

# Rapid Phenotypic Antimicrobial Susceptibility Testing

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EUROSTAR-gänget

# Rapid phenotypic AST

- **Direct susceptibility testing**
  - AST directly on sample (mostly Urine)
  - AST directly from the blood culture bottle

**Cuts 24 hours off the standard procedure**

- **Potential problems:**
  - **Inoculum??**
  - **Purity of culture??**

# Rapid phenotypic AST

- **“Early Reading” susceptibility testing**
  - Reading disk diffusion, or gradient tests, or automated testing earlier than stipulated in the standard method.

**Cuts 24 hours off the standard procedure**

- **Potential problems:**
  - **Some resistance mechanisms may not have time to express themselves phenotypically in 6, 7 or 8 hours**
  - **Not standardised, but could be standardised by EUCAST**

## Why rapid ID and rapid AST?

To increase the chance of patient survival, save time and limit costs.

- Species ID determines the course of treatment, not only choice of antibiotic, but of many non-antimicrobial decisions.
- Empiric antimicrobial therapy will fail more often as resistance increases.
- To improve antibiotic stewardship – fewer cocktails, fewer “to be on the safe side”, fewer “trials-and-errors”
- Saves time
- Saves cost (Garcia-Vazquez SJID 2013)
- Because now we can!

- Every hour is important in the treatment of sepsis (septic shock) (Kumar CCM 2006)
- Inappropriate AB therapy → 2 days longer hospital stay (Shorr CCM 2011)
- More Rapid Species ID and AST improves AB-therapy (Kerremans JAC 2008)

# The lives saving diagnostic chain in blood stream infections

- The 24/7 rapid transportation (2 h) of blood culture bottles from patient to cupboard (incubation).
- The 24/7 access to cupboards for incubation and growth detection.
- Laboratory staff action on positive signals.
- Rapid ID – direct MT on bottles within 60 minutes of positive signal.
- Direct inoculation of AST from blood culture bottles.
- Early reading of AST – 6 to 8 hours of positive signal.
- Immediate alert and transfer of result.

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# No EUCAST recommendation on direct AST

- EUCAST does not recommend:
  - Direct inoculation of AST from blood culture bottles.
  - Early reading of AST – 6 to 8 hours of positive signal.

Since the inoculum is not standardised and the standard method is validated for 16 – 20 h incubation.

Urine - cultures are mixed and the pH variability may affect antimicrobial activity.

# EUCAST

## ...considers...

- validating "direct inoculation from blood culture bottles" and "early reading" of disk diffusion.

## ...will not consider...

- validating "direct inoculation from urines"

# Direct AST of urines

Each laboratory needs to

- Validate - confirm with standard methods
- Restrict to certain agent/species combinations
  - E.coli, Klebsiella pneumoniae
  - Primary UTI agents (Piv-MEC, NIT, CDR, TRI, CIP)
- Define when to repeat/confirm result.
- Only report if pure culture and acceptable inoculum.

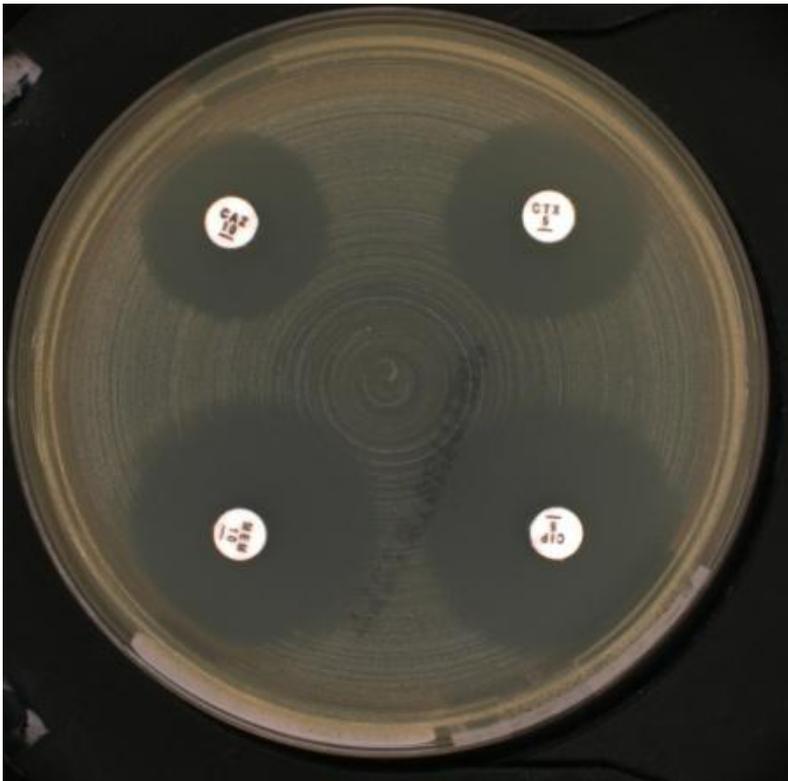
# Direct AST from blood culture bottles

- 22/26 Swedish laboratories perform Direct AST from positive blood cultures
- Early reading (after 6-8h incubation) is performed by 10 of 22
  - Disk diffusion
  - Vitek2

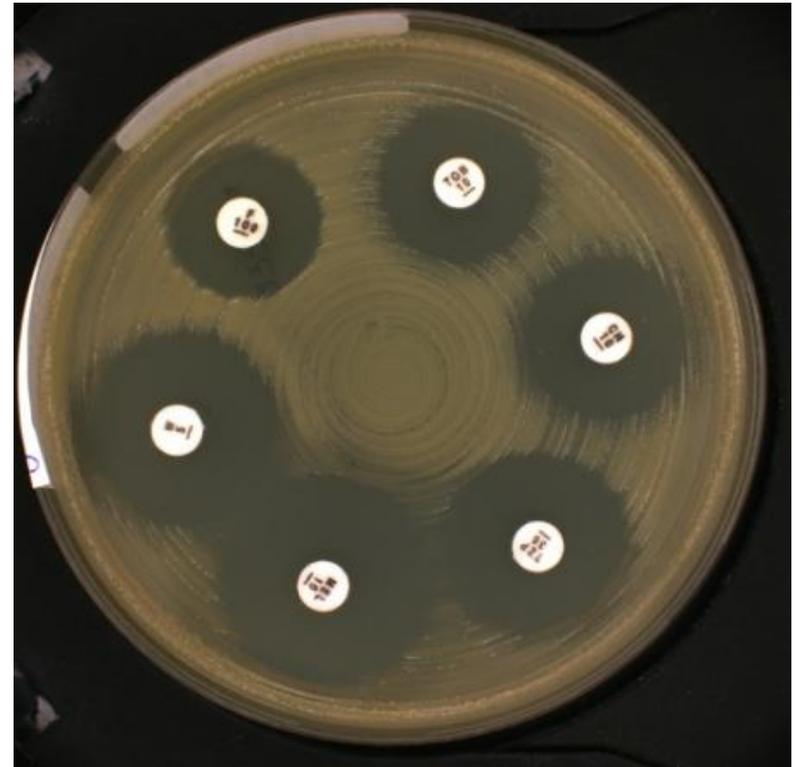


# *E. coli* ATCC 25922 early reading

6h



18h



# “Early reading – strategies”

1. Get it right – complete correlation - repeat testing not necessary.
2. Err on the side of caution – overcall resistance; use ECOFFs; repeat testing necessary
3. Identify “safe sensitive” – give the clinician a few safe alternatives; repeat testing necessary

# EUROSTAR RAPID DISK

## Metod

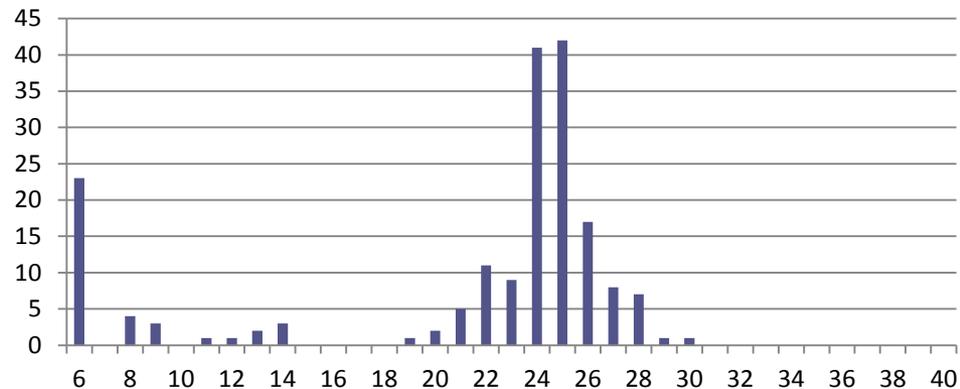
- EUCAST method with short incubation time
- 6h
  - *E.coli*
  - *K.pneumoniae*
  - Enterococci
- 8h
  - *P.aeruginosa*
  - *S.aureus*
- 93% could be read after 6 or 8h

## Lab

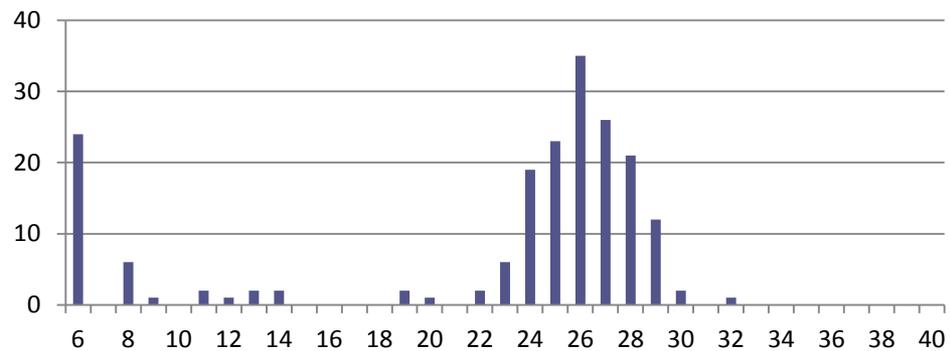
- Växjö
  - Manual reading
- Hvidovre
  - Manual reading
- AMC
  - Camera (Kiestra) with manual adjustment. Reading every hour.

# E. coli vs. cefotaxime 5 $\mu$ g at 6, 8 and 18 h

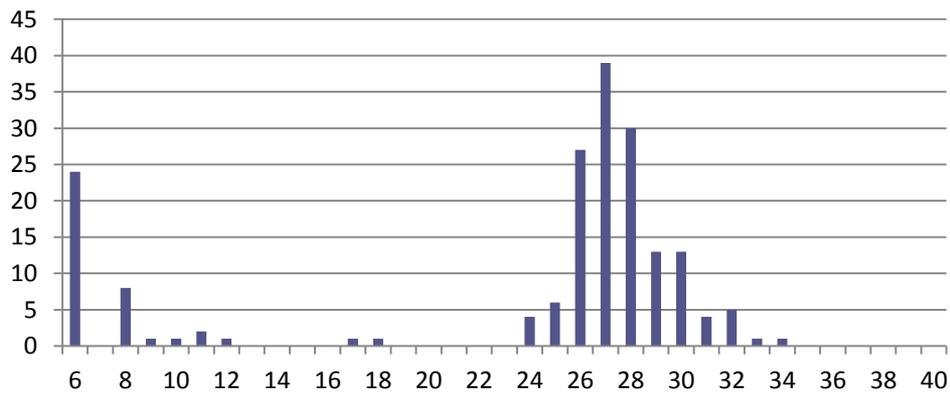
## 6 h



## 8h

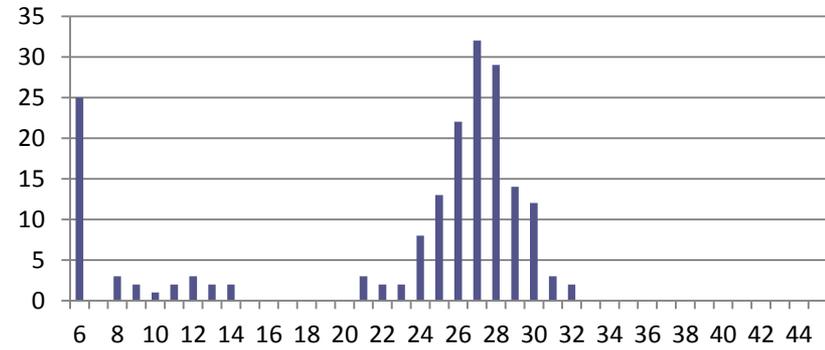


## 18 h

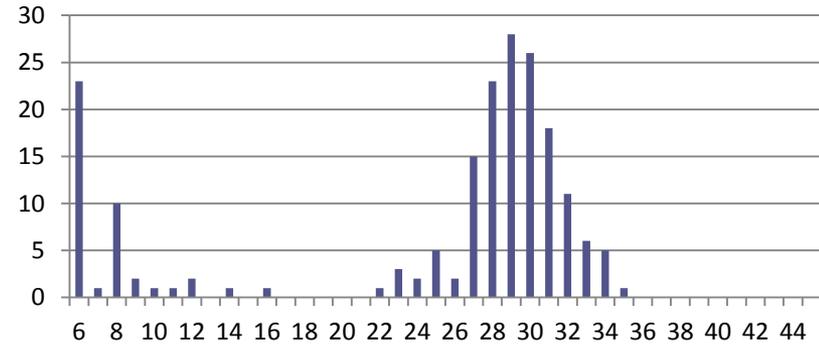


*E. coli* vs. ciprofloxacin 5  $\mu\text{g}$   
at 6, 8 and 18 h

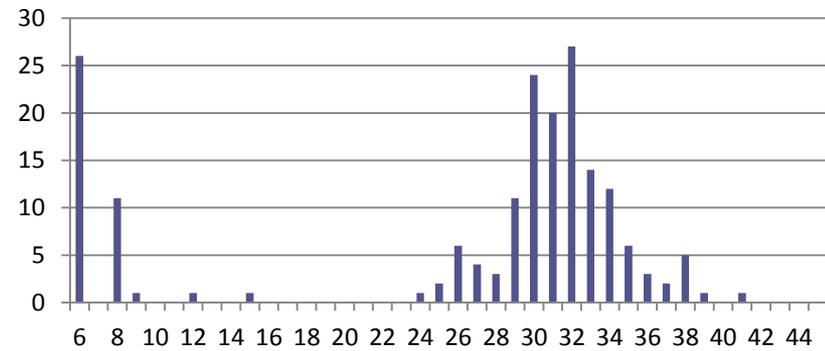
**6 h**



**8h**

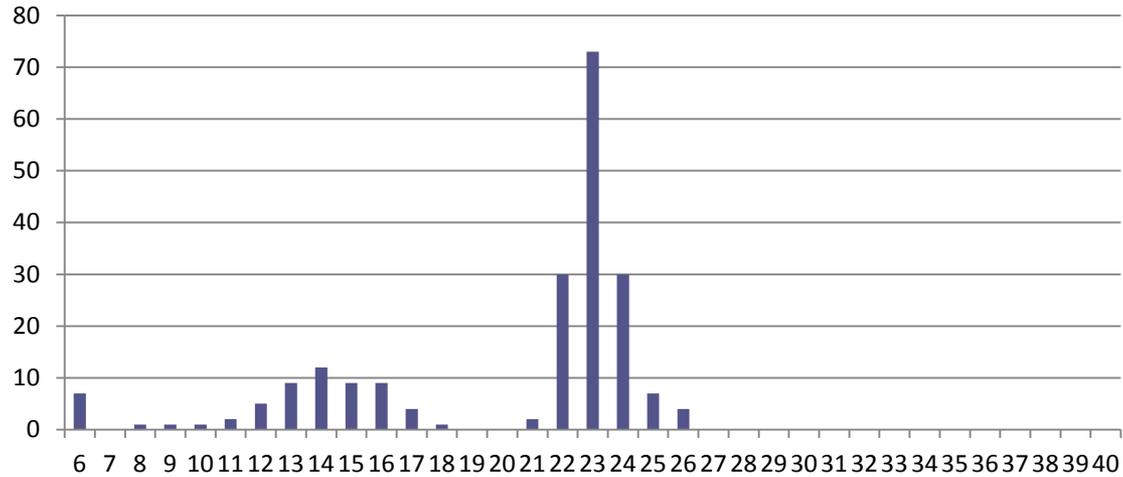


**18 h**

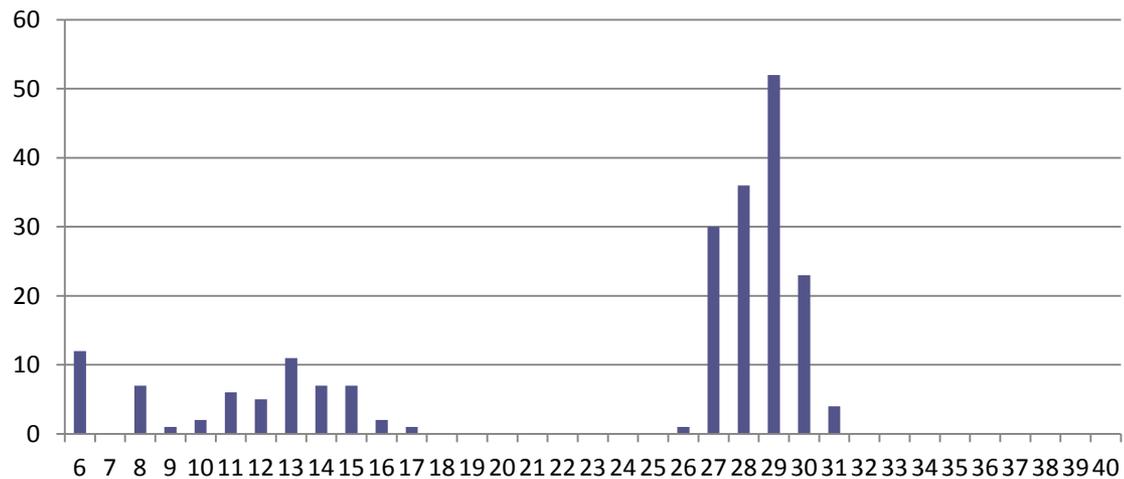


# *S. aureus* vs. cefoxitin 30 µg

8h



18h



# Disk diffusion – early reading

- After 6 – 8 hours
  - Zone diameters for sensitive organisms are smaller than after 16-20 hours and
  - Zone diameters for resistant organisms are bigger than after 16-20 hours
  - The margin for errors shrink with 6-8h readings.

**DIRECT AST DIRECTLY FROM URINE**

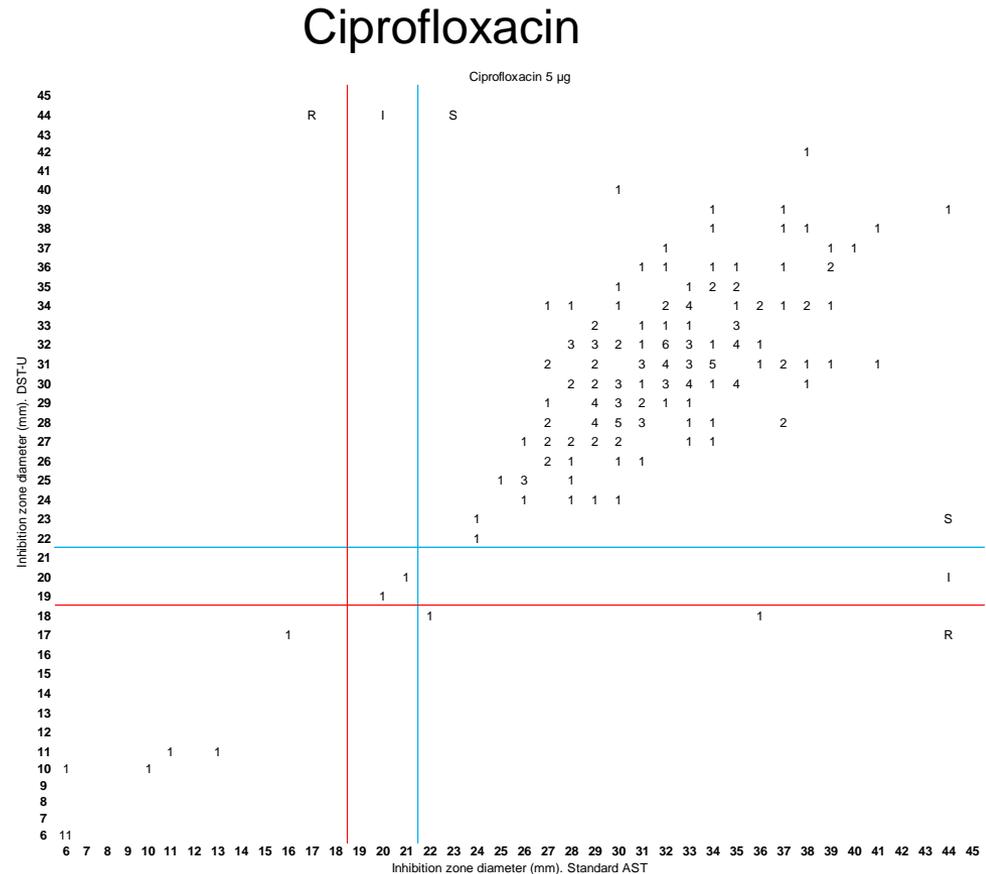
# DAST Urine

- Every day routine in many laboratories
- Increased usefulness with MALDI-TOF (direct?)
- Increased performance if pre-screening is performed?



# DAST Urine

- $\geq 10^5$  cfu/mL
- Singel species only
- 177 samples, 123 *E.coli*
- EUCAST CBP
- 855 tests
  - 3 VME
  - 5 ME
  - 7mE



# DAST urine, Routine

Örebro University Hospital april 2013

- 470 prov
  - 53% Neg
  - Gram pos 9%
  - 10% Mixed cultures
  - DAST recorded on 25%
- 98 *E.coli* (87%)
- 2 ME (TRI och MEC)
  - 2/2 ESBL were found
- 10 *Proteus* spp
- 1 mE
- Other Enterobacteriaceae
- No errors
  - Rapid Species ID important

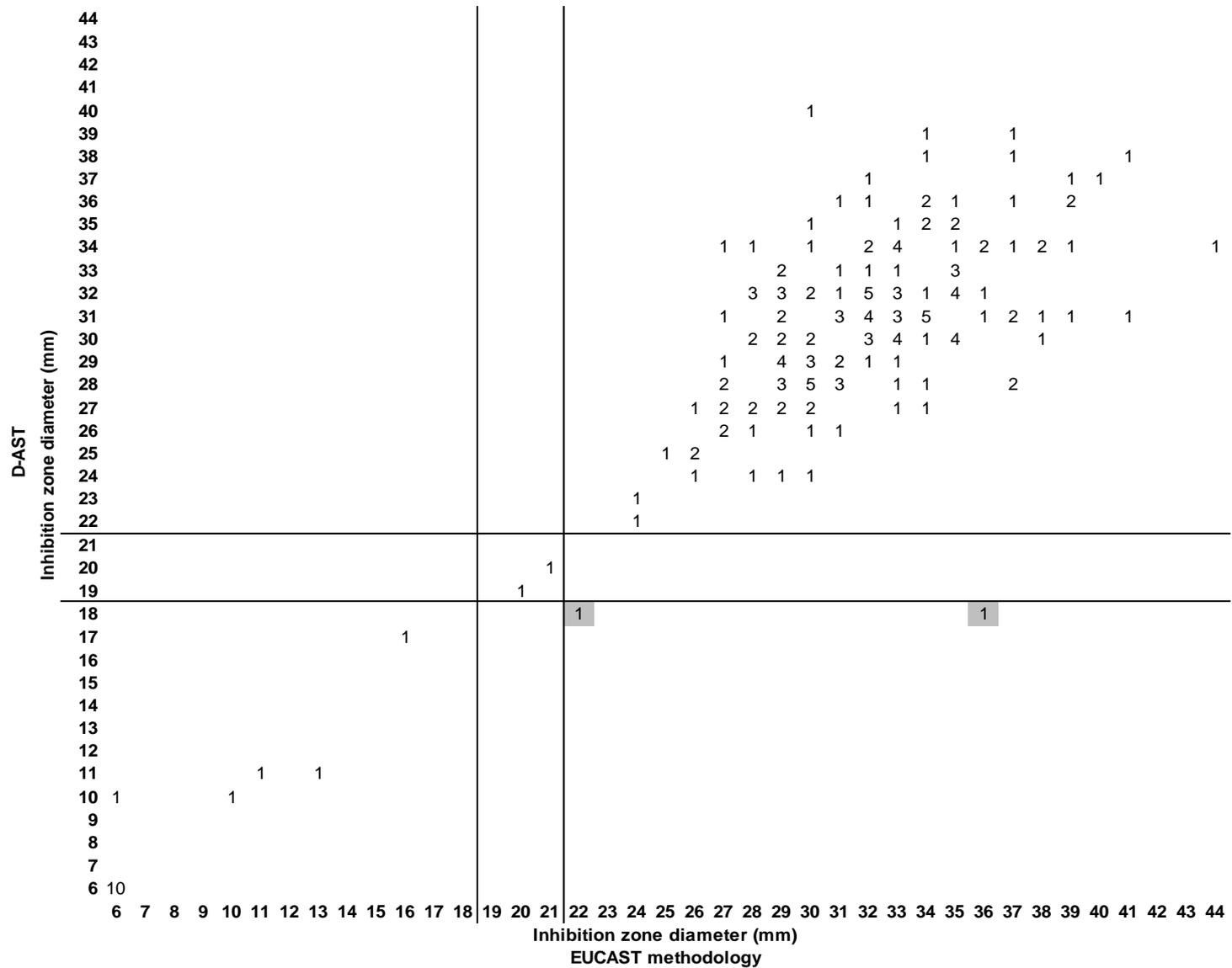
Antimicrobial Susceptibility Testing Directly from Urine Samples – a Comparison between Standardised and Direct Disk Diffusion Testing together with Direct Species Identification using Matrix assisted laser desorption/ionisation time of flight.

Jonas Olafsson

Klinisk mikrobiologi, Växjö  
Linné-universitetet

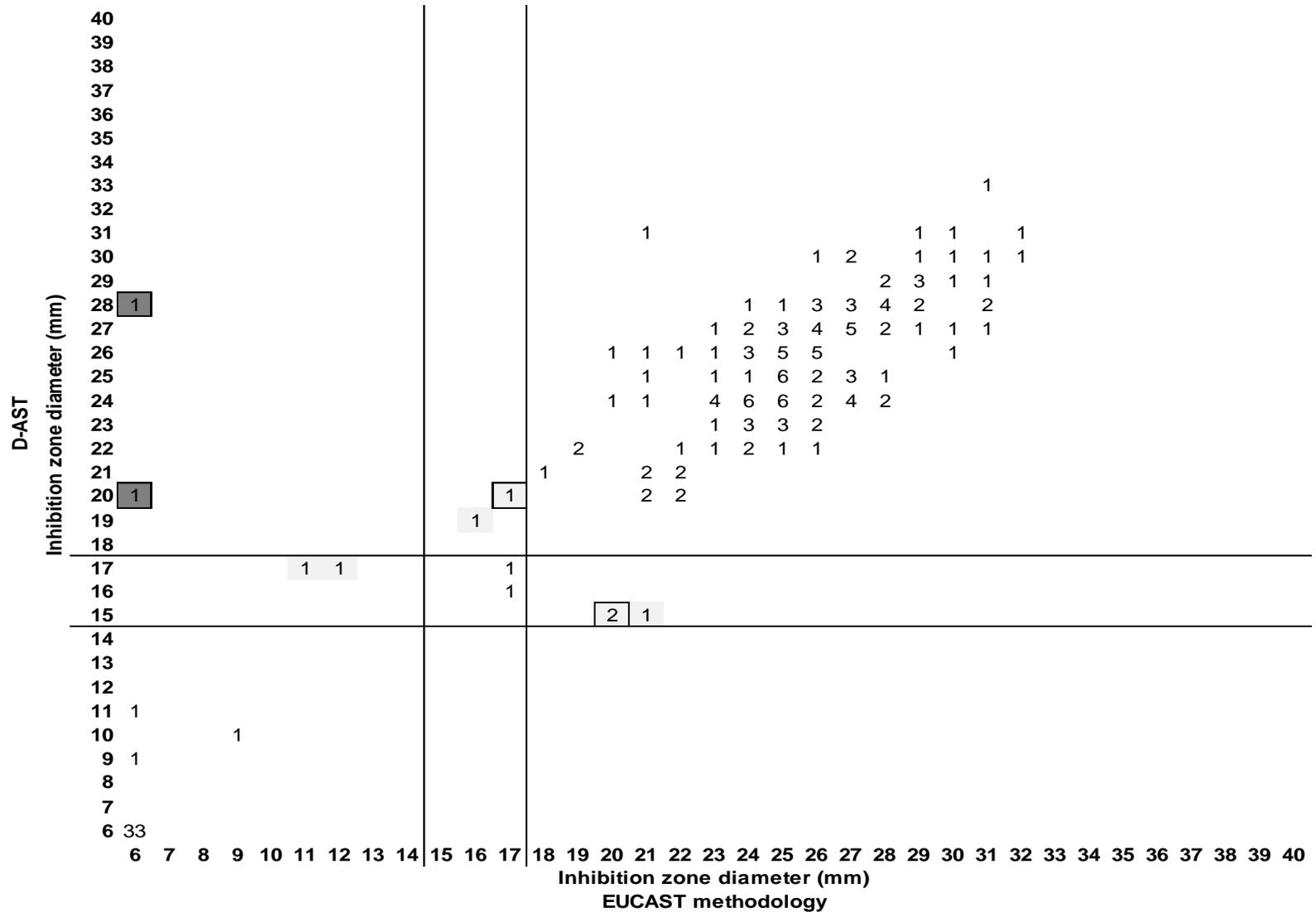
Poster ECCMID 2014

# Ciprofloxacin 5 µg (n=188).

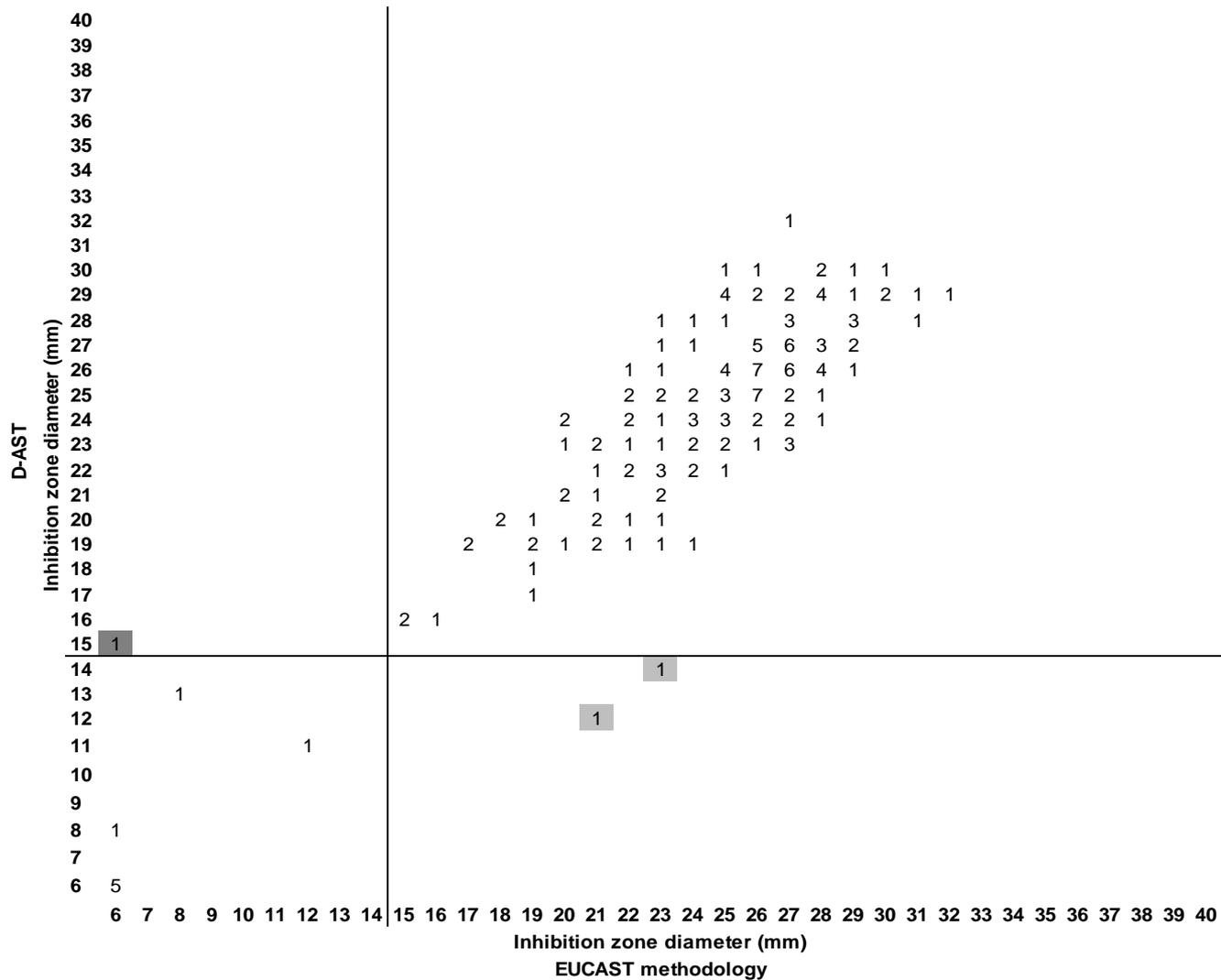


# Trimethoprim 5 µg (n=188).

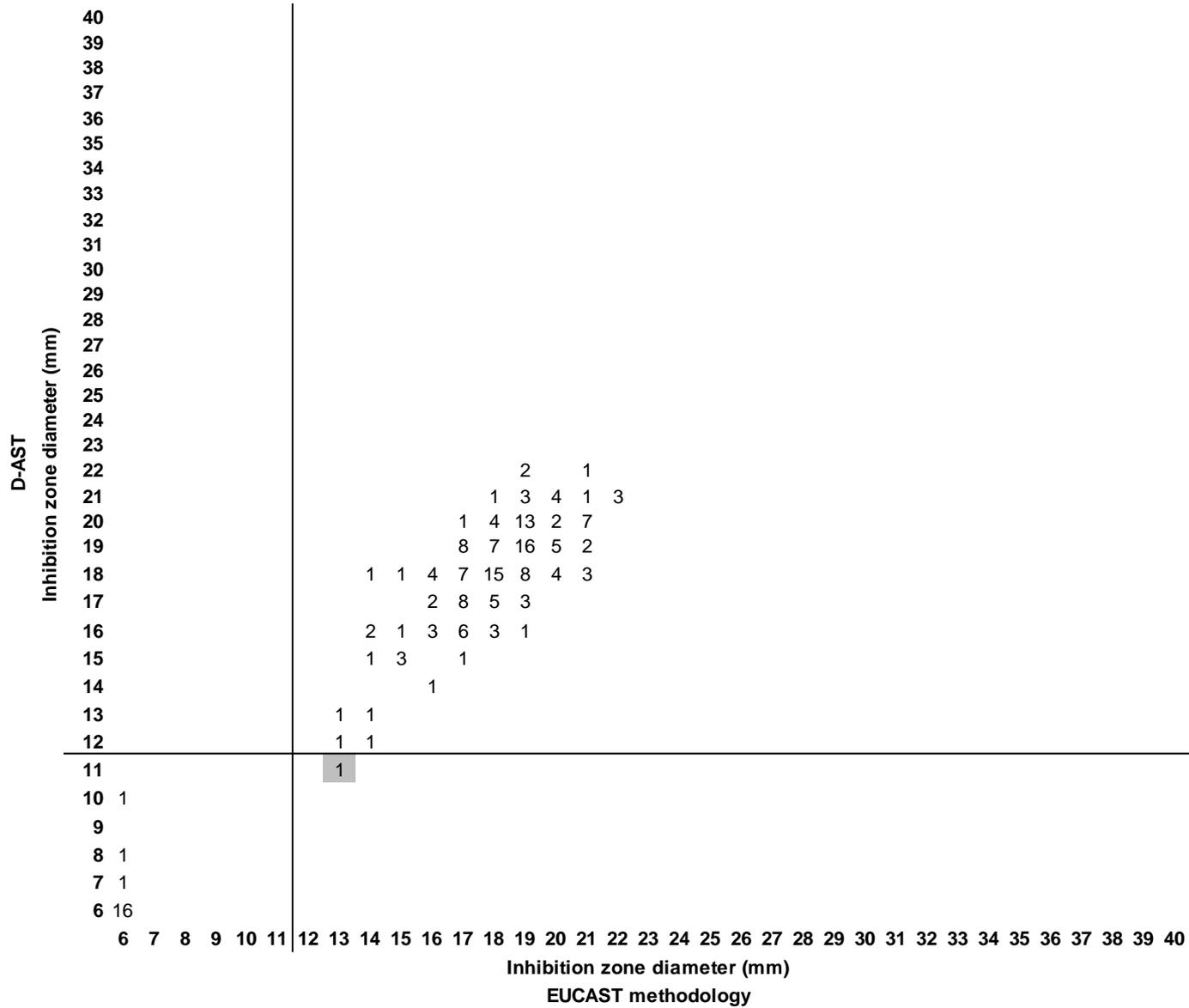
Errors for *P. mirabilis* are marked with black boxes.



# Mecillinam 10 µg (*E. coli*, *Klebsiella* spp. and *P. mirabilis*, n=172).



# Cefadroxil 30 µg (n=188).



Direkt resistensbestämning på urinprover för  
andra gramnegativer än *Escherichia coli* och  
*Klebsiella pneumoniae*

Nadia Vickius

Oskar Ekelund

Poster ECCMID 2015

Klinisk mikrobiologi för Kronoberg  
och Blekinge

# Upplägg

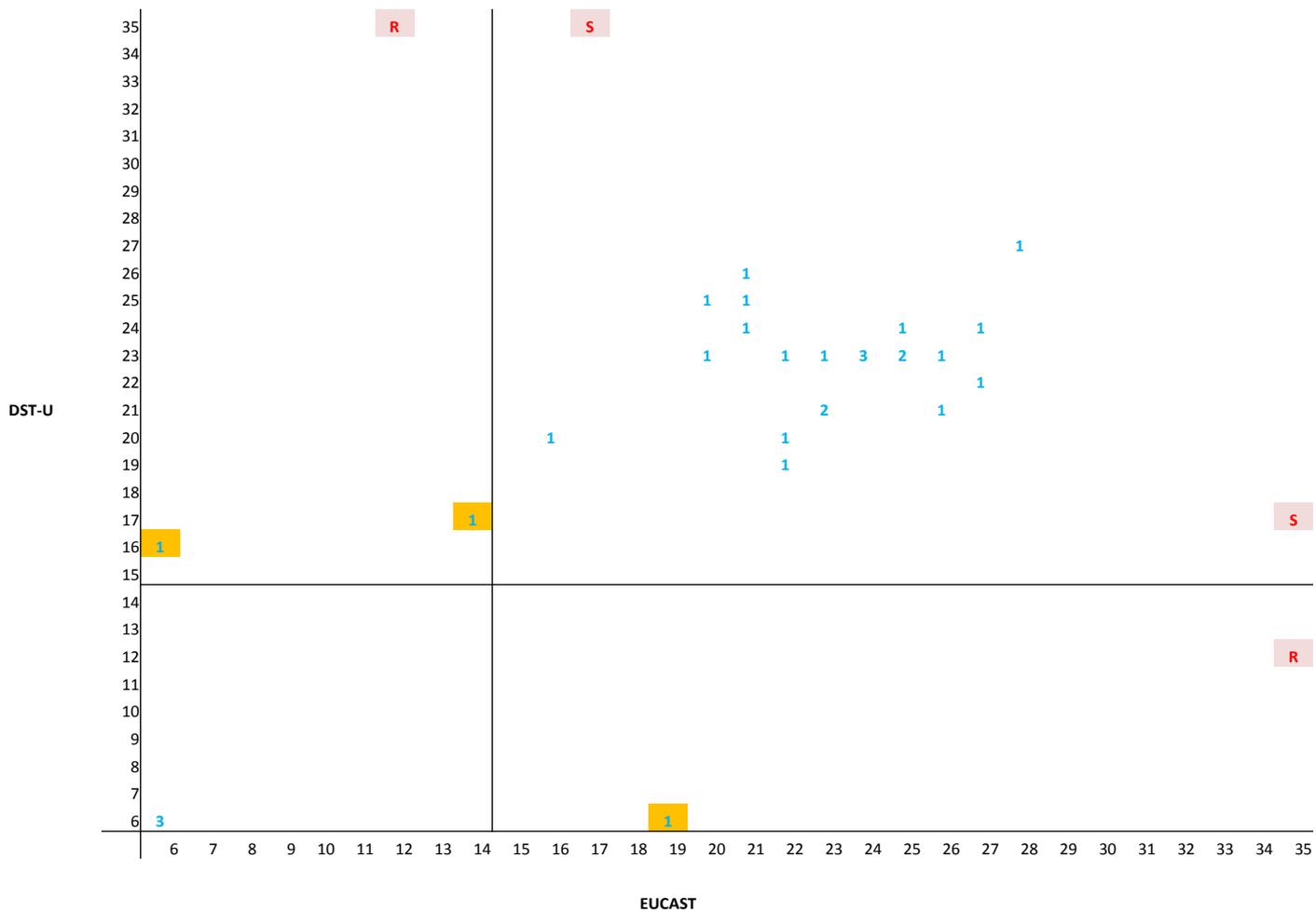
Konsekutiva urinprover med signifikant bakteriuri med någon av följande arter:

|                            |         |
|----------------------------|---------|
| <i>P. mirabilis</i>        | (n=29)  |
| <i>Enterobacter spp.</i>   | (n=17)  |
| <i>Citrobacter spp.</i>    | (n=22)  |
| <i>K. oxytoca</i>          | (n=29)  |
| <i>Serratia marcescens</i> | (n=3)   |
| <br>                       |         |
| Totalt                     | (n=100) |

Studien utförs under perioden mars 2013 - augusti 2014 (18 månader). Provsättning och avläsning utförs av på urinavdelningen tjänstgörande BMA.

# D-AST vs. EUCAST, endast *P. mirabilis*

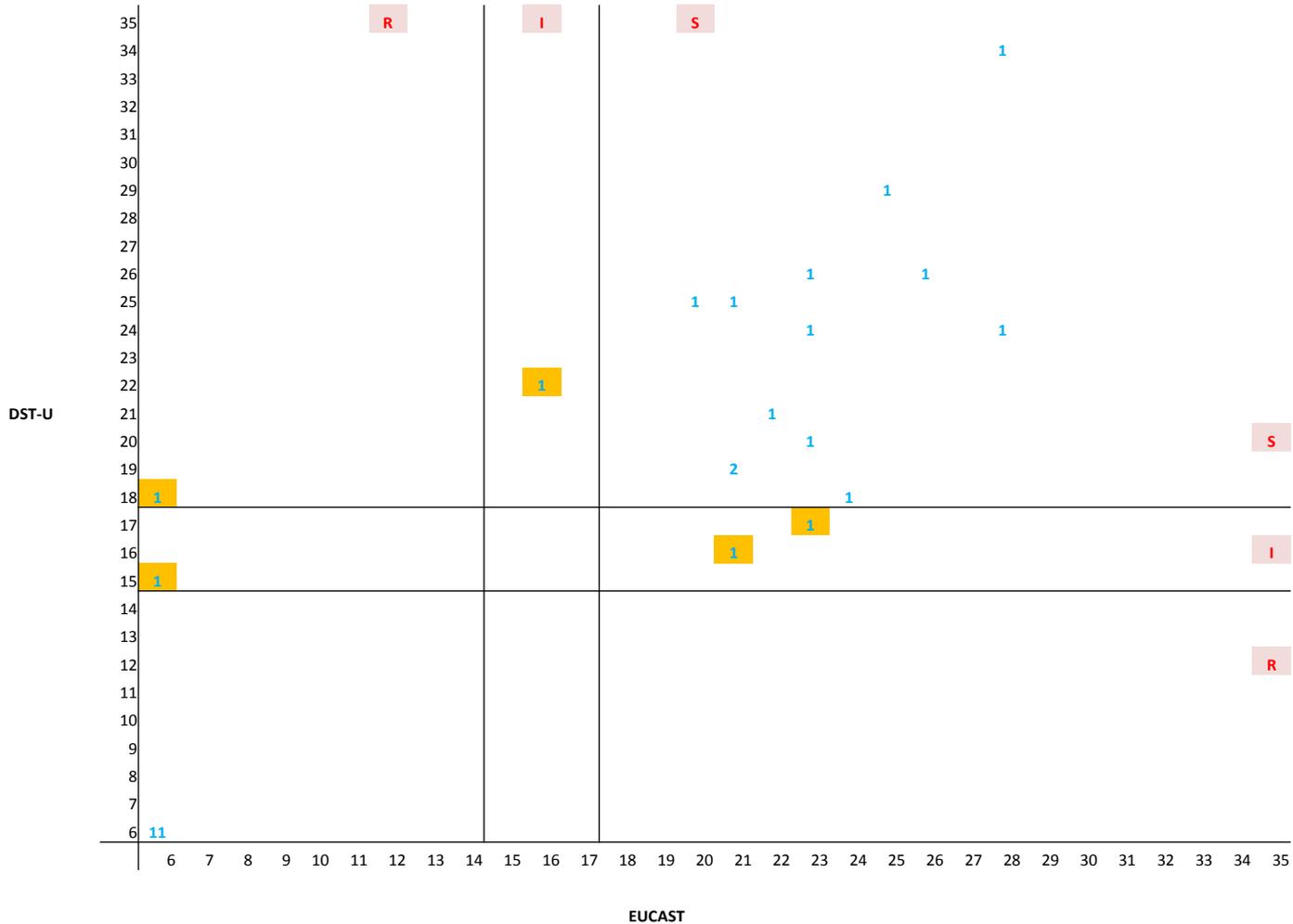
## Mecillinam 10 µg



Felklassificeringar av *P. mirabilis* (3/29 = 10.3%)

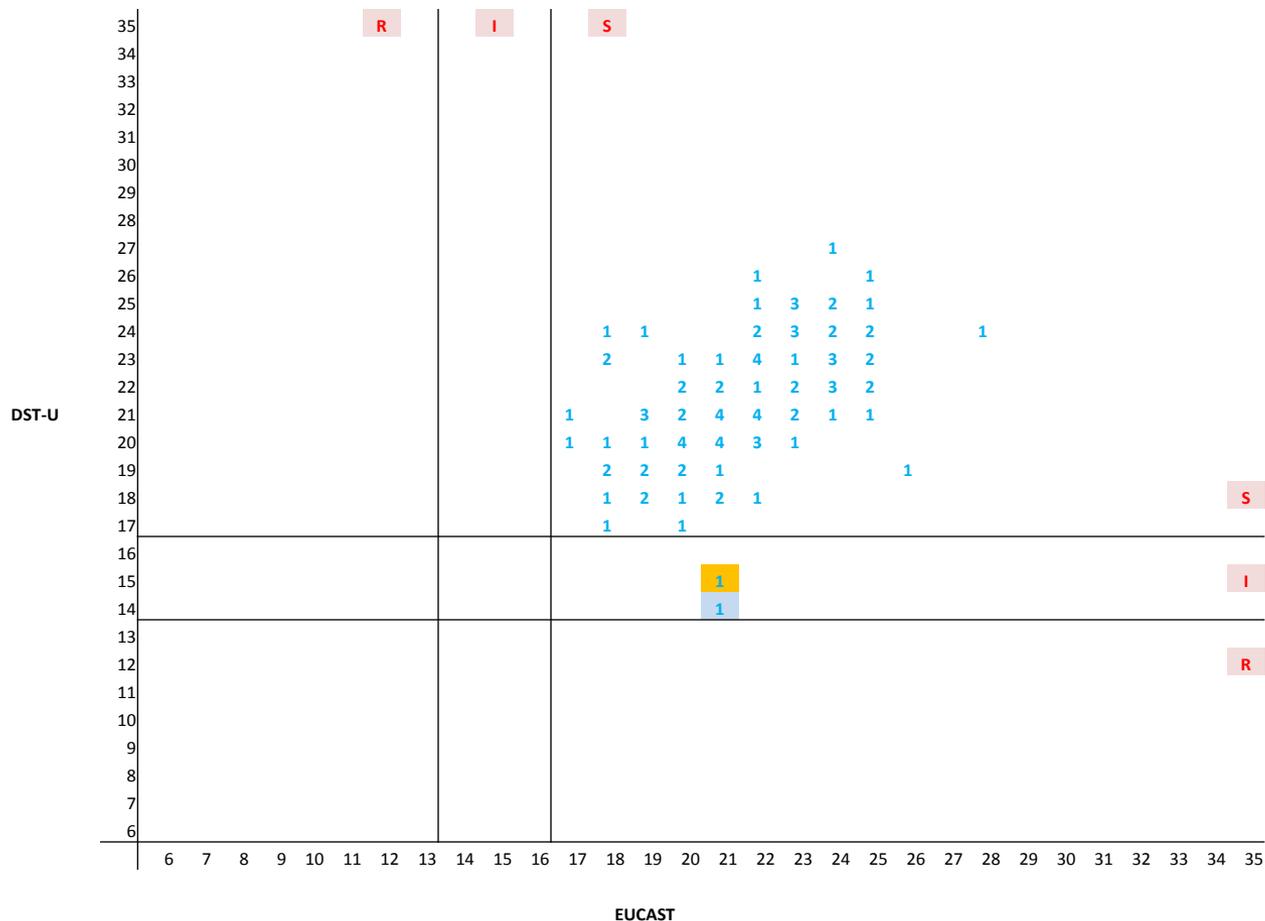
# D-AST vs. EUCAST, endast P. mirabilis

## Trimetoprim 5 µg



Felklassificering av *P. mirabilis* (5/29 = 17%)

# D-AST vs. EUCAST, Gentamicin 10 µg



■ Felklassificering av *P. mirabilis*  
■ Felklassificering av *K. oxytoca*

# Problems with DAST on Urine

- Errors probably not a major problem if restricted to *E.coli* and *Klebsiella pneumoniae*
  - Other species need more validation
- High cost if performed on every sample
  - Pre-screening ??
  - On demand/request



**IN PIPELINE**

# Resistance detection using MALDI-TOF

- Detection of resistant subtypes (i.e. subtyping)
  - CfiA +/- B.fragilis (Wybo 2011, Johansson JMM 2014)
- Detection of specific peaks associated with resistance (i.e. subtyping)
  - Porin loss in K.pneumoniae (Cai JCM 2012)
- Detection of degradation of antibiotics (i.e. Beta-lactamase detection)
  - TEM, ESBL, Carbapenemases (Sparbier JCM 2012)
  - Carbapenemases (Hrabak 2011-2014)

# Susceptibility testing using MALDI-TOF

- Detection of changes in mass-spektra in the presence of breakpoint concentrations of antibiotics (i.e. quantitative MALDI)
  - Incubate in BHI-broth +/- antibiotics 1h
  - Centrifugation steps and extraction with standard
  - Load on MALDI-tray and cover with HCCA
  - Analyse and compare AUC
  - SIR can be predicted using Breakpoint concentrations

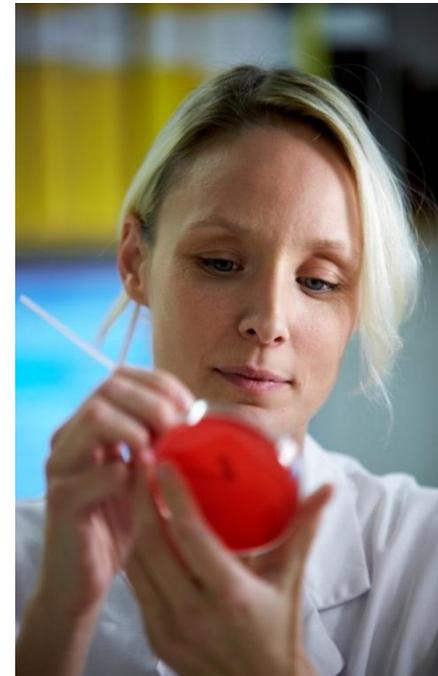
# AST and MALDI (so far...)

## Advantage

- Rapid
- Broth
- Not thorough validated (only one study)
- Might be useful for specific "hard to test" bacteria

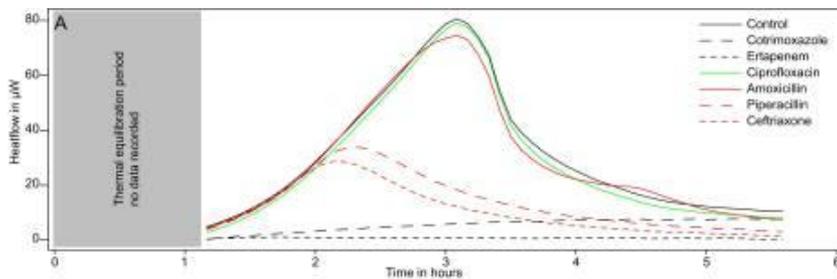
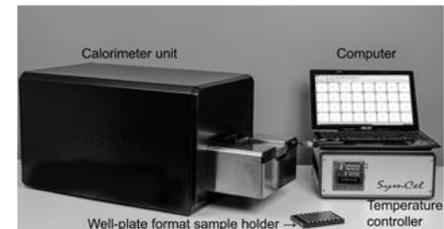
## Drawback

- Laborious
- Not quantitative
- Not automated
- Breakpoint concentrations

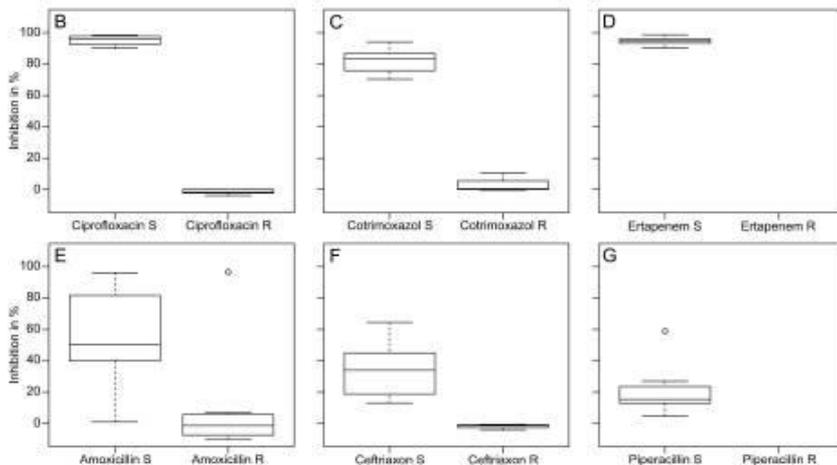


# Microcalorimetry

- Measurement of Isothermal Microcalorimetry
- Breakpoint concentrations, 6h
- Urine as medium



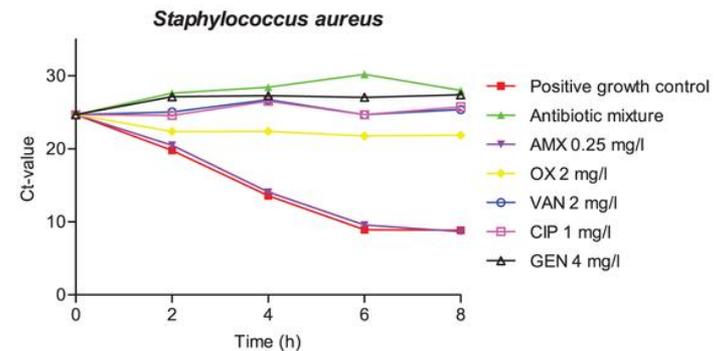
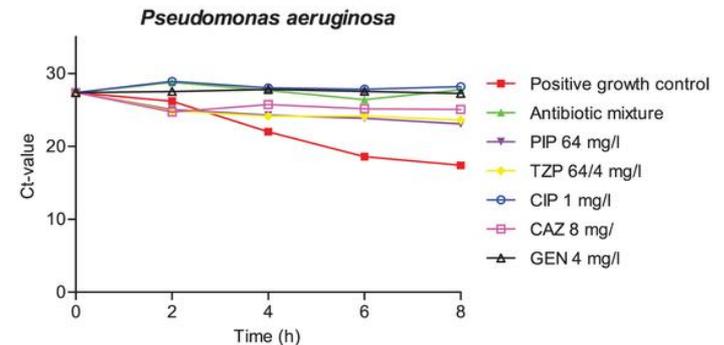
*E.coli* resistant to ampicillin and ciprofloxacin



Inhibition Index

# Combined Broth Dilution and PCR (FAST)

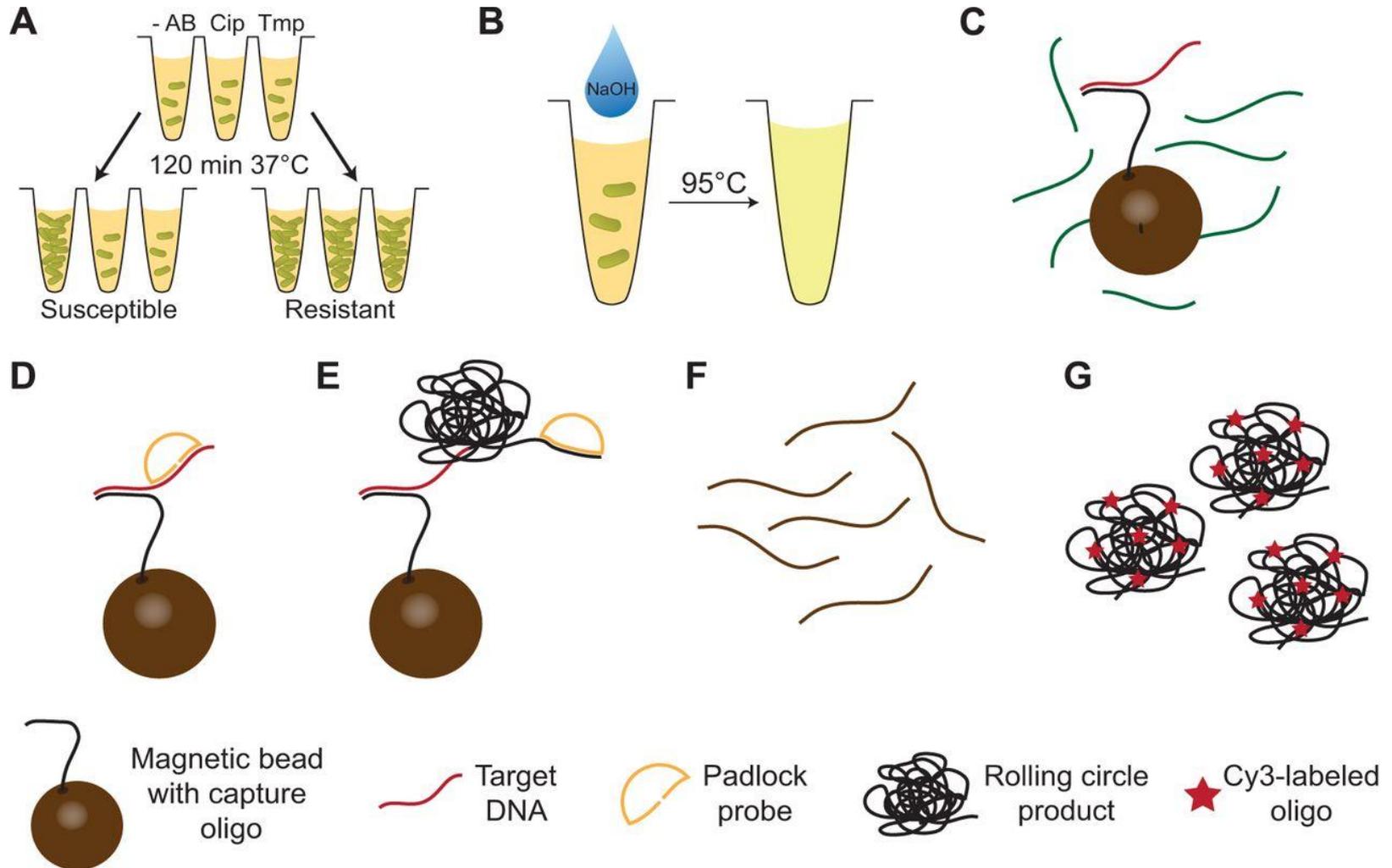
- 5ml BC broth centrifuged
- Suspension in MH +/-ab
- Breakpoint concentrations
- Incubation 0-8h
- 16SrRNA qPCR
- Meltcurve → species
- Good results
- 9h
- 50.7 vs 66.3h



# Combined Broth Dilution and using PadLock probes and RCA

- Short (0.5-2h) preincubation of urine in LB-broth +/- ab
- Breakpoint concentrations CIP and TRI
- Addition of species specific PadLock Probes
- RCA
- Differences in "Growth curves" → SIR
- Good correlation

## Schematic illustration of the method.



Anja Mezger et al. *J. Clin. Microbiol.* 2015;53:425-432

Journal of Clinical Microbiology

# Future perspectives

- Molecular based detection from whole blood
  - Black box
  - Random access
  - Affordable
  - If accomplished: preliminary report in 6-8h?
- Culture (usually positive within 20h)
  - MALDI-TOF + rapid DD → definitive report in <30h?

# But, to be rapid we need "Microbiologistics":

- Blood culture cabinets (also decentralized) available 24/7/365
- Microbiology open 24/7/365
- Skilled staff 24/7/365
- Not to forget other samples from the critically ill patient
- Take control of the transport organization and the referrals!

# Thanks to

## Växjö



- **Emma Jonasson**
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