	498	264	50	6	11	17	94	179	91	6	0	0	1.0
Streptococcus thermophilus	0	0	0	0	0	0	24	46	27	17	0	0	0	7	7	0	0	0	0	ND
Streptococcus, viridans group	0	0	0	0	0	1	12	98	164	63	23	17	18	25	71	60	20	4	0	2.0
Yersinia enterocolitica	0	0	0	0	0	0	0	0	2	171	267	20	1	0	0	0	0	1	4	4.0
Yersinia spp	0	0	0	0	0	0	0	0	0	11	37	4	0	0	0	0	1	0	0	ND

For more details

Kahlmeter - ECOFFs, ECOFFs, ECOFFs



The setting of a PK/PD cutoff

ORIGINAL ARTICLE BACTERIOLOGY

The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach

J. W. Mouton[1], D. F. J. Brown[2], P. Apfalter[3], R. Cantón[4], C. G. Giske[5], M. Ivanova[6], A. P. MacGowan[7], A. Rodloff[8], C.-J. Soussy[9], M. Steinbakk[10] and G. Kahlmeter[11]

The setting of a PK/PD CO

Step1

Selection of a PK/PD index predictive of clinical efficacy and/or prevention of resistance

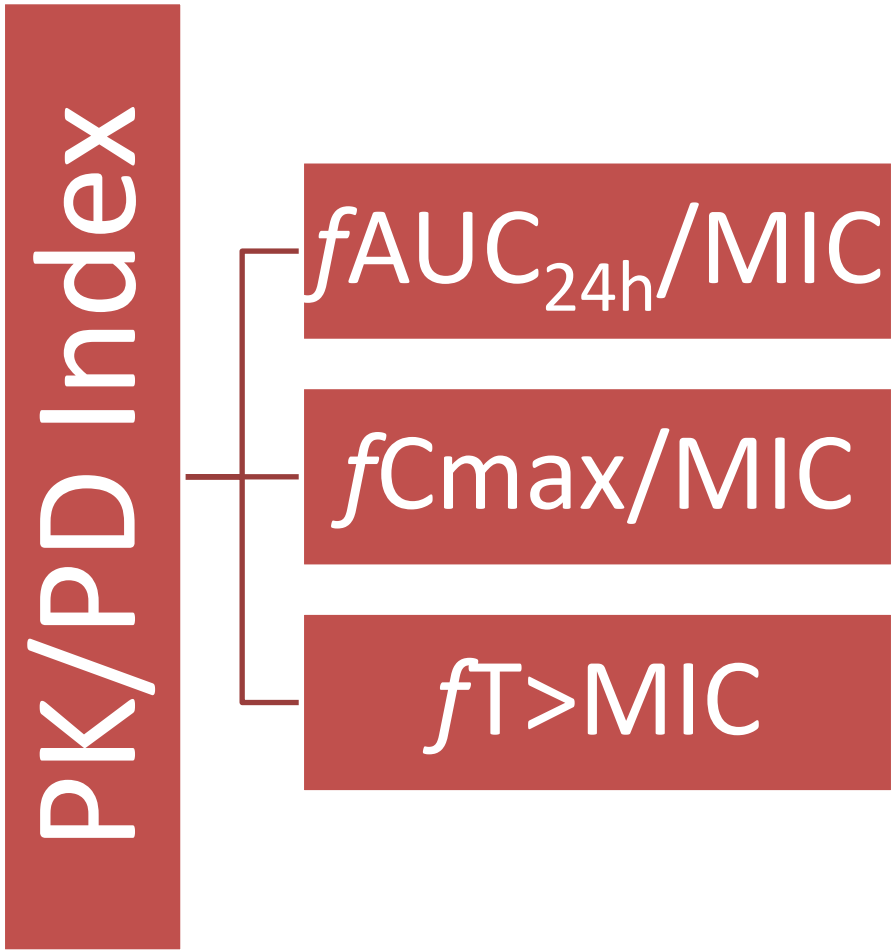
Step 2

Determination of the critical value (size) of the selected PK/PD index

Step 3

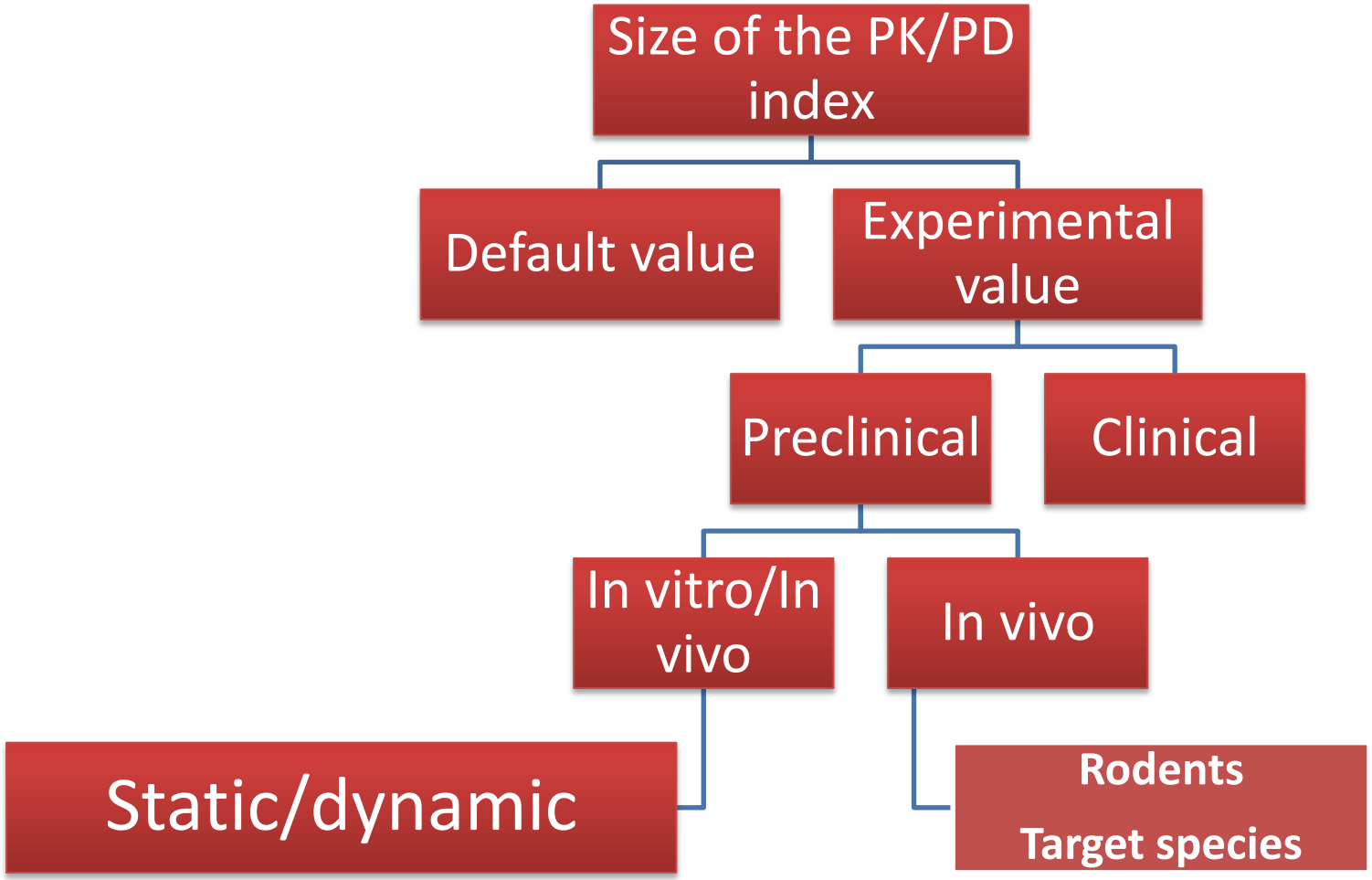
Computation , for a given animal species and for all **possible** (not probable) **MICs** of the percentage (proportion) of animals able to achieve the critical value of the selected PK/PD index (computation of so-called Target Attainment Rates (or TAR)

Step 1:Selection of a PK/PD index



Rem: it was shown that for all drugs/formulations having a long half-live that AUC/MIC is the appropriate index (AU/MIC: universal index?)

Step 2: Determination of the critical value (size) of the selected PK/PD index



A-Clinical determination of the numerical target value for the $CO_{PK/PD}$

Logistic regression analysis

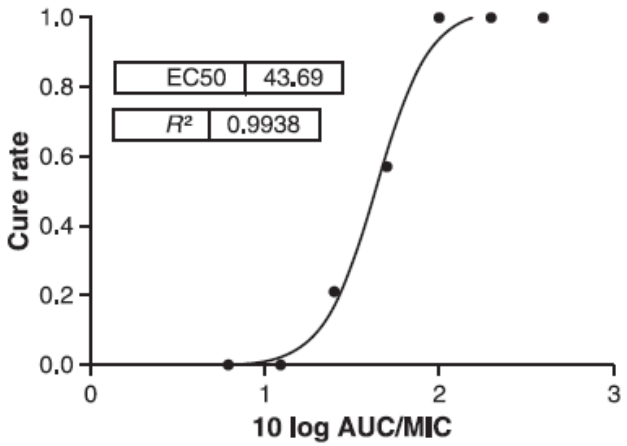


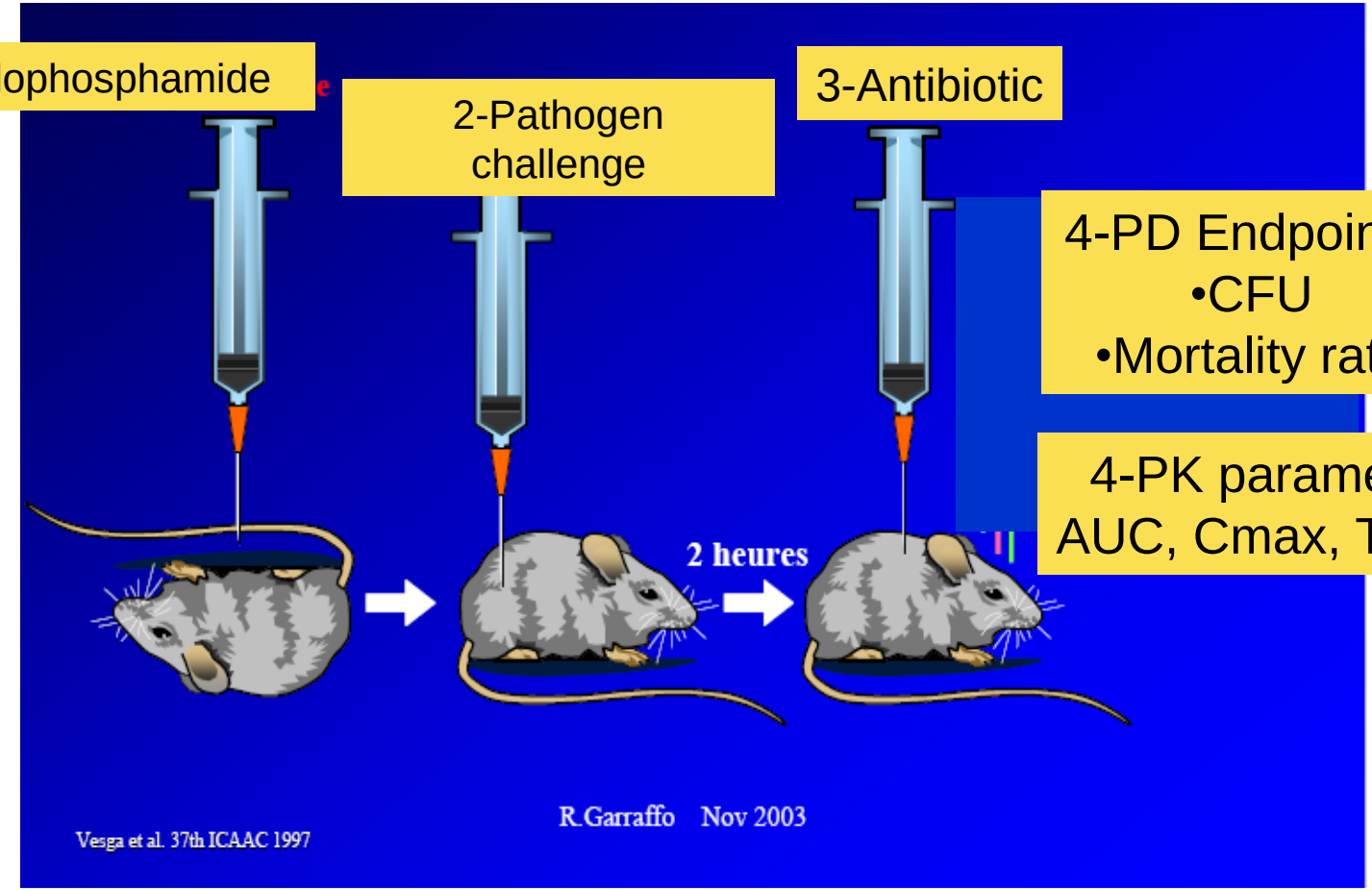
FIG. 1. Cure rate after treatment with fluconazole in patients (n = 132) with oropharyngeal candidiasis. From these patients, the MIC distribution of the *Candida albicans* strains causing the infection was determined. Patients received different doses of fluconazole and the area under the curve (AUC) was estimated in each patient. There were seven different *f*AUC/MIC ratio values, resulting in seven groups. The proportion of patients cured in each group was plotted against the AUC/MIC ratio and the relationship was determined using the E_{max} model. The figure clearly shows the proportionality between *f*AUC and cure rate, whereas the MIC is inversely proportional to the cure rate [13].

B-Preclinical determination of the numerical target value for the $CO_{PK/PD}$

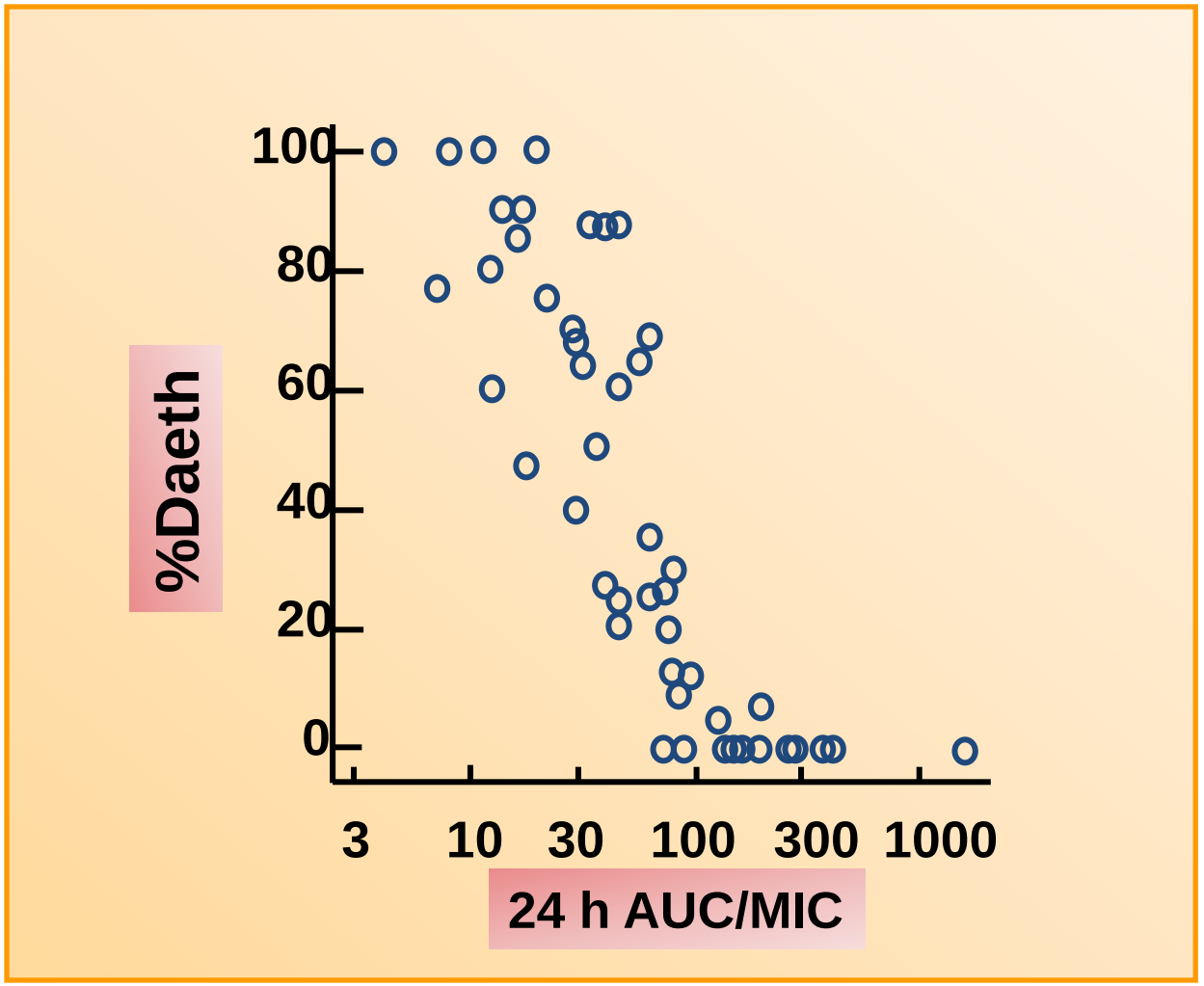
Preclinical determination of $CO_{PK/PD}$

- **In vivo: (target) animal models**
  - mice that are rendered neutropenic and infected with an inoculum of 10_6CFU/mL of microorganisms in the thigh or lung.
  - Treatment is then initiated and after 24 or 48 h the total bacterial count is determined for each organ.
  - Using different doses and dosing intervals, ranges of exposure are obtained and are subsequently plotted against the number of CFU to establish exposure–response relationships.
- **in vitro: killing curves & hollow fibres infection models**

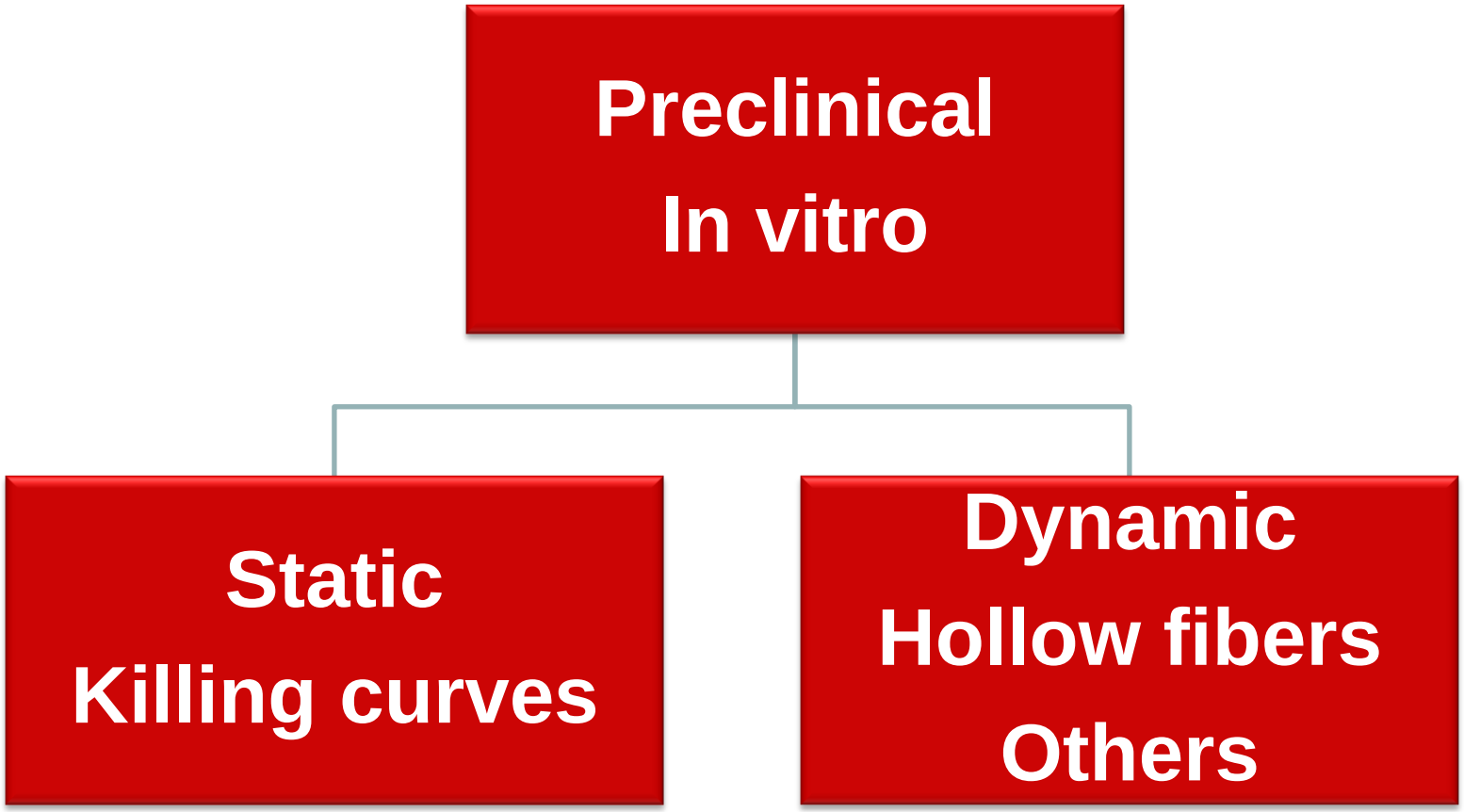
How were established these indices?



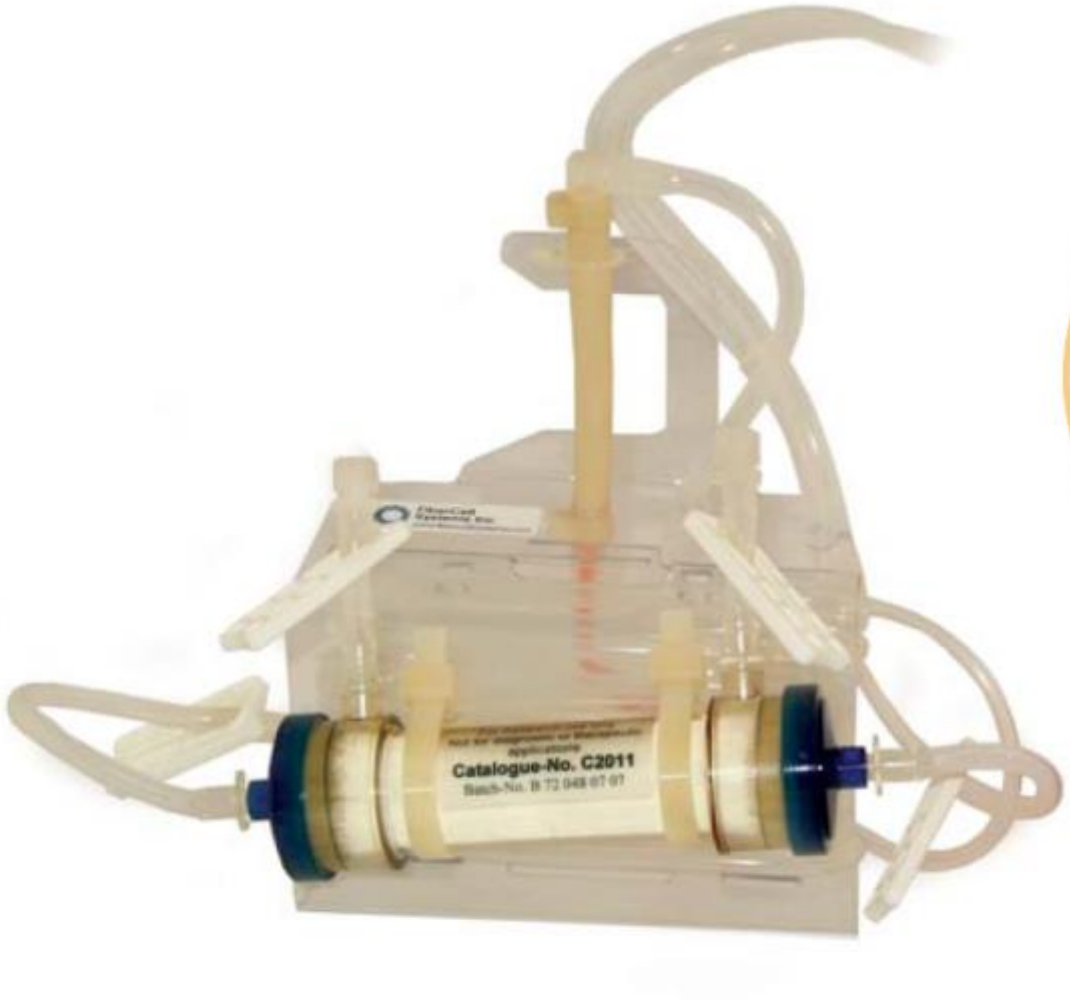
Relationhip between AUC/MIC et % of death for a FQ against a G-

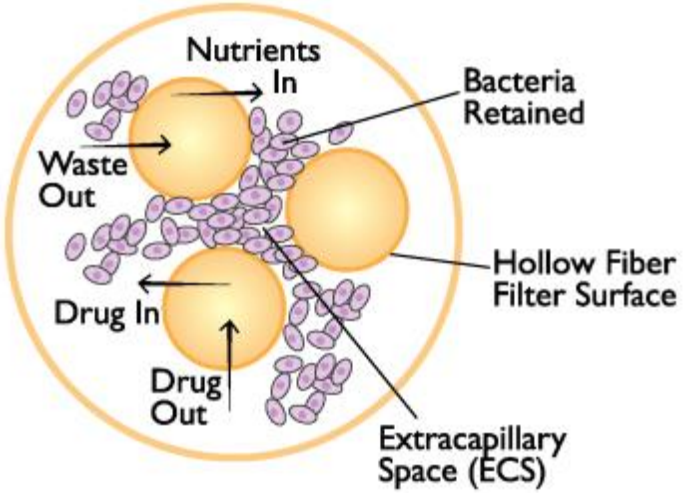


Preclinical determination of $CO_{PK/PD}$



The hollow fiber





The setting of a PK/PD CO

Step1

Selection of a PK/PD index predictive of clinical efficacy and/or prevention of resistance

Step 2

Determination of the critical value (size) of the selected PK/PD index

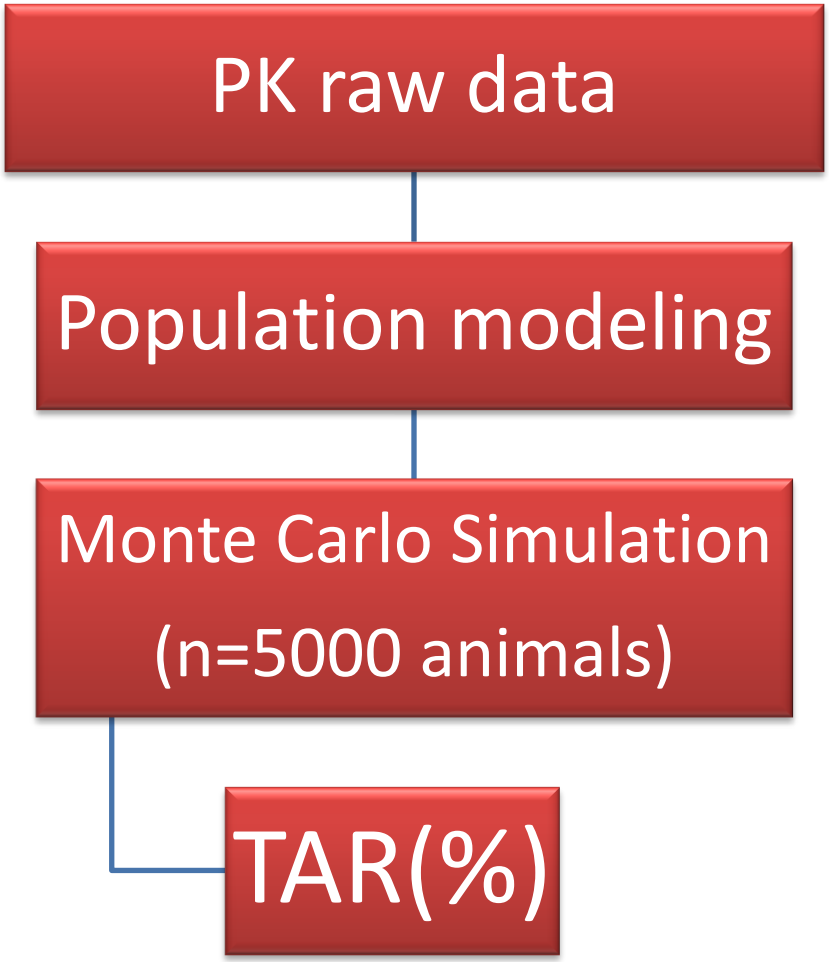
Step 3

Computation , for a given animal species and for all possible (not probable) MICs of the percentage (proportion) of animals able to achieve the critical value of the selected PK/PD index (computation of so-called Target Attainment Rates (or TAR)

Step 3: Computation of the TAR (%) for the critical value of the selected index for the different possible MICs (TAR are stratified by MIC)

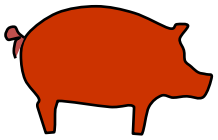
- More literally the question is:
  - E.g. What is the critical (maximal) MIC for which we can guarantee that plasma drug concentration will be:
    - above a possible MIC for X% of the dosage interval (often 50 or 80%) for T>MIC
    - or≥ to a given AUC/MIC
  - for the intended dosage regimen in at least 90% of subjects of the targeted population

Step 3: Computation of the TAR (%) for the critical value of the selected index for the different possible MICs (TAR are stratified by MIC)

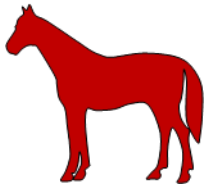


See next presentation

Use of Monte Carlo simulation to determine pharmacodynamic cutoffs of amoxicillin to establish a breakpoint for antimicrobial susceptibility testing in pigs

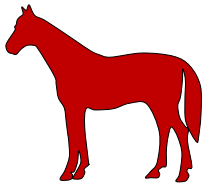


Julien F. Rey, DVM; Céline M. Laffont, PhD; Siska Croubels, Pharm D; Patrick De Backer, DVM; Claudine Zemirline, DVM; Eric Bousquet, DVM; Jérôme Guyonnet; Aude A. Ferran, DVM; Alain Bousquet-Melou, DVM; Pierre-Louis Toutain, DVM



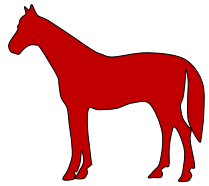
Thre case of marbofloxacin in the horse

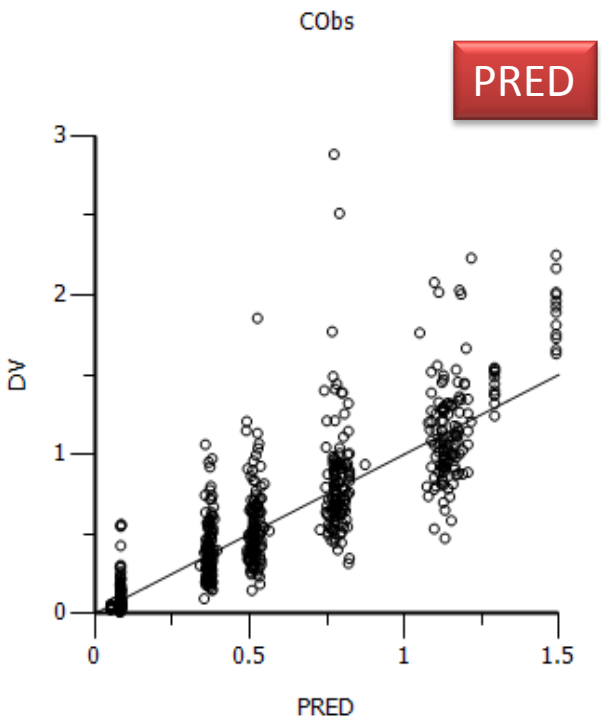
Population investigation

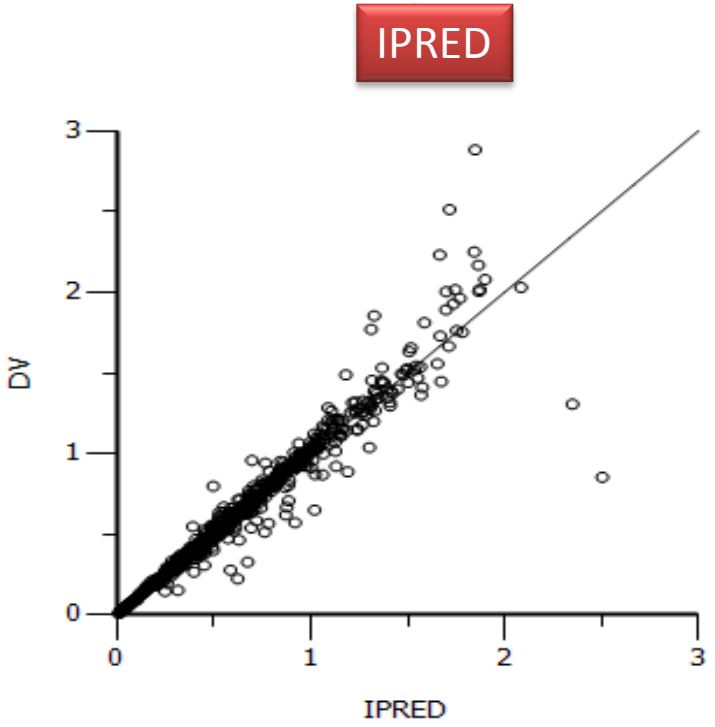


Caracteristics		Montréal	Toulouse1	Utrecht	Vienne	Toulouse2
Population (n=131)		44	58	9	8	12
Sex	Female , (n=68)	31	23	3	4	7
	gelding (n=48)	11	25	6	1	5
	Male (n=13)	2	8	0	3	0
Status	healty(n=60)	21	18	9	1	12
	hopital (n=70)	23	40	0	7	0
Age (Yrs) (n=97)		9.9	10.5	12.9	8.1	15.8
BW(Kg) (n=127)		482	397	596	401	564

Marbofloxacin



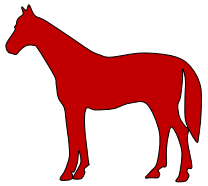




PRED (population predicted concentration) vs observed concentrations (µg/mL). Data are evenly distributed about the line of identity, indicating an appropriate structural model could be found for most individuals

IPRED (Individual predicted concentrations) vs. observed concentrations (µg/mL). IPRED were obtained by setting random effects to the 'post hoc' or empirical Bayesian estimate of the random effects for the individual from which the DV observation was made.

Marbofloxacin: $CO_{PK/PD}$



	AUC/MIC (h)					
MIC	24	48	72	96	125	250
2	0	0	0	0	0	0
1	0.712	0	0	0	0	0
0.5	21.1	0.712	0	0	0	0
0.25	78.2	21.1	4.2	0.712		
0.125	**98.8**	78.2	44.9	21.1	8.4	0.051
0.0625	100	98.8	**92.3**	78.2	58.6	8.4
0.03125	100	100	99.8	98.8	**95.6**	58.6

This table provide the percentage of horses able to achieve AUC/MIC of 24, 48…250h for MIC ranging from 0.03125 to 2µg/mL

Assessment of the currently used dosage regimen

The goal is for the committee to have an **opinion** on the **validity** of the current dosage regimen (especially for old drugs)to discuss the **value** of its AST, to manage the situation where several different dosage regimen are existing in the EU, to prioritise bugs for which we need urgently an AST …

Required information: a model to compute the dose and ad hoc parameters (PK) and data sets (PD) for the Monte Carlo Simulations

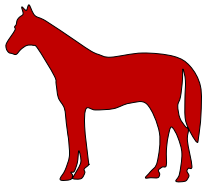
PK

(the same as for step 3) to generate population **parameters**

PD: MIC distributions

(the same as for the establishment of the ECOFF

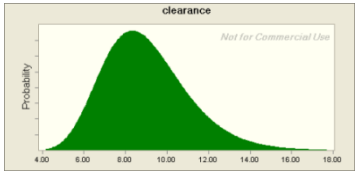
MIC Marbofloxacin



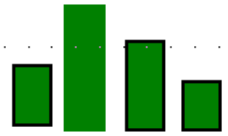
Strains	MIC values (µg/mL)														MIC50 (µg/mL)	MIC90 (µg/mL)
	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32		
Escherichia coli			2	15	1								2		0,022	0,038
Pantoea agglomerans (Enterobacter agglomerans)				37	3										0,022	0,029
Rhodococcus equi								3	17						0,665	0,922
Streptococcus zooepidemicus									29	9	2				0,806	1,714

Computation of the dose

Log normal distribution of CL/F (pop PK)

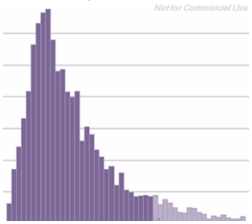


Observed MIC distributions



BP 125h

$$Dose = \frac{Clearance\ \textbf{(per hours)} \times \left(\frac{AUC}{MIC}\right)_{BP} \times MIC}{F\% * fu}$$



Monte Carlo simulation: applied to PK/PD models

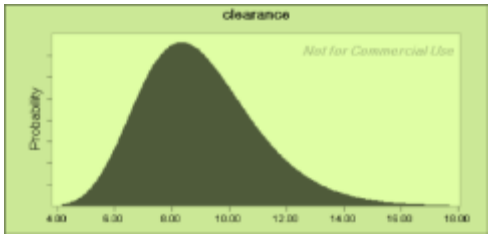
Generate random AUC and MIC values
across the AUC & MIC distributions that **conforms to their probabilities**

Calculate a large number of AUC/MIC ratios

Plot results in a probability chart

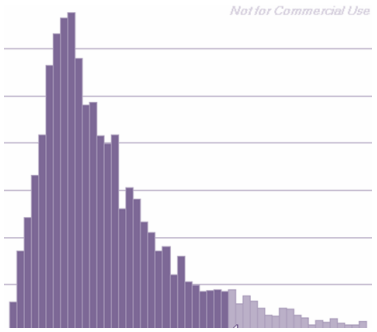
Model: AUC/MIC

PDF of AUC



PDF of MIC

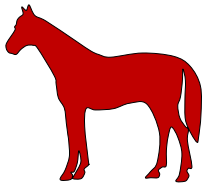
PDF of AUC/MIC

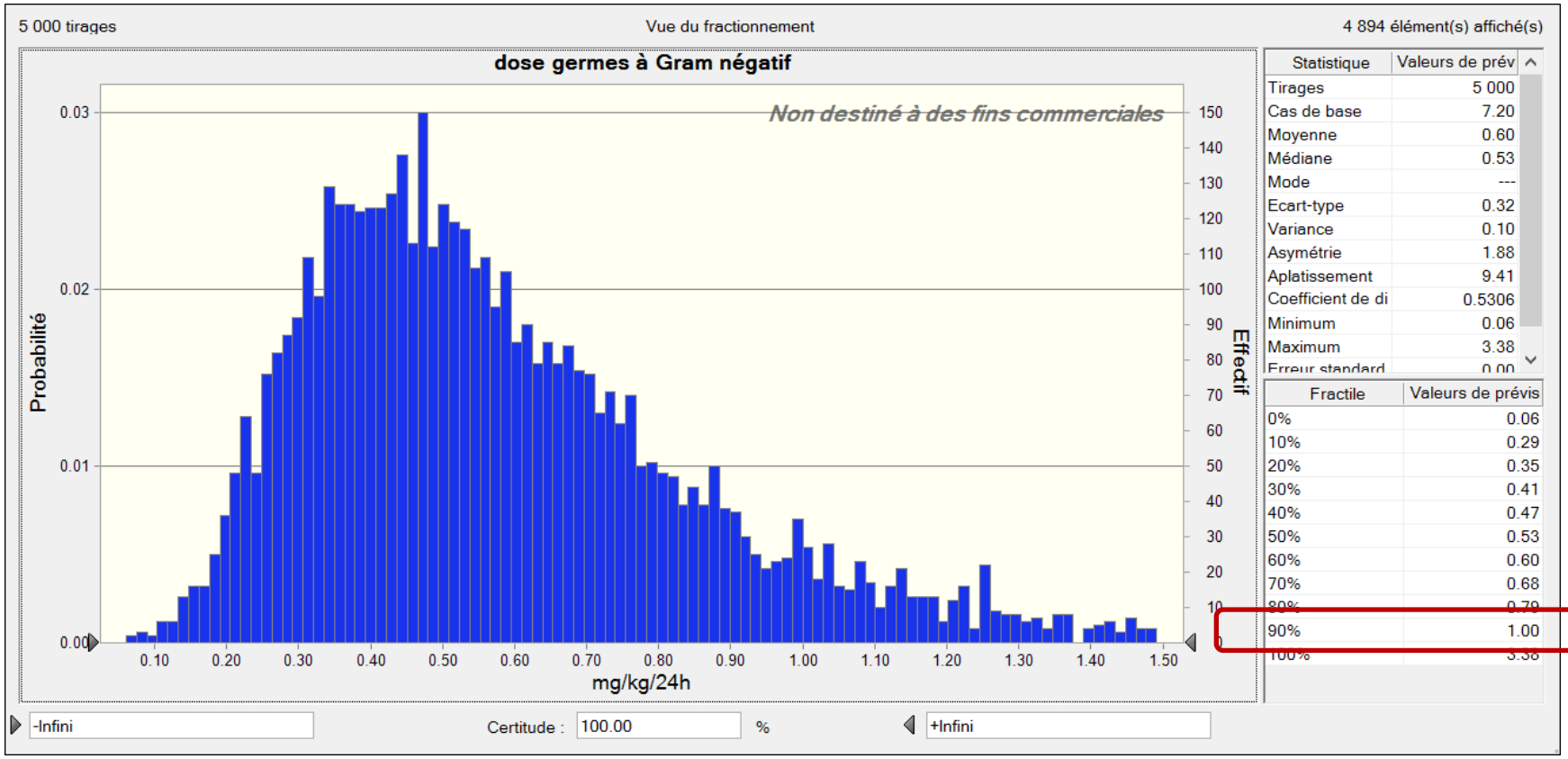


% target attainment

Adapted from Dudley, Ambrose. Curr Opin Microbiol 2000;3:515−521

Marbofloxacin
Dose for Gram negative



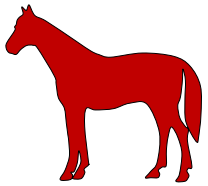


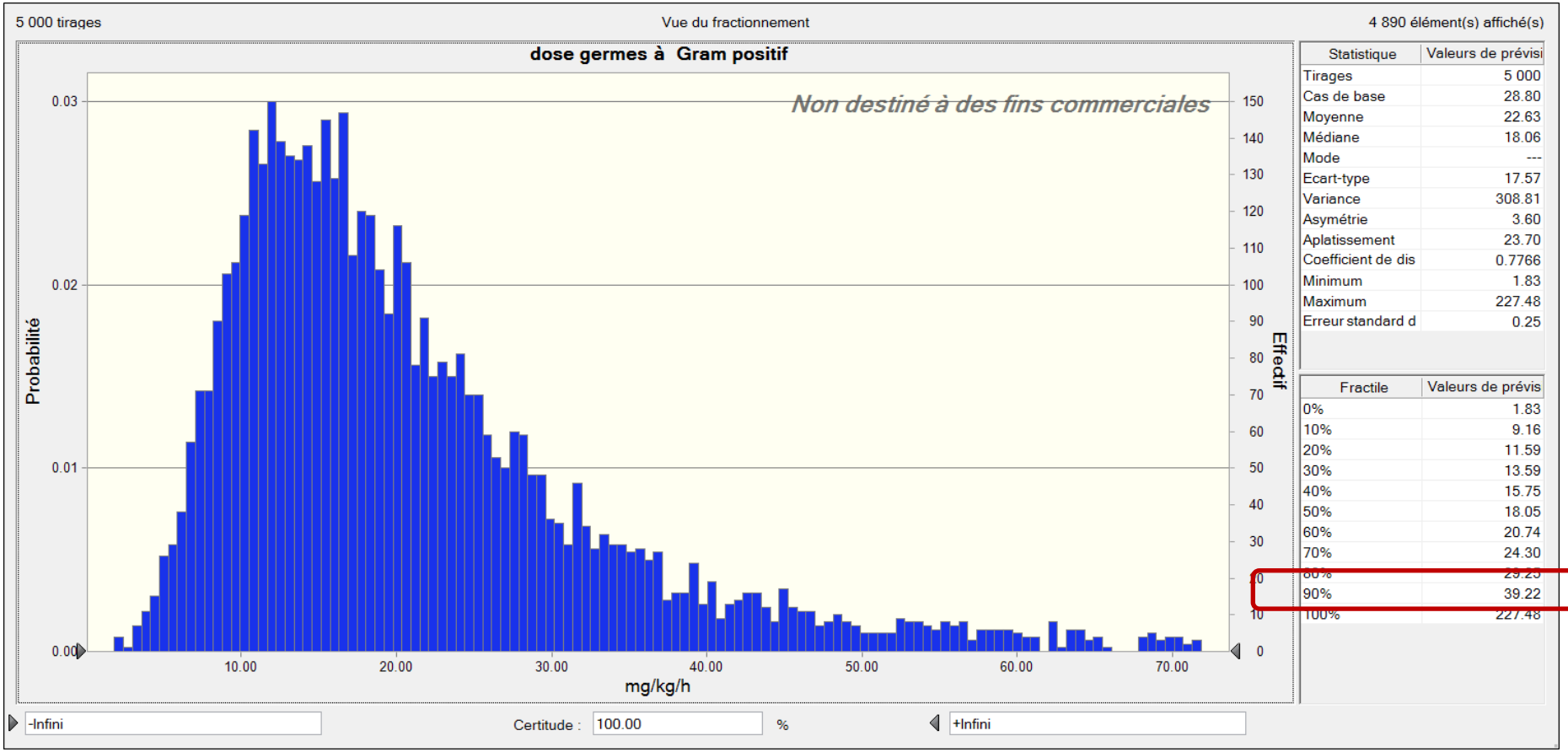
Statistique	Valeurs de prév
Tirages	5 000
Cas de base	7.20
Moyenne	0.60
Médiane	0.53
Mode	---
Ecart-type	0.32
Variance	0.10
Asymétrie	1.88
Aplatissement	9.41
Coefficient de di	0.5306
Minimum	0.06
Maximum	3.38
Erreur standard	0.00

Fractile	Valeurs de prévis
0%	0.06
10%	0.29
20%	0.35
30%	0.41
40%	0.47
50%	0.53
60%	0.60
70%	0.68
80%	0.79
90%	1.00
100%	3.38

<<lower than the Marketed dose: 2mg/kg

Marbofloxacin
Dose for Gram positive





Statistique	Valeurs de prévisi
Tirages	5 000
Cas de base	28.80
Moyenne	22.63
Médiane	18.06
Mode	---
Ecart-type	17.57
Variance	308.81
Asymétrie	3.60
Aplatissement	23.70
Coefficient de dis	0.7766
Minimum	1.83
Maximum	227.48
Erreur standard d	0.25

Fractile	Valeurs de prévis
0%	1.83
10%	9.16
20%	11.59
30%	13.59
40%	15.75
50%	18.05
60%	20.74
70%	24.30
80%	29.25
90%	39.22
100%	227.48

much higher than the marketed dose of 2mg/kg

Conclusions for marbofloxacine in horses

- The current dose is able to cover 100% of horses for gram negative pathogens
  - (rather seldom in horses)
- The current dose is unable to treat a gram positive infection in horses

- **Thus no AST for horses is required**

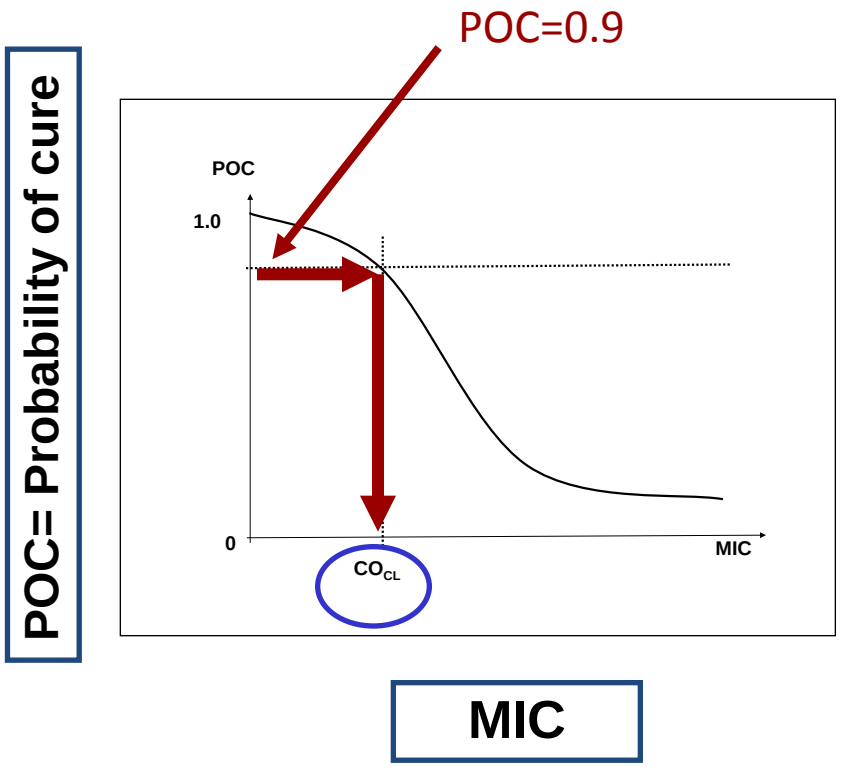
Clinical cutoff

The clinical CO (CO_{CL})

The **CO_{CL}** is based upon the collection of isolates obtained during the clinical effectiveness studies.

CO_{CL} reflects the upper limit of the MIC values associated with a high likelihood of clinical success [probability of cure (POC)].

There is no set method for establishing the **CO_{CL}**, and no hard target for POC.



Probability of cure (POC)

- Logistic regression can be used to link MIC values (as independent variable) to the probability of a clinical success

$$POC = \frac{1}{1 + e^{-a+bf(MIC)}}$$

Dependent variable

Ceiling response

sensitivity

Independent variable (here collected MICs during clinical trials) *+other covariables*

2 parameters: **a** (ceiling effect) & **b** (slope of the MIC-effect curve)

Logistic modelling: difficulties

- Scarse an unbalanced data (not enough failure)
- Possible international project of research?

Example of clinical *cutoff* higher than the epidemiological CO

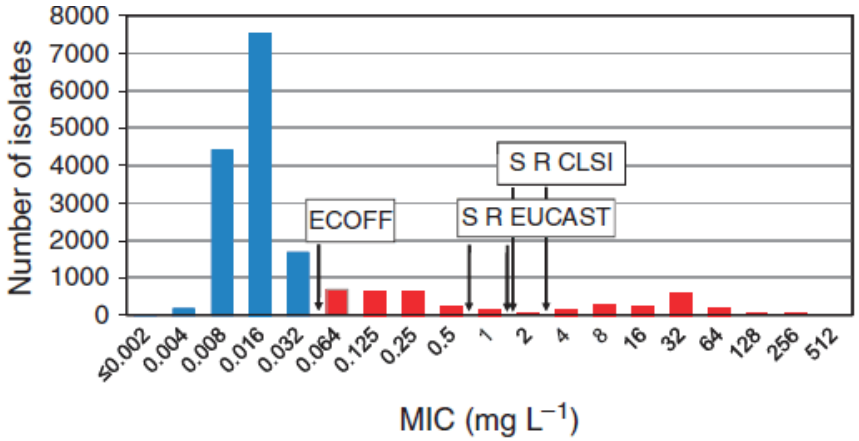


Fig. 1. Ciprofloxacin MIC distribution of *Escherichia coli* isolates (http://www.eucast.org). Epidemiological cut-off (ECOFF) values and clinical susceptible (S) and resistant (R) breakpoints from CLSI and EUCAST committees are indicated. The clinically susceptible population (below the clinical susceptible breakpoint) includes part of the microbiologically resistant population (low-level resistant bacteria, presumably expressing *qnr*-like genes or other PMQR mechanisms or first step *gyrA* mutations) and the wild-type population (below the ECOFF value and presumably without resistance mechanisms). The clinically resistant population (beyond the clinical resistant breakpoint) includes isolates with high-level resistance mechanisms (most probably double-step *gyrA* mutants or a combination of *gyrA* with *parC* mutations).

Clinical breakpoint

Setting the breakpoint

- A risk management exercise
- Not a scientific exercise but should be scientifically acceptable
- CO and others consideration
  - Harmonization (e.g different dosage regimen across EU, no splitting of the wild distribution…

Decision tree to select a BP from the three CO

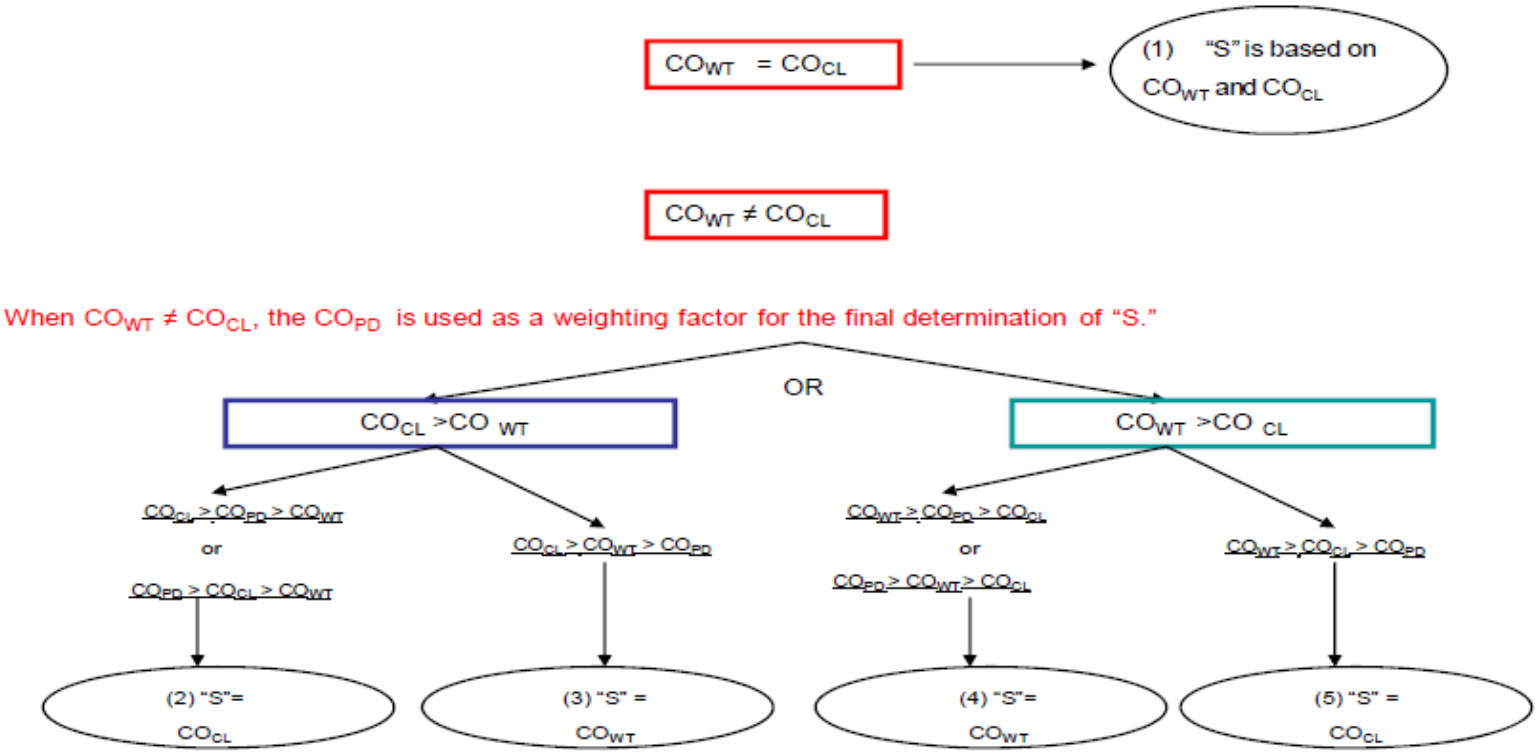


Figure C2. Susceptibility Breakpoint (SBPT) Decision Tree

Clinical breakpoint

- **CLSI: vote**
- **EUCAST: consensus**

Conclusion

- Veterinary medicine needs specific breakpoints
- International cooperation is possible to settle the epidemiological cutoff
- Currently, no well established approach to select a clinical cutoff
- The value of AST has to be supported by computing their predictive value and diagnostic gains