



**EUCAST**

EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

# Decision made to switch to EUCAST: How to implement EUCAST methodology in your lab

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# Implementation of EUCAST methodology -General

- Liaise with the NAC
- Identify all AST methods used in the laboratory
- Identify support systems that may be affected
  - LIS, accreditation etc
- Identify a “champion” among laboratory staff
- Liaise with a laboratory which has already implemented EUCAST breakpoints and methods

# Implementation of EUCAST methodology -General

- Identify and inform all stakeholders
  - Laboratory staff, customers/users, surveillance programmes, EQA programmes and distributors
- Make sure that necessary “AST materials” will be available

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# Compliance of manufacturers of AST materials and devices with EUCAST guidelines

- Data are based on questionnaires to manufacturers of materials and devices for antimicrobial susceptibility testing.
- The tables will be updated when manufacturers report changes (contact [erika.matuschek@ltkronoberg.se](mailto:erika.matuschek@ltkronoberg.se)).
- The accuracy of data in these tables is not verified by EUCAST and the inclusion of any materials or devices does not indicate endorsement by EUCAST.

**Last updated 15 August 2014**

# Implementation of EUCAST methodology -General

- Identify and inform all stakeholders
  - Laboratory staff, customers/users, surveillance programmes, EQA programmes and distributors
- Make sure that necessary “AST materials” will be available
- Set up a 3-6 month educational programme within the laboratory
- Consult when necessary with EUCAST



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## Implementation of EUCAST Disk Diffusion Test - guidelines

Below are documents to help in the implementation of EUCAST susceptibility testing. A checklist developed and tried by several laboratories will assist in the introduction of the EUCAST disk diffusion test in the clinical laboratory.

[Check list for implementation of EUCAST Susceptibility Testing](#)  
(v. 1.0 May 28, 2010)

For translations to other languages - see Translations.

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... Disk diffusion implementation

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# Implementation of EUCAST methodology -Disk diffusion

1. Educate laboratory staff
2. Perform reading exercises
3. Let laboratory staff prepare their own AST plates
4. Monitor QC data

# Disk diffusion implementation

## 1. Educate laboratory staff

- Teach all staff about inoculum preparation, streaking of plates and reading of plates
- An implementation guide and a tutorial slide show is available at [www.eucast.org](http://www.eucast.org)
  - Available in several languages





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### EUCAST Disk Diffusion Test Methodology

EUCAST has developed a disk diffusion test based on MH media and calibrated to EUCAST clinical breakpoints. Updates are published regularly.

- [EUCAST Disk Diffusion - Manual \(v 4.0, 19 June, 2014\)](#)
- [EUCAST Disk Diffusion - Slide Show \(v 4.0, 19 June, 2014\)](#)
- [EUCAST Disk Diffusion - Reading Guide \(v. 4.0, 19 June, 2014\)](#)

For translations to other languages - see [Translations](#).

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### EUCAST documents translated to other languages

- [Documents in Czech](#)
- [Documents in German](#)
- [Documents in Italian](#)
- [Documents in Scandinavian languages](#)
- [Documents in Spanish](#)
- [Documents in Turkish](#)
- [Documents in French \(see below\)](#)

#### Translations in French (v 3.0):

- [Implementation guideline](#)
- [Media preparation](#)
- [EUCAST Disk Diffusion - Manual](#)
- [EUCAST Disk Diffusion - Slide Show](#)
- [EUCAST Disk Diffusion - Reading Guide](#)

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# Disk diffusion implementation

2. Let all laboratory staff read zones from the same plate

Choose two strains with four disks respectively for each occasion.

- Repeat reading for the same organism twice
- Compare results and discuss with all staff
- Repeat the exercise with the same organisms until everyone gets the same results (mean  $\pm$  1 mm)

# Disk diffusion implementation

Suggested strains for reading exercises:

1. *E. coli* ATCC 25922 + *S. aureus* ATCC 29213
2. *H. influenzae* ATCC 49766 + *S. pneumoniae* ATCC 49619
3. *P. aeruginosa* ATCC 27853 + *E. faecalis* ATCC 29212
4. Clinical isolates, including  $\beta$ -haemolytic streptococci

# Reading exercise in Växjö

## *Staphylococcus aureus*, clinical isolate 1st reading occasion

Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacin	Rifampicin
26	27	26	29	20	30
26	28	28	29	22	31
26	29	28	29	25	31
29	28	28	30	23	31
26	27	27	28	19	30
26	28	29	30	23	32
26	29	29	30	21	31
26	27	27	29	23	29
25	27	26	29	20	32
25	25	26	28	25	32
28	27	26	27	22	30

Mean

26

27

27

29

22

31

SD

1.2

1.1

1.2

0.9

2.0

1.0

> 1 mm above mean

> 1 mm below mean

# Reading exercise in Växjö

## *Staphylococcus aureus*, clinical isolate After analysis and discussion

Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacin	Rifampicin
27	24	25	26	18	28
27	25	26	28	18	29
27	25	26	27	17	27
27	25	26	27	16	27
27	25	26	27	19	28
28	24	24	26	17	25
27	26	27	29	19	29
27	24	26	28	18	28
27	25	23	26	18	27
28	25	26	27	18	30
27	24	24	25	17	27

Mean

27

25

25

27

18

28

SD

0.4

0.6

1.2

1.1

0.9

1.3

> 1 mm above mean

> 1 mm below mean

# Reading exercise in Växjö

## *Haemophilus influenzae* NCTC 8468 1st reading occasion

Ampicillin	Cefotaxime	Tetracycline	Nalidixic acid	Trim-sulfa
22	32	30	30	31
24	34	32	31	33
24	34	32	32	35
25	35	34	33	36
23	33	32	31	33
25	35	33	32	34
24	34	32	31	33
24	33	30	32	34
24	34	32	31	32
23	34	32	30	34
22	33	30	30	33

<b>Mean</b>	<b>24</b>	<b>34</b>	<b>32</b>	<b>31</b>	<b>33</b>
<b>SD</b>	1.0	0.9	1.3	1.0	1.4

> 1 mm above mean

> 1 mm below mean

# Reading exercise in Växjö

## *Haemophilus influenzae* NCTC 8468 After analysis and discussion

Ampicillin	Cefotaxime	Tetracycline	Nalidixic acid	Trim-sulfa
24	35	33	32	36
24	35	33	33	37
24	35	34	33	38
24	36	33	32	36
24	34	34	33	34
25	36	35	34	38
25	37	35	34	38
23	36	33	34	36
24	34	33	33	36
23	34	34	33	36
25	37	35	34	38

<b>Mean</b>	<b>24</b>	<b>35</b>	<b>34</b>	<b>33</b>	<b>37</b>
<b>SD</b>	<i>0.7</i>	<i>1.0</i>	<i>0.9</i>	<i>0.8</i>	<i>1.2</i>

> 1 mm above mean

> 1 mm below mean



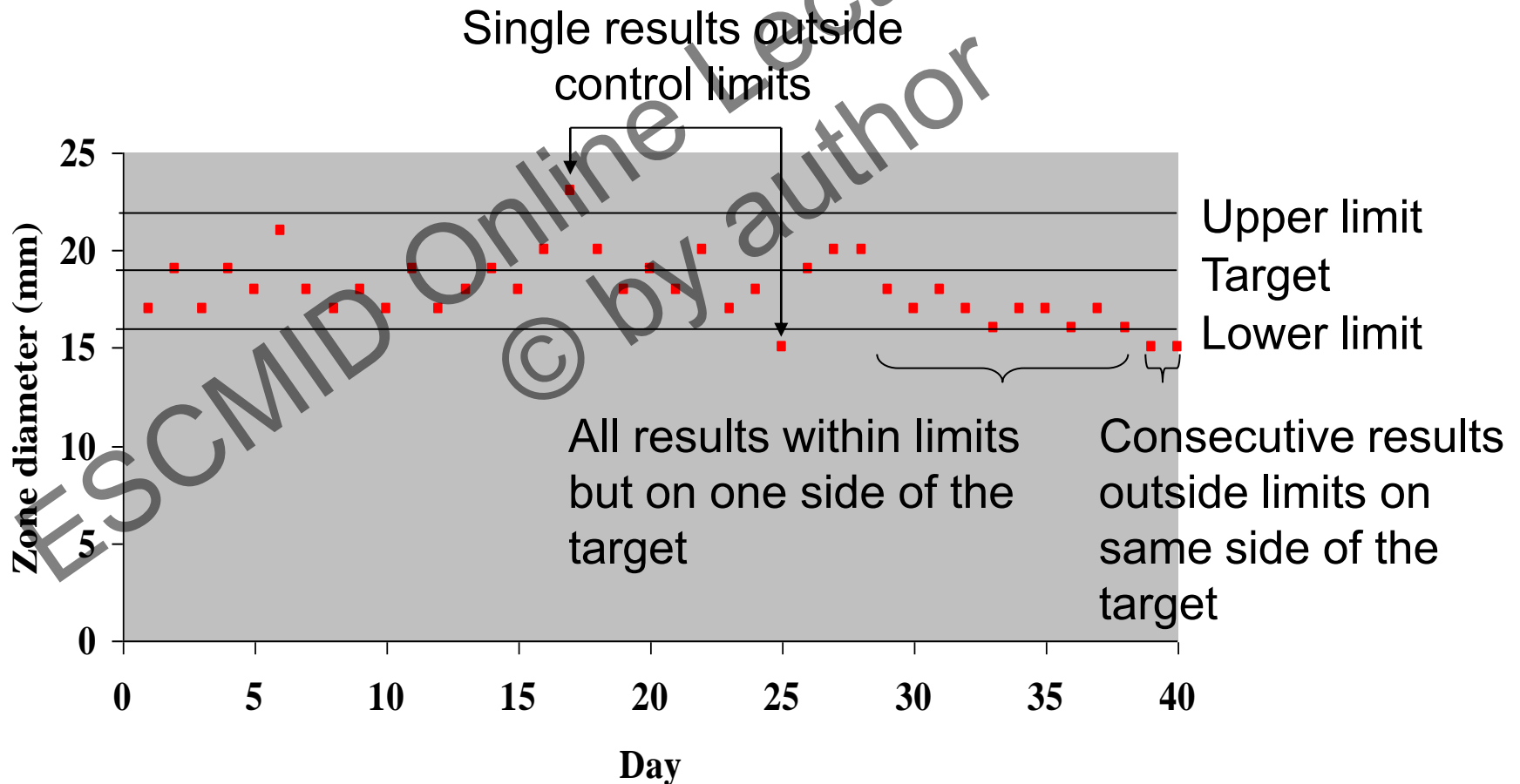
# Disk diffusion implementation

3. Let all staff prepare their own inoculum and streak plates for all EUCAST QC strains.

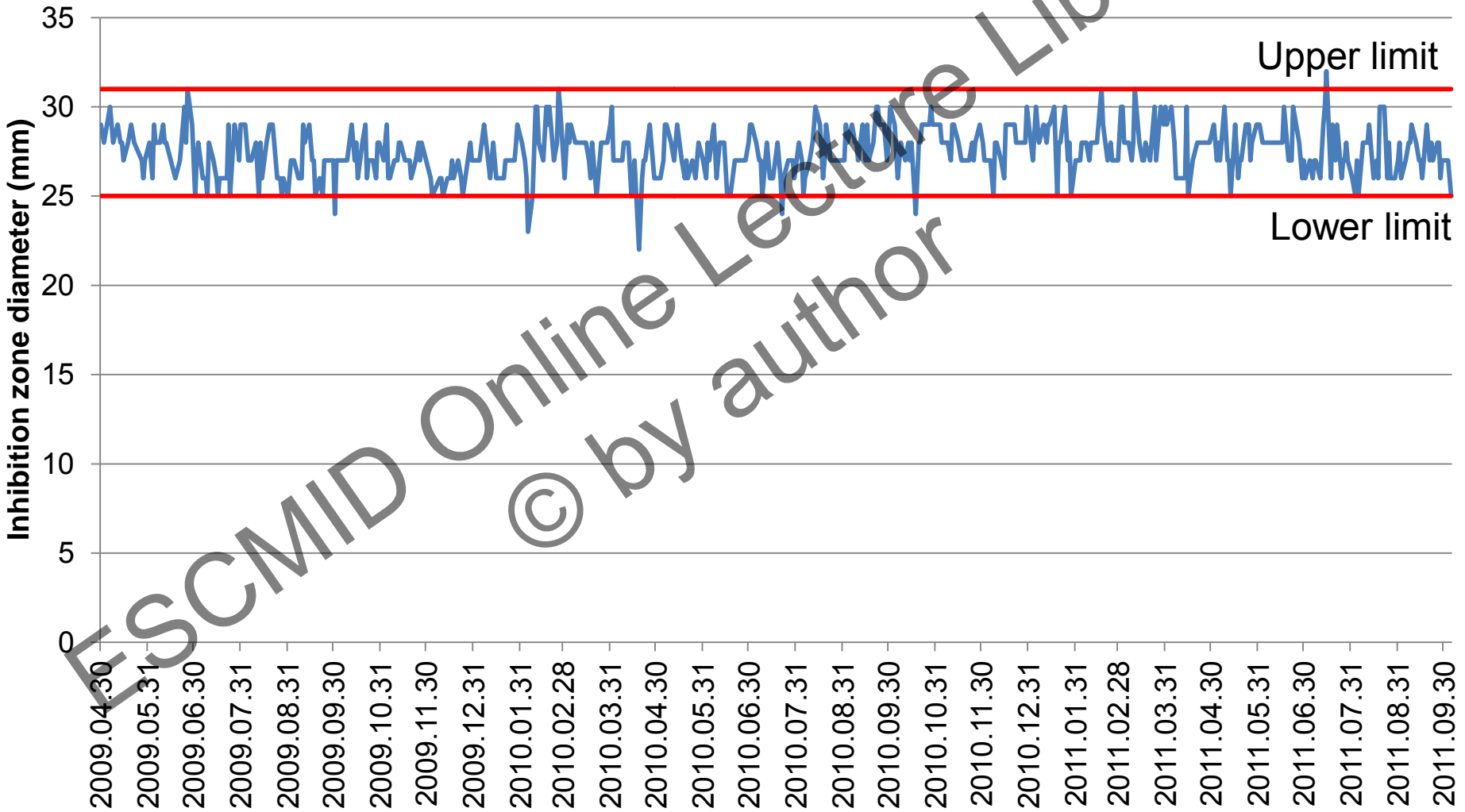
Make sure that the inhibition zones are within the QC ranges.

# Disk diffusion implementation

## 4. Monitor QC data

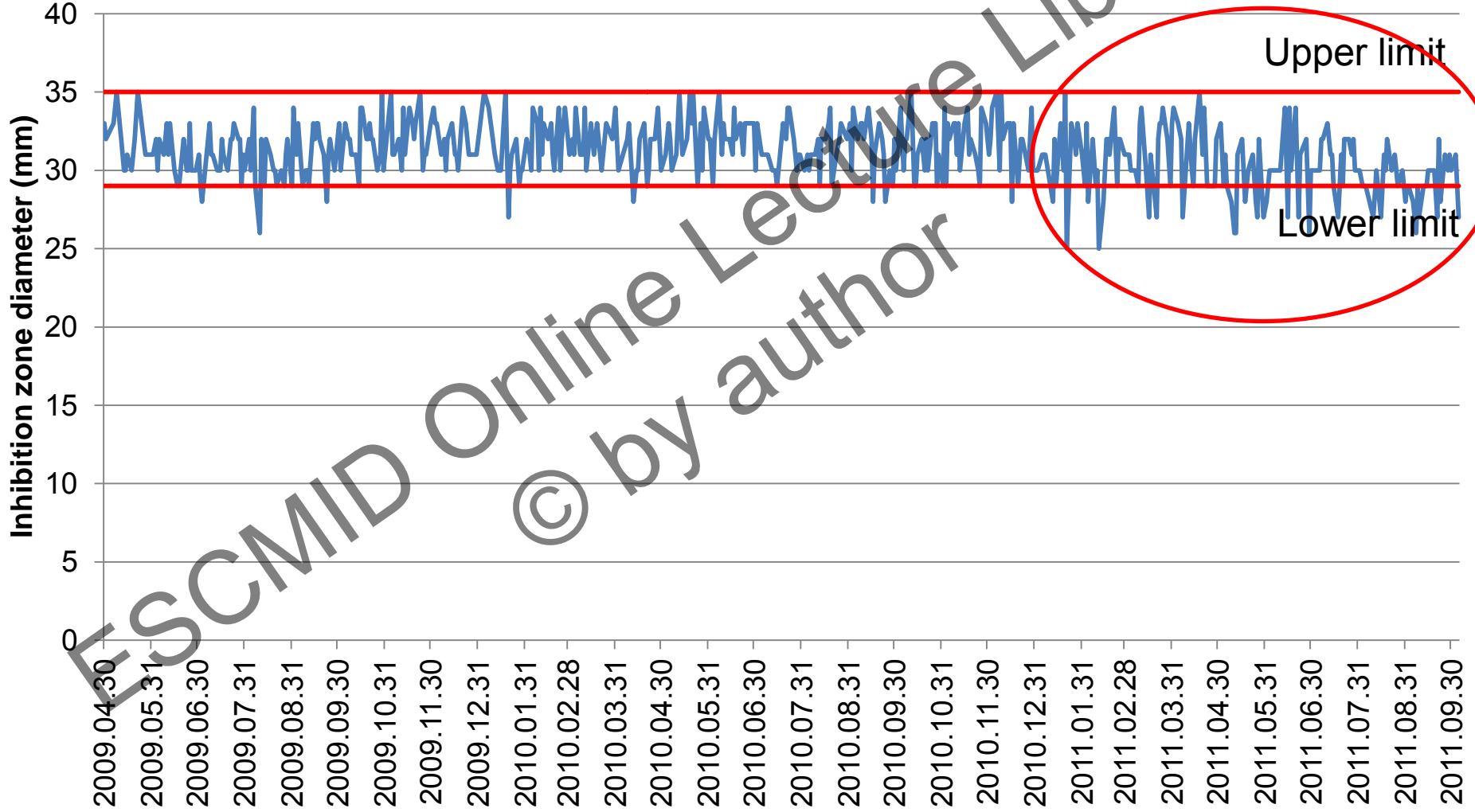


***E. coli* ATCC 25922 with cefotaxime 5 µg**



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***H. influenzae* NCTC 8468 with cefotaxime 5 µg**



BAKT. : STAPHYLOCOCCUS AUREUS

ANTIB. : ERYTHROMYCIN 15

3163 st. stammar.

S >= 21mm    R <= 17mm

S = 95.7%

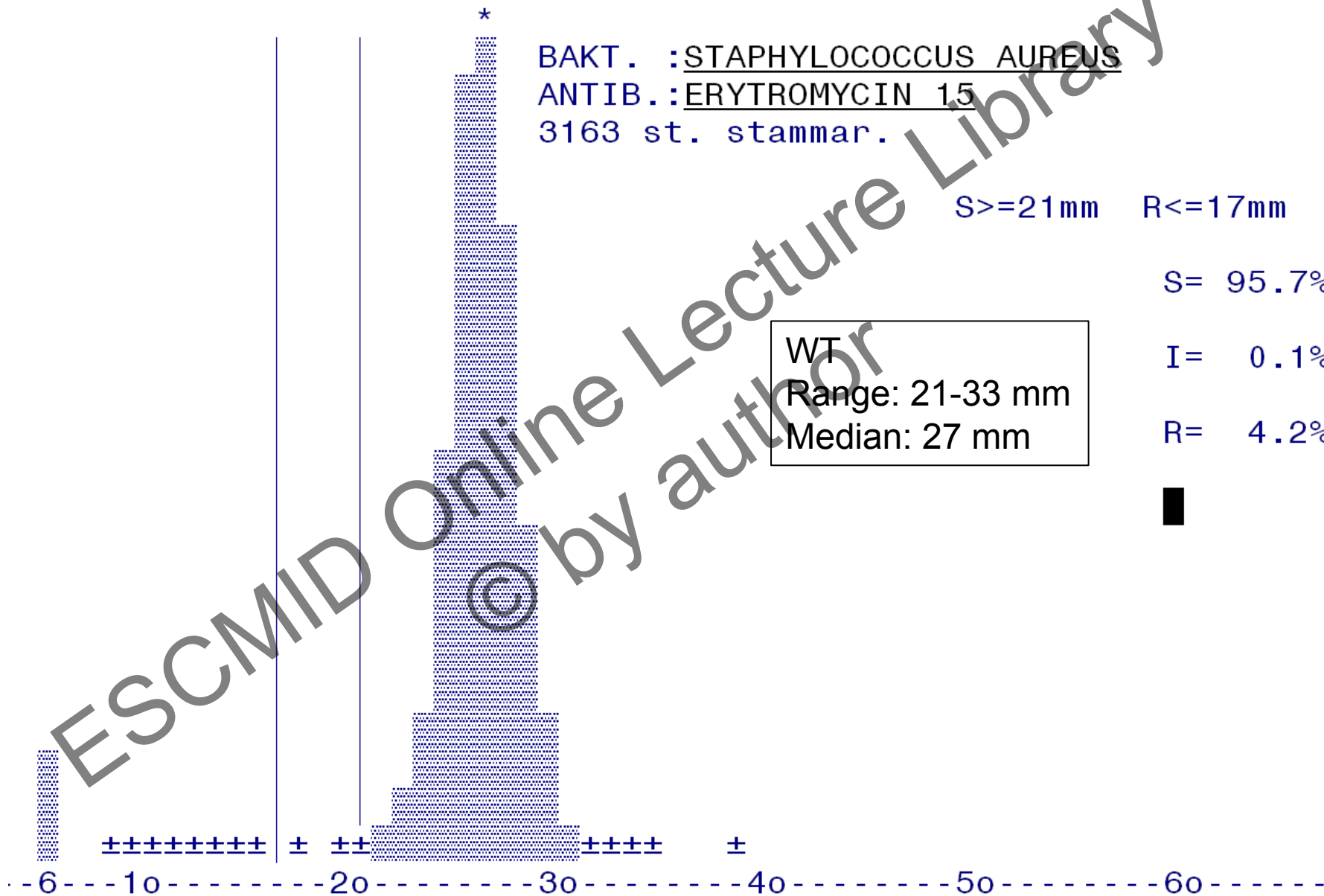
I = 0.1%

R = 4.2%

WT

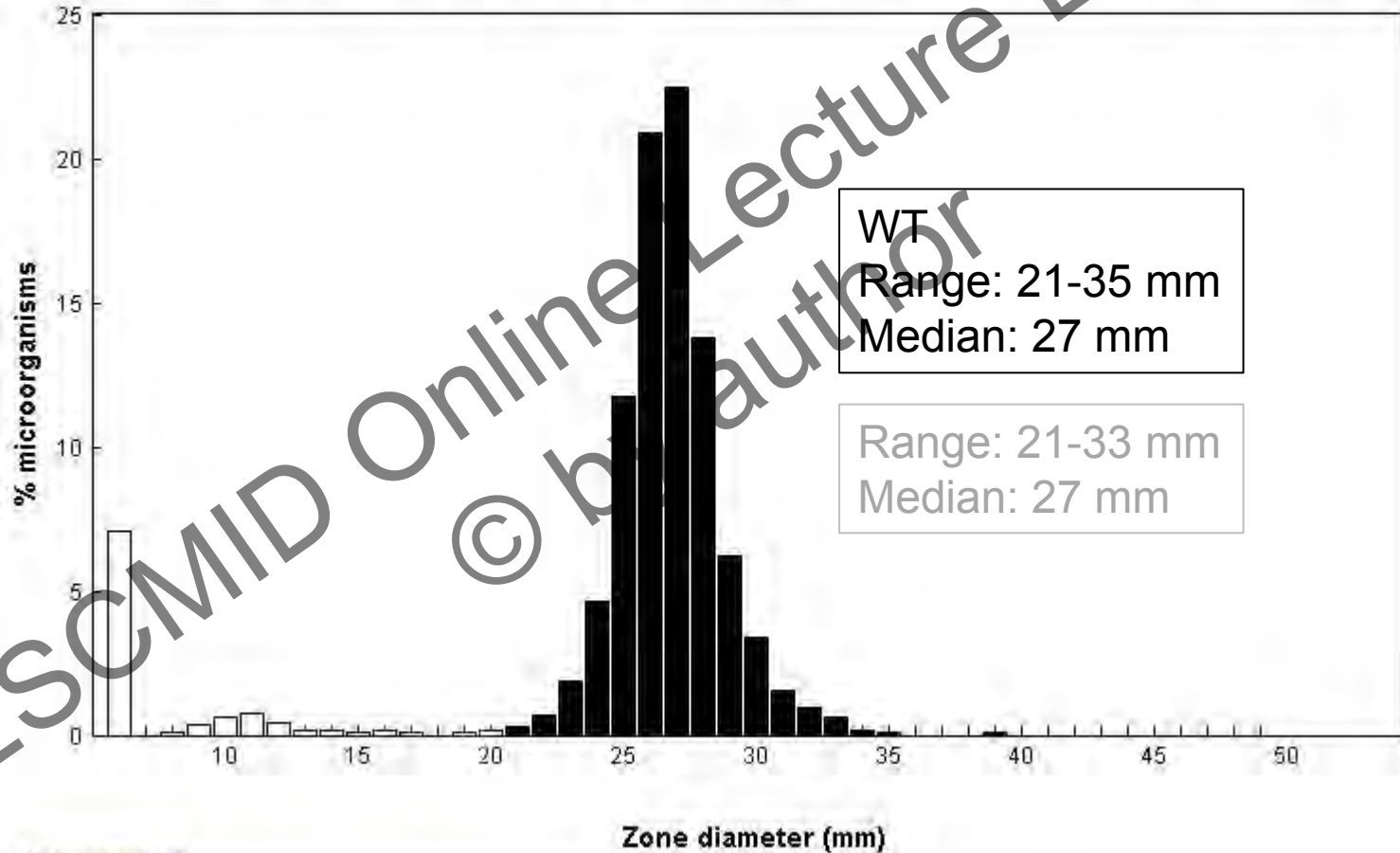
Range: 21-33 mm

Median: 27 mm



**Erythromycin / *Staphylococcus aureus***  
**International wild type zone diameter distribution - Reference database 2014-09-09**  
**EUCAST disk diffusion method**

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 15  
Epidemiological cut-off (ECOFF): 21 mm (MIC = 1 mg/L)  
Wildtype (WT) organisms:  $\geq$  21 mm (MIC = 1 mg/L)

11242 observations (9 data sources)

# EUCAST Ranges and Targets

- **Range**

- Mean value  $\pm 2 \times \text{SD}$  (at least mean  $\pm 3 \text{ mm}$ )
- Used to allow occasional variation

- **Target**

- Mean value or median of distribution
- Mean values from repeated measurements should optimally be on target  $\pm 1 \text{ mm}$

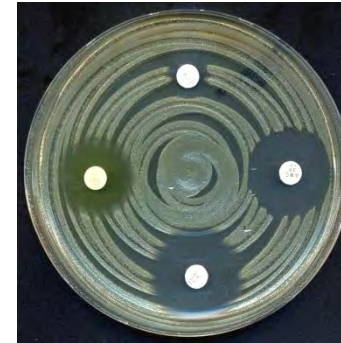
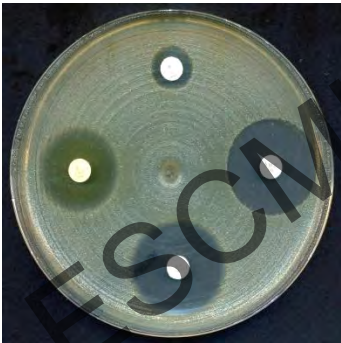
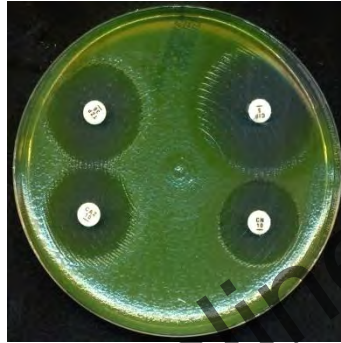
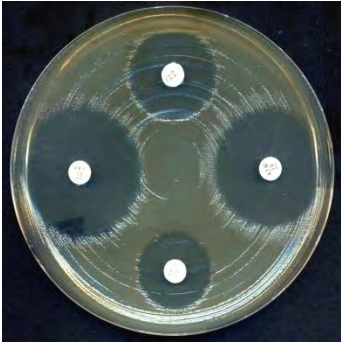
Some significant details for a  
correct disk diffusion result



# Confluent and even growth

- The inoculum should be evenly spread over the agar surface to get uniformly circular inhibition zones and reproducible zone diameters.
  - Regardless of if plates are inoculated by hand or with a plate rotators

The growth should be confluent and evenly spread over the plate



**Plates should look like this..**

**..and NOT like this!**

# Don't over inoculate plates!

- It is important not to inoculate plates too heavily, which will result in smaller zone diameters.

Enterobacteriaceae, Pseudomonas and Haemophilus	Remove excess fluid
Staphylococci and streptococci	Allow the swab to be more humid

# Follow the "15-15-15-minutes rule"

- Use the inoculum within **15 minutes** of preparation – and never beyond 60 minutes.
- Apply disks within **15 minutes** of inoculating plates.
- Start incubation within **15 minutes** of application of disks.

# Follow reading instructions

- Read zone edges at the point where no obvious growth is detected by the unaided eye.
- Read MH plates without supplements from the back of the plate against a black background.
- Read MH-F plates from the front with the lid removed.

# Questions?

Please contact us!

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