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**Surveillance of resistance in *M. tuberculosis* and
potential impact of epidemiological cut-off values**
The critical proportion is important

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Objectives of performing drug susceptibility testing of *M.tuberculosis* (DST-MTB)?

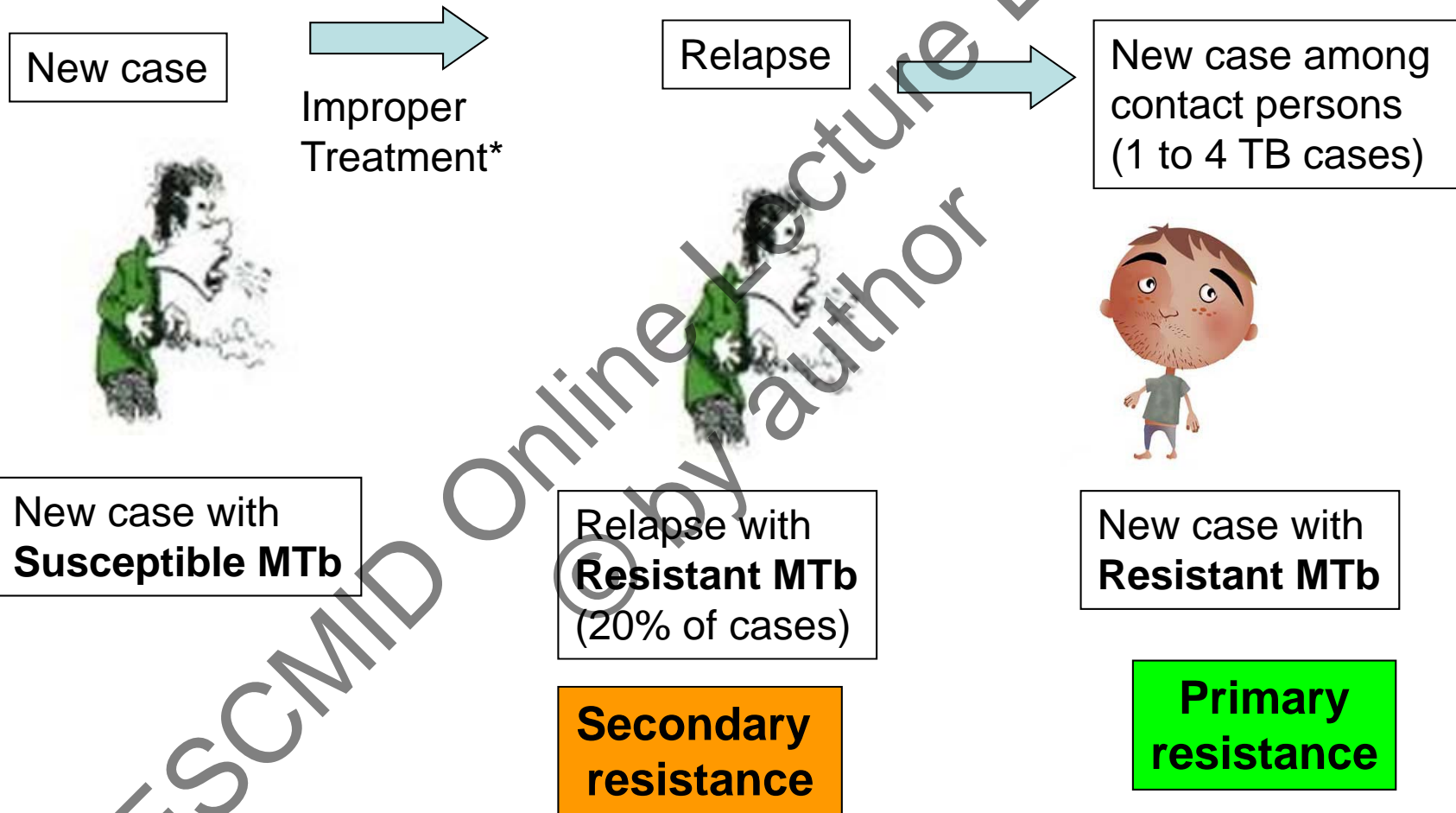
1. Individual treatment management of a tuberculosis case
2. Drug-resistance surveillance at the scale of a hospital, city, region or country

Definitions

- **Resistance** : **decrease in sensitivity** compared to wild type strains (never came into contact with the drug).
 - unlikely to show clinical responsiveness to the drug
- **Susceptibility** : level of sensitivity not significantly different from wild type strains
 - likely to show clinical responsiveness to the drug

Heifets L, ARRD 1988
Mitchison DA, 1968

Primary and secondary resistance

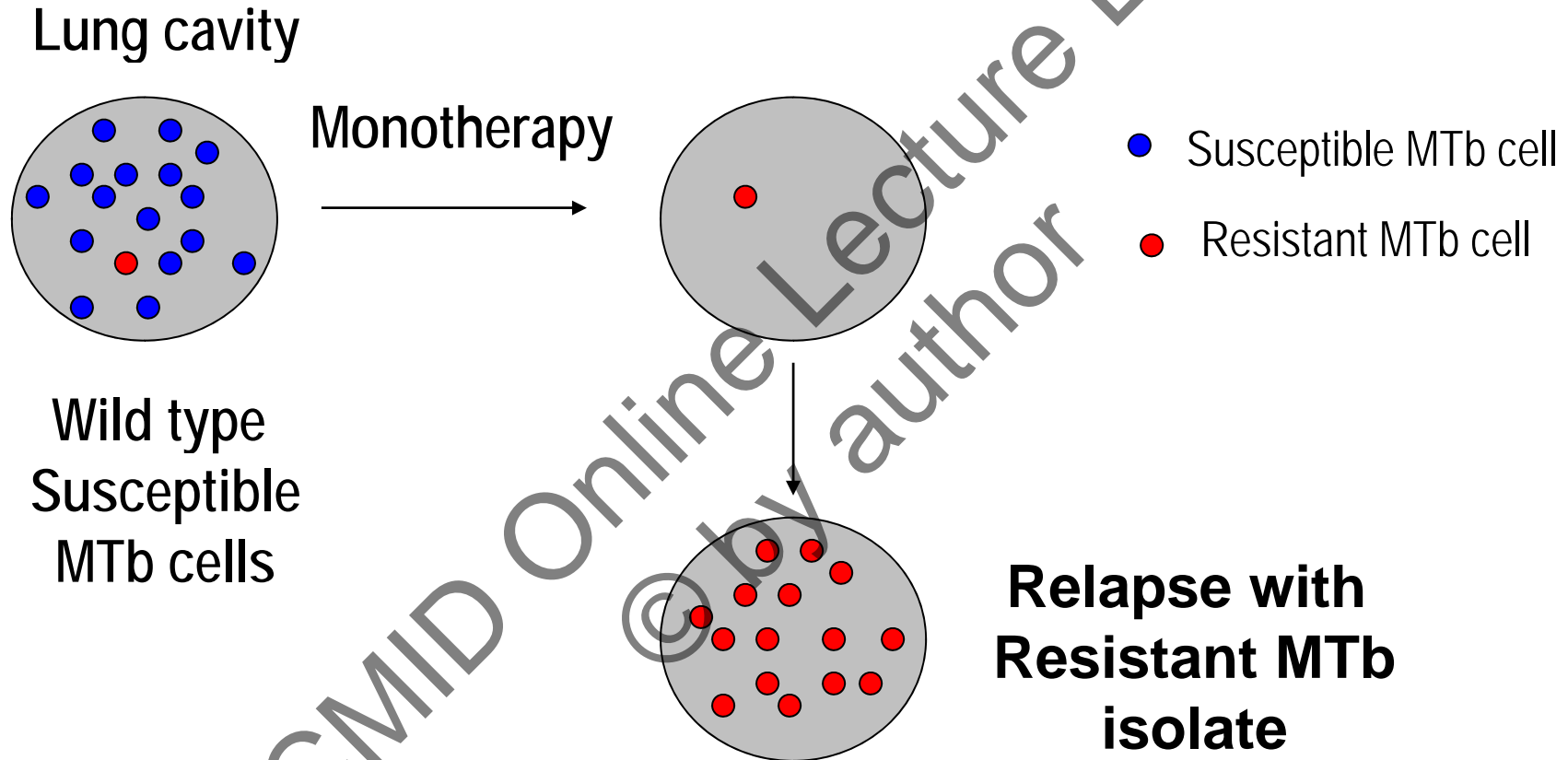


*compliance, dosage, prescription

Secondary and Primary resistance

- Secondary resistance or acquired resistance
 - Drug resistance appearing during treatment
 - Results of selection and multiplication of resistant mutant isolates pre-existing in the tubercle bacillus population before therapy
 - Resistance rate observed in re-treatment cases
- Primary resistance
 - Drug resistance observed before treatment,
 - Consequence of exposure to a drug-resistant source of infection
 - Resistance rate observed in new cases

How to explain secondary resistance

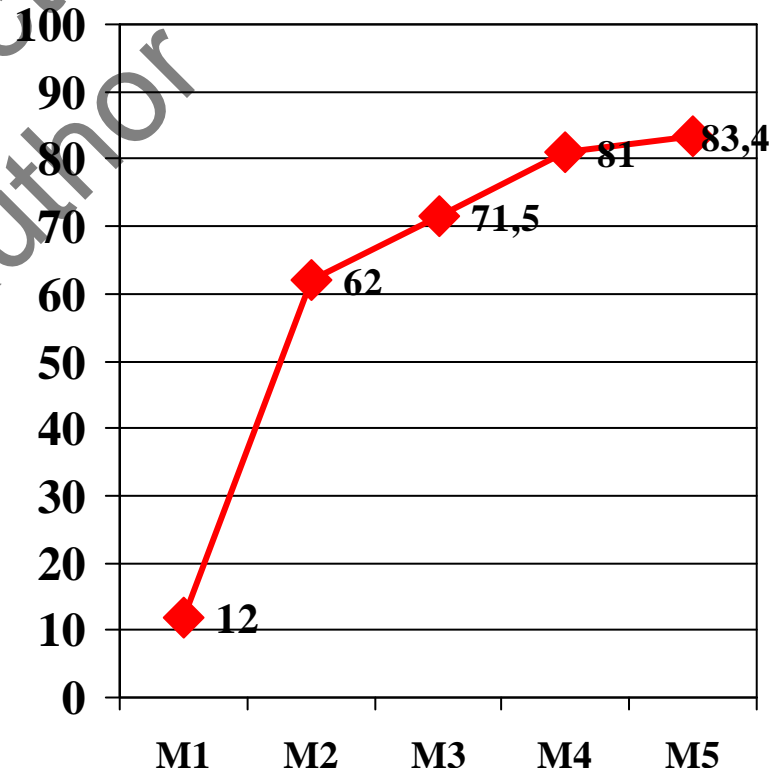


= selection of resistant mutants during monotherapy

First study with Streptomycin therapy

Medical Research Council Investigation 1947-48

- 107 pulmonary TB cases
- Streptomycin 2 g/d
- For 4 months
- Mortality : 7 % vs 27 % control untreated cases
- Failure and relapse for 42 cases with cavitory TB
- MIC x 10 to 1000

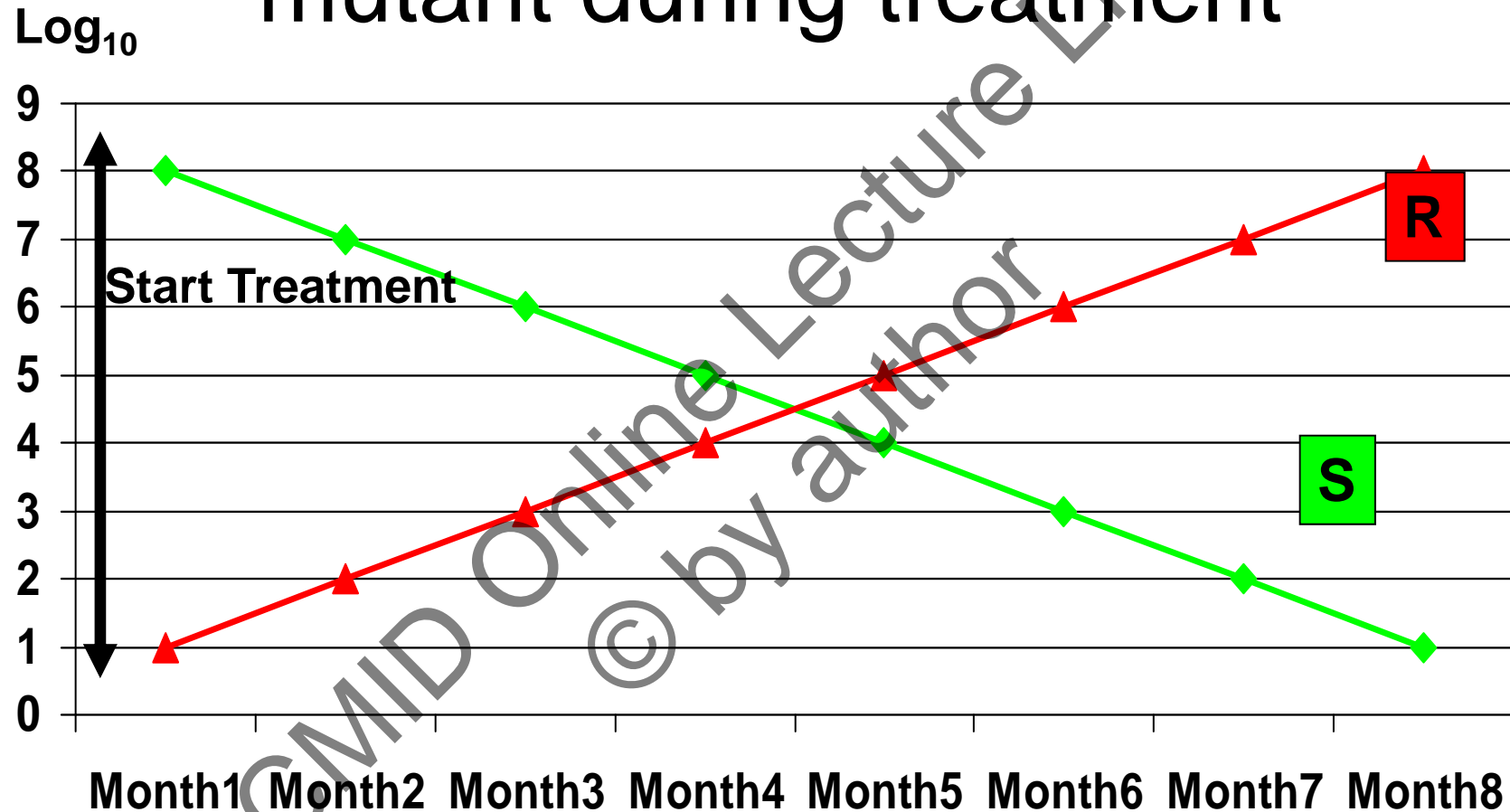


Further studies with monotherapy

Year of use	Drug (N patients treated)	Relapse cases (N months therapy)					
		1	2	3	4	5	>6
1946	Streptomycin (42)	12	63	73	82	85	-
1960	Isoniazid (87)	10	-	36	-	-	52
1972	Rifampicin (85)	-	-	-	48	-	-
1985	Ofloxacin (19)	-	5	26	58	63	-

BMRC 1947; Fox 1953; Tsukamura 1968; Tsukamura 1985

Scheme of selection of resistant mutant during treatment



◆ Susceptible Population ▲ Resistant mutant

Genetic support of acquired resistance in *M. tuberculosis* complex

= chromosomal mutation in genes involved
in the mode of action

1. Point mutation (ex *rpoB* and rifampicin)

TCG ---> TTG
Serine ---> Leucine

2. Deletions (ex *katG* and high level isoniazid resistance)

ACGCCTAGAT ----> AC TAGAT

3. Insertions ACGCCTAG ----> ACCTTGCCTA

Proportion of resistant mutants in wild-type strains of *M.tuberculosis*

Drugs	Range of proportion of mutants 10^{-x}	N resistant Mtb cell in cavitory lung (10^8 cell)
Streptomycin	10^{-6} to 10^{-7}	100
Isoniazid	10^{-5} to 10^{-6}	1000
Rifampicin	10^{-7} to 10^{-8}	10
Ofloxacin	10^{-7} to 10^{-8}	10
TMC-207	10^{-7} to 10^{-8}	10

Canetti 1963, Tsukamura 1968, Cambau 1994, Andries 2005

Performances of DST-MTB methods

- **Assess susceptibility**
=> No false resistance:
the patient will not benefit from the standard treatment
- **Detect resistance**
=> No false susceptibility:
the patient may fail and relapse

Kim et al. 2004

Tools to detect for resistance and assess for susceptibility

- **Critical concentration:** the lowest drug concentration at which wild type strains did not grow

Proportion of resistant mutants in wild-type strains of *M.tuberculosis* with regard to the drug concentration

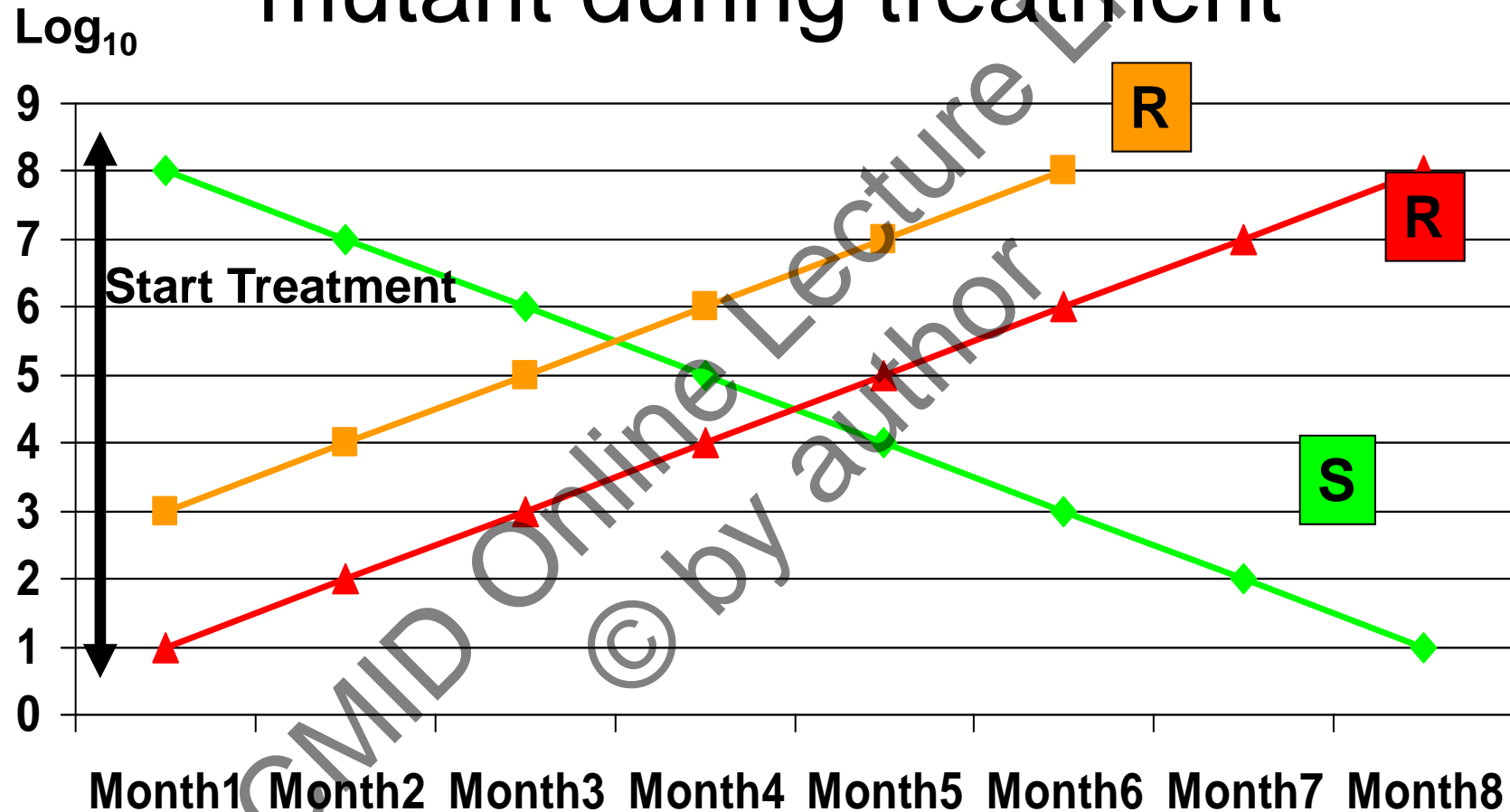
Drugs	Max value of proportion of mutants in % (10^{-x})	Drug concentration
Isoniazid	0.02% (10^{-4})	0.1
	0.004% (10^{-5})	0.2
Kanamycin	30% (10^{-1})	10
	0.5% (10^{-1})	20
	0.05% (10^{-1})	30
Ofloxacin	0.0001 (10^{-6})	MIC
TMC-207	0.00001 (10^{-7})	2x MIC
	0.00001 (10^{-8})	4 x MIC

Canetti, Rist and Grosset 1963, Canetti G. AMJRD 1965, Cambau 1996, Andries 2005

Susceptible strains of MTb

- **False resistance can be observed if the inoculum is too high**
- -> need to monitor the inoculum
- => need to determine the total viable population
- => need to compare numbers of resistant cells to susceptible ones
= **Proportion of resistant %**

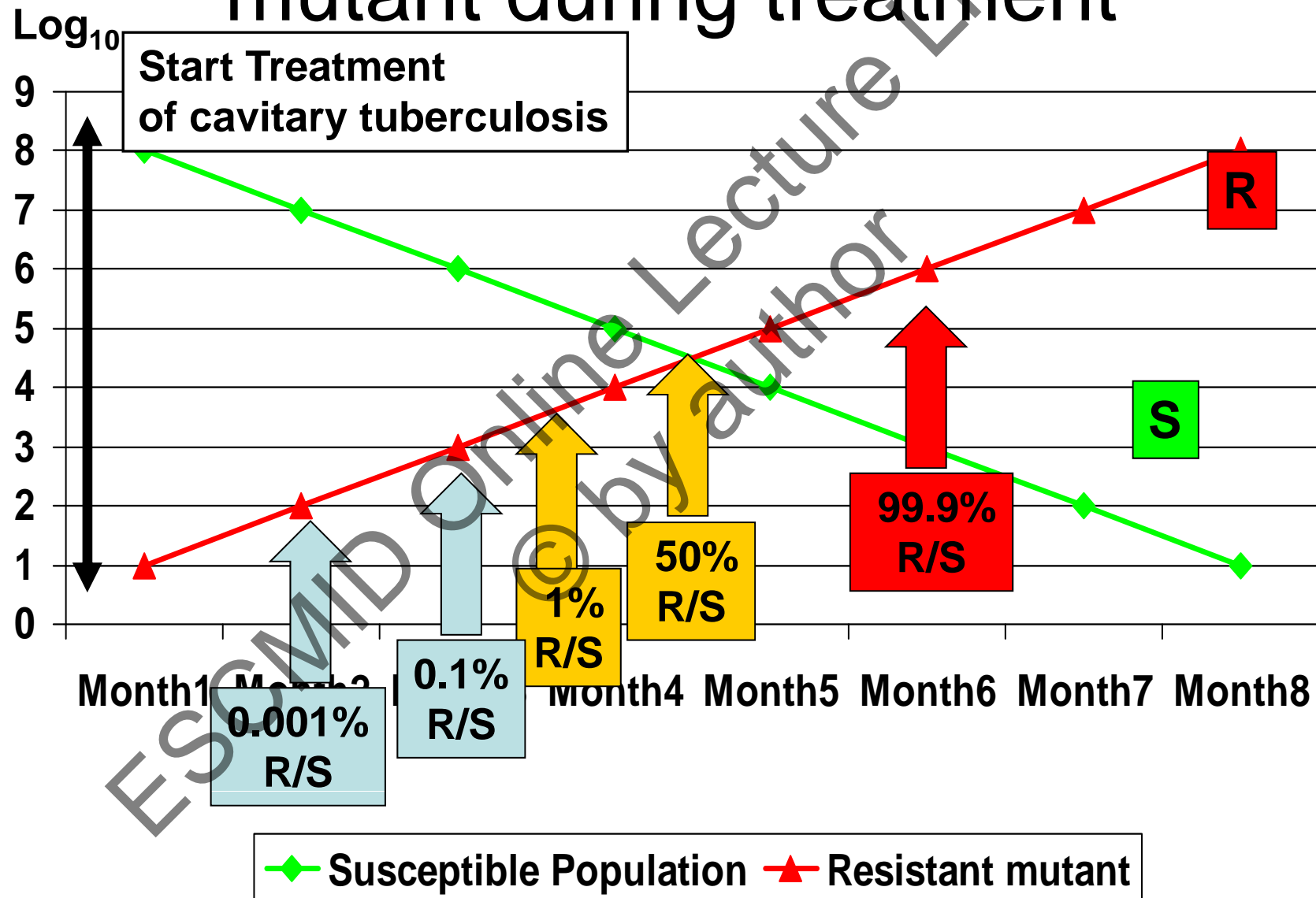
Scheme of selection of resistant mutant during treatment



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◆ Susceptible Population ■ Resistant mutant 10-5
▲ Resistant mutant 10-8

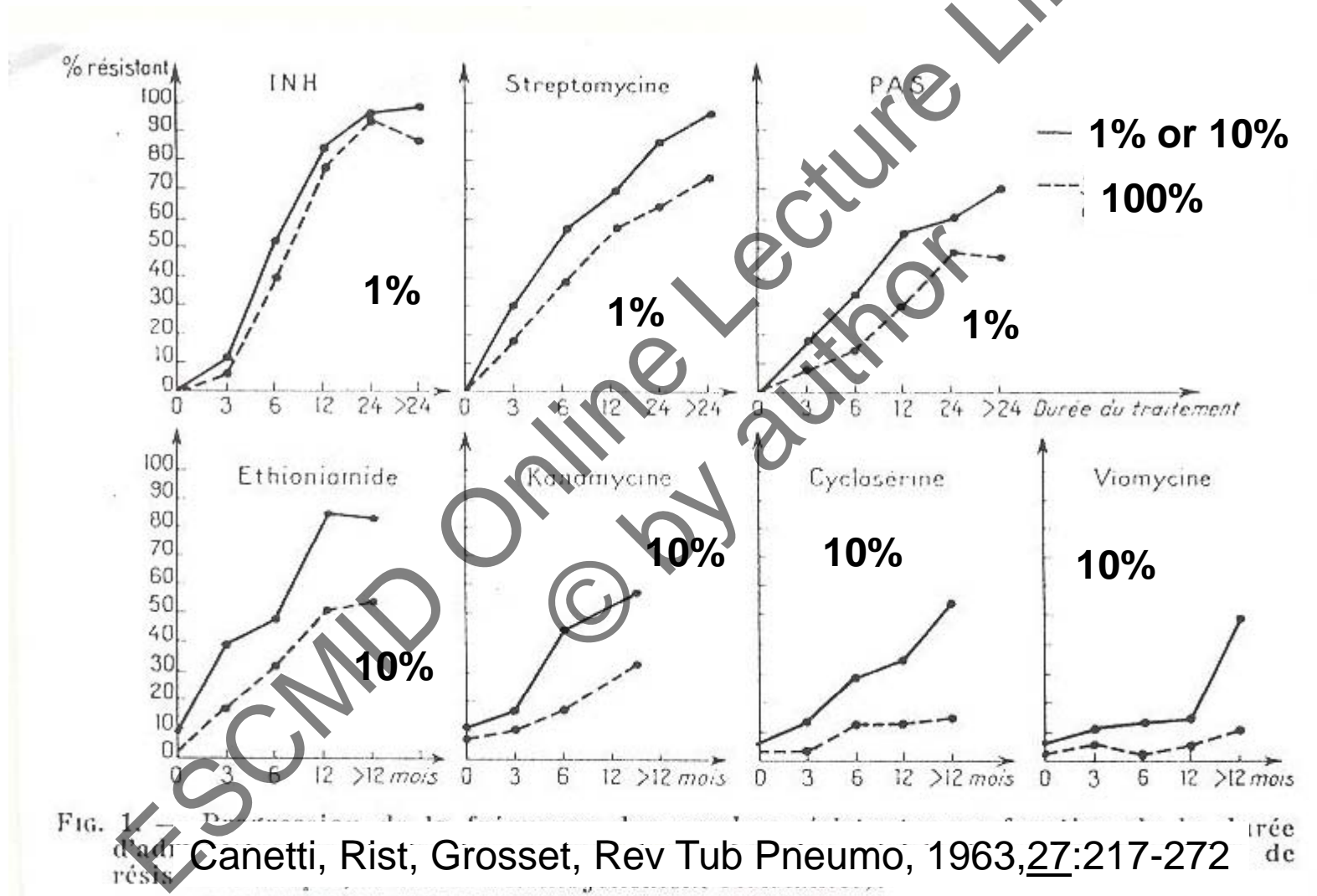
Scheme of selection of resistant mutant during treatment



Critical Proportions

- Russel and Middlebrook , J Clinical Invest, 1959
- Canetti G. AMJRD 1965
- Cabetti, Rist and Grosset Study on 546 patients with
 - 450 relapse and failure (lung surgery)
 - 100 sputum (new cases or non failure cases)
- isoniazid, streptomycin, kanamycin, ethionamide, PAS

Correlation of proportion of resistant cells with outcome (failure)



Resistant strains of MTb

- Significant proportion of resistant mutants is predictive for clinical failure
 - 1% for major drugs
 - 10% for minor drugs
- False susceptibility can be observed if the resistant cells are not « visible »
 - => Ratio R/S is lower than 100%

Tools to detect for resistance and assess for susceptibility

- **Critical concentration:** the lowest drug concentration at which wild type strains did not grow
- **Critical proportion:** The lowest percentage of a wild type strain population that may grow at the critical concentration

The Proportion Method

- LJ medium, and then modified for liquid medium
- Calculates the proportion of resistant bacilli present in a strain at critical concentrations
- Growth controls: 2 to 3 inocula (10-fold diluted) growing in absence of drug

Proportion method on LJ

3 inocula if you do not want to miss one



Variations of the inocula according to the strain

Total N in a 1 mg/ml suspension	Number of colonies in 0.2ml of 10 ⁵ dilution			
	INH-S n :203	INH-R n : 141	Total n : 344	Same strain (55 times)
>10 ⁸	4	1	3	5
5.10 ⁷ - 10 ⁸	10	8	9	22
10 ⁷ - 5.10 ⁷	56	40	49	56
5.10 ⁶ - 10 ⁷	11	16	13	7
10 ⁶ - 5.10 ⁶	12	23	17	4
<10 ⁶	7	12	9	5

Canetti Rev Tub Pneumo, 1963,27:217-272

Determine the proportion of resistant mutant

- Ratio of Resistant bacterial cells / Susceptible bacterial cells
- = number of colonies on drug medium
- / number of colonies on control medium
- X 100%

T 10⁻¹

T 10⁻³

**INH 0,2
10⁻¹**

R 10⁻¹

0.01%

< 10⁻⁴

**> 500
(10 000)
col**

100 col

1 col

0 col

S

S

T 10^{-1}

H
(1mg/l)
 10^{-1}

R
 10^{-1}

E
 10^{-1}

50%

100%

0.5%

200 col

100 col

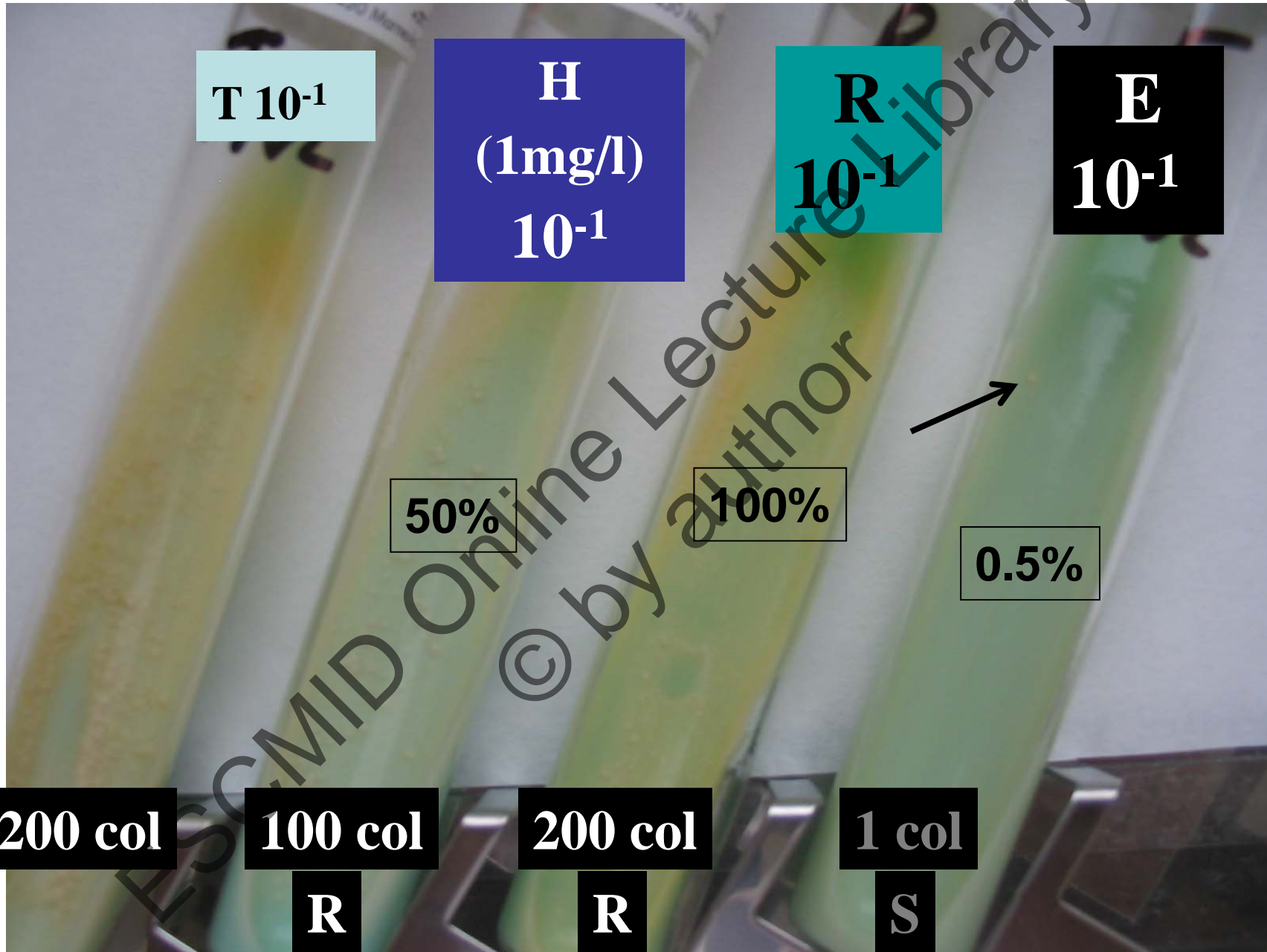
200 col

1 col

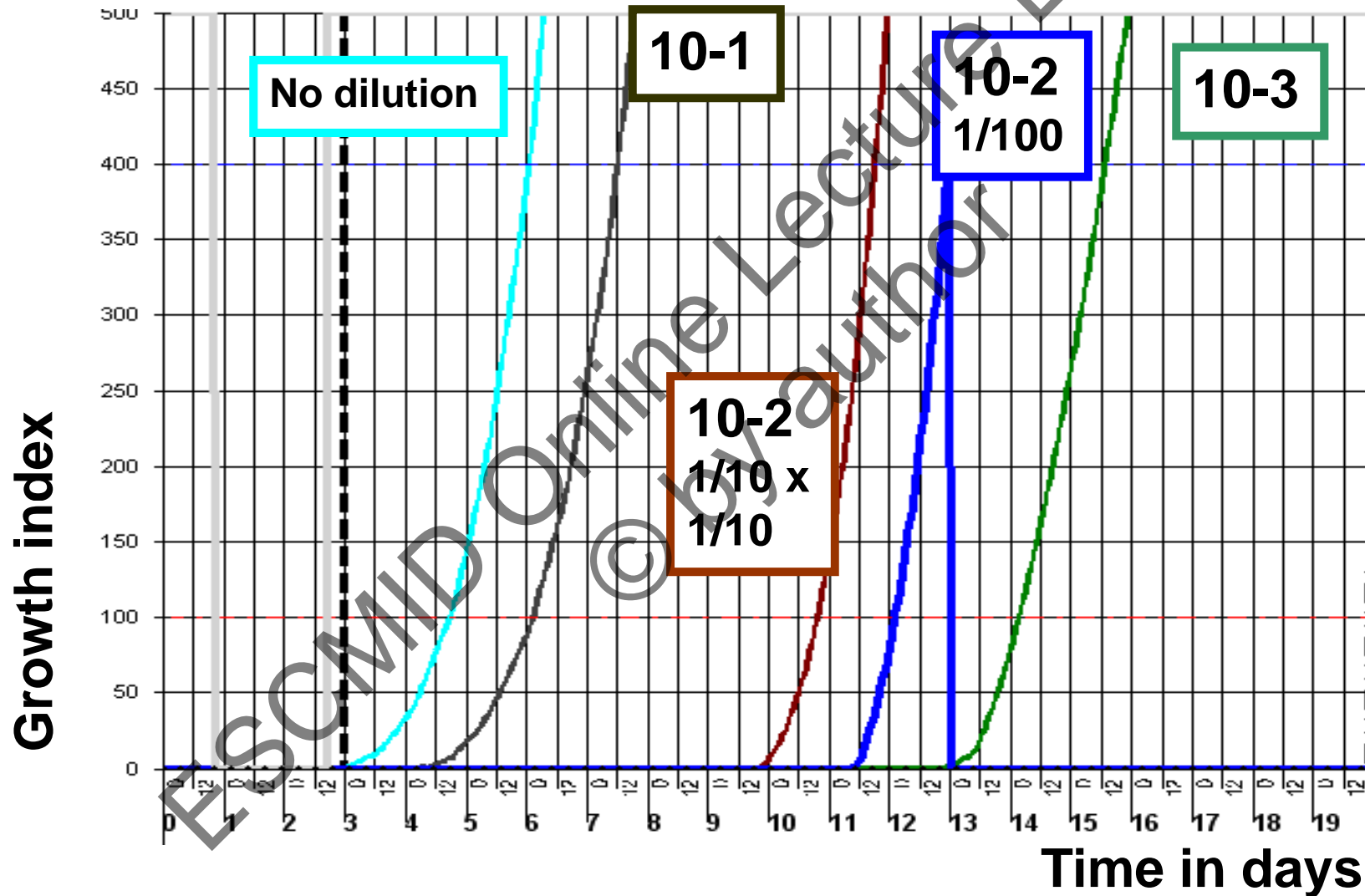
R

R

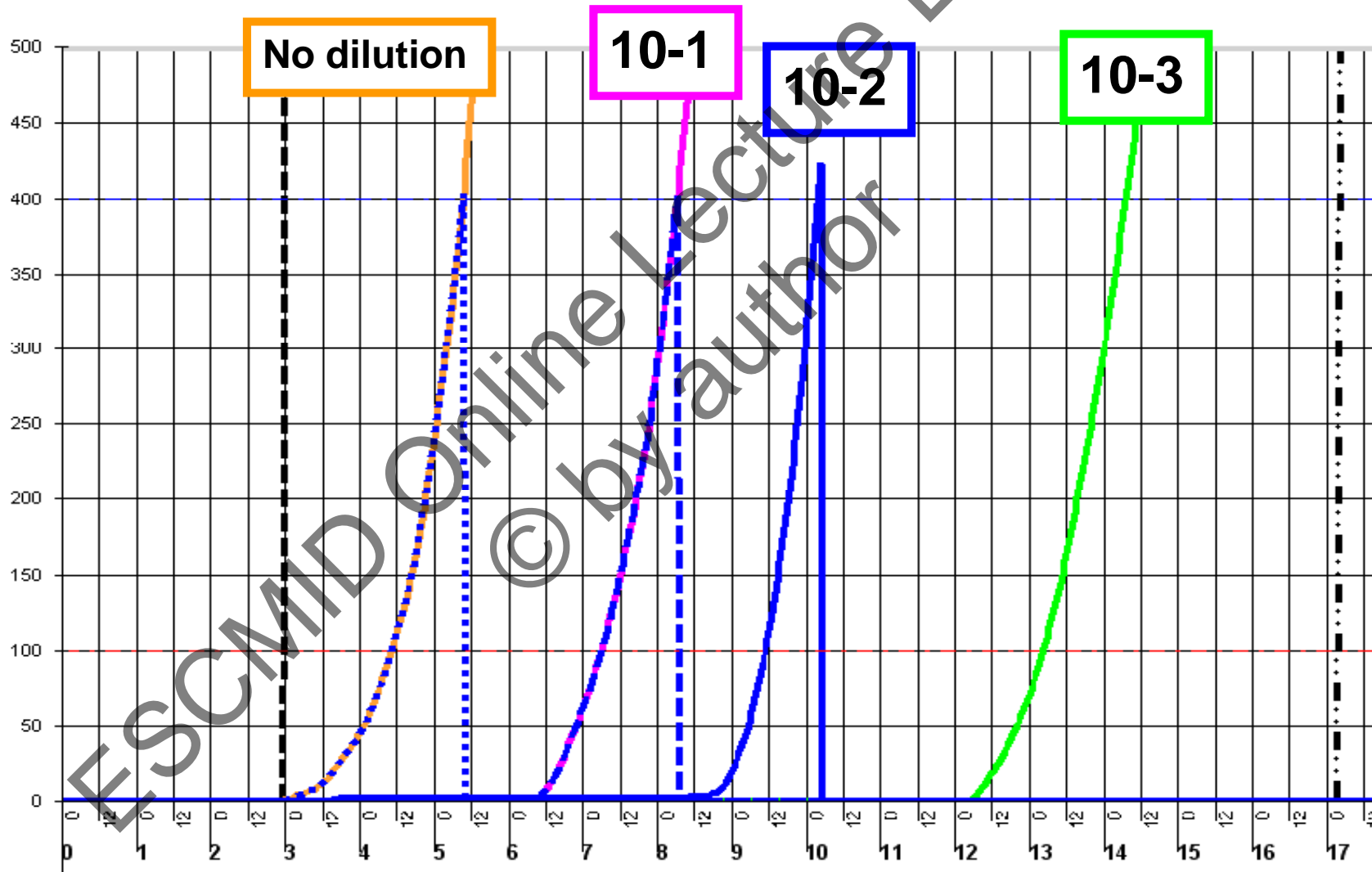
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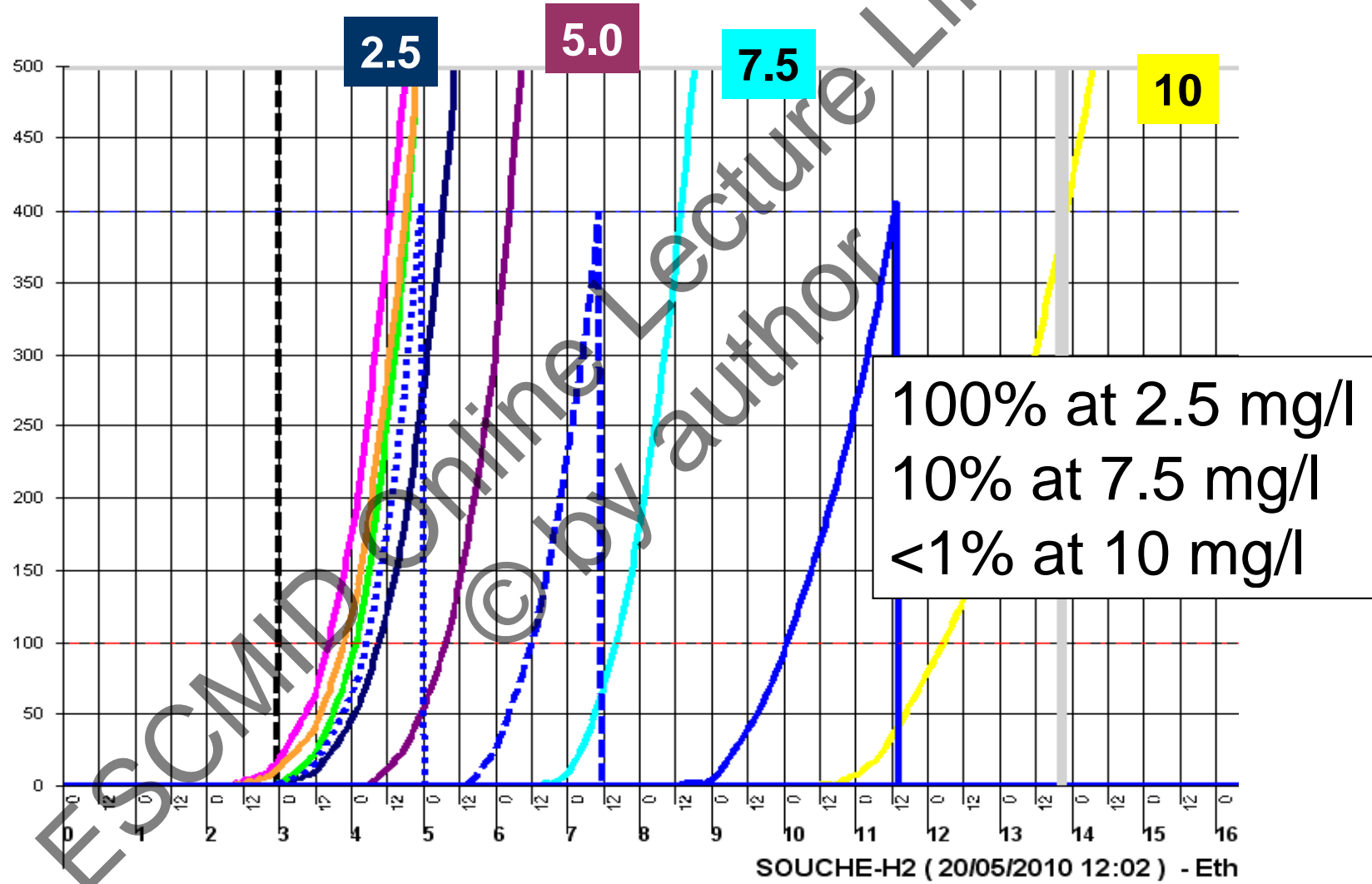
Measuring the proportion of bacterial cells in MGIT with TBExist



Integrating the proportion of bacterial cells



Strain resistant to ethambutol



Conclusions

- Methods for drug susceptibility testing (DST) on *M. tuberculosis* have been developed in relation with clinical outcome of tuberculosis
- Defined critical concentrations and critical proportions which are both necessary for accurate DST
- DST results is highly relevant for MDR-TB cases
 - => justifies on going work in the future
 - for MDR strains
 - Second line drugs
 - new drugs