WELCOME TO THE 33rd

ASM CDS WORKSHOP

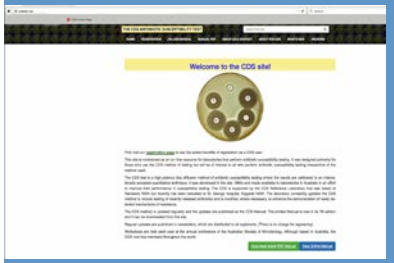
Canberra 2015

THE CDS TEAM

What's Been Happening with the CDS ?

The CDS laboratory has moved to Kogarah

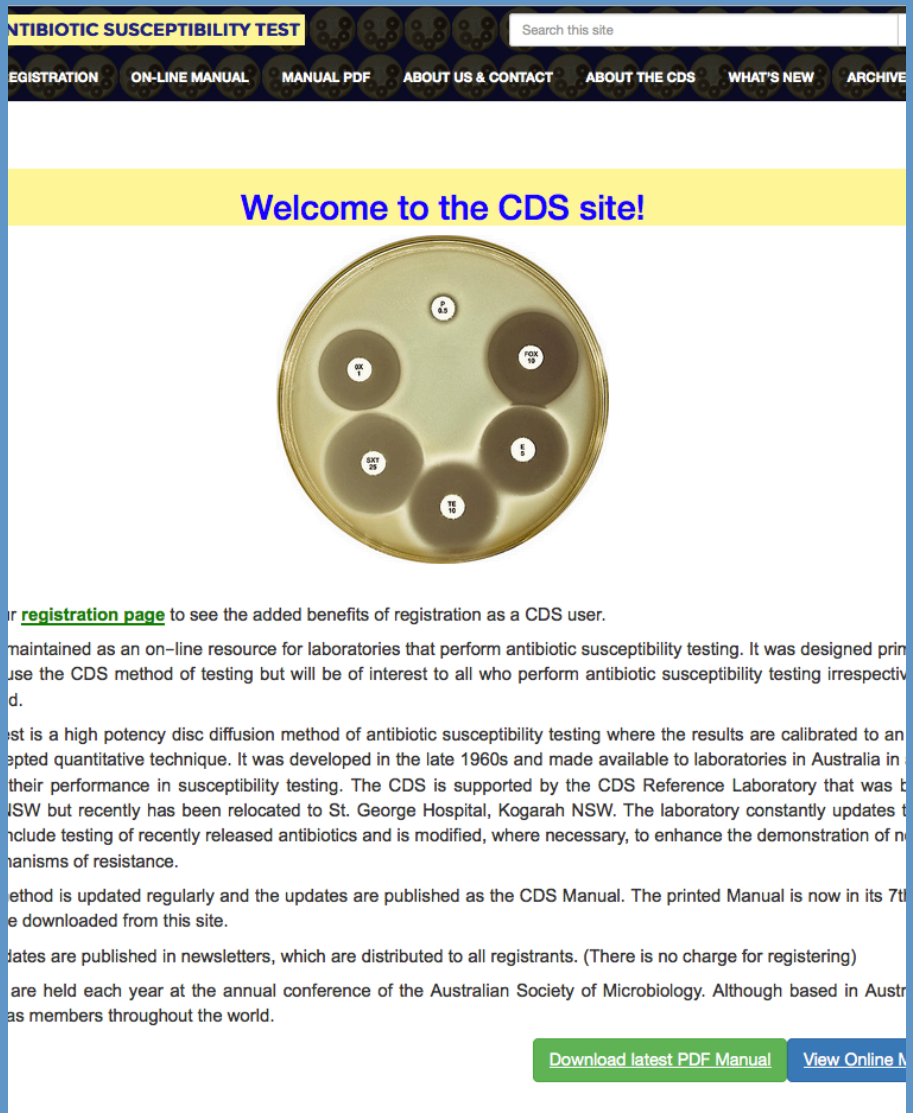
Dianne Rafferty has returned

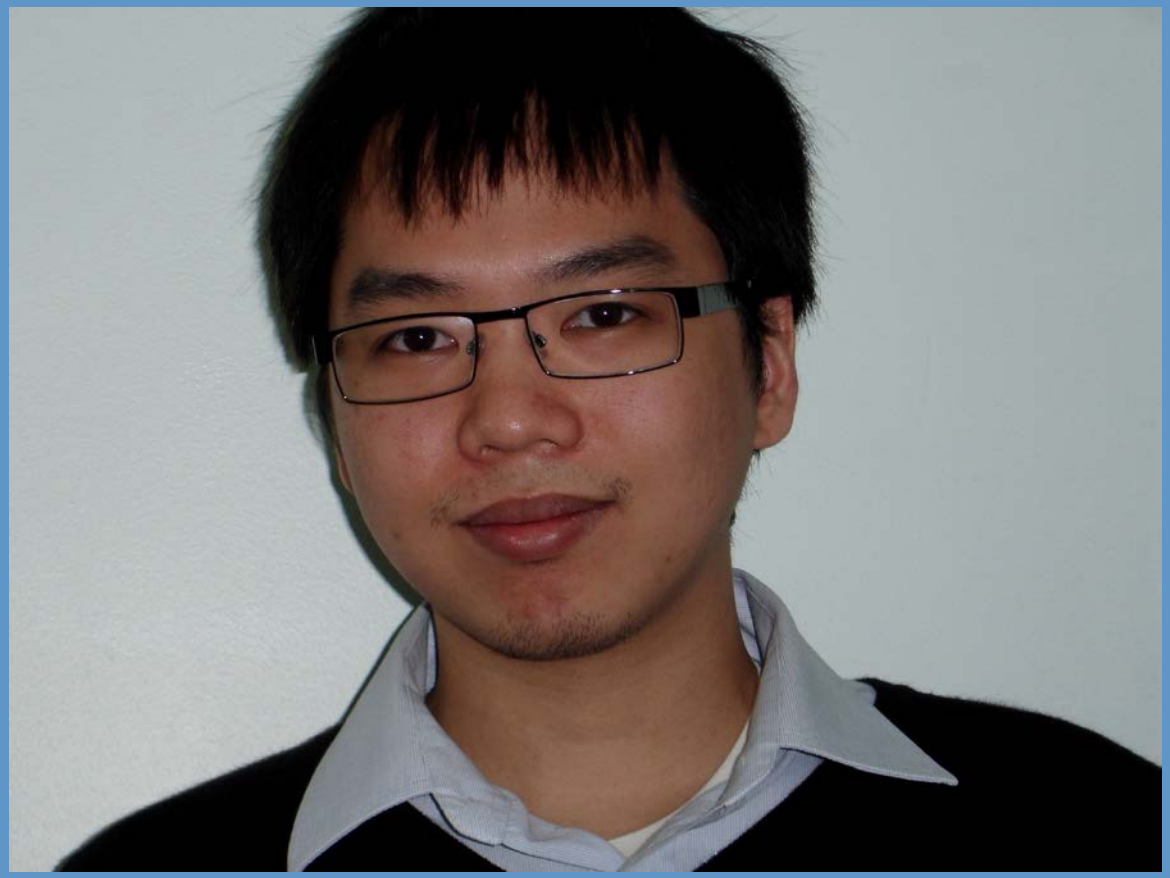


The CDS web site relocated & rebuilt

The CDS Web Site has Changed

//web.med.unsw.au/cds ⟶ //cdstest.net





Ryan Pratama

What's Next with the CDS ?

- Update the web site including the Manuals

  (CDS Users- help please)

- Reconcile Agar MICs and broth MICs

  (Putting the ISO furphy to rest)

- Harmonising Breakpoints

Investigation of *Staphylococcus intermedius* group (SIG) and the reporting of Methicillin susceptibility

Canberra 2015

Dianne Rafferty

SEALS South

NSW GOVERNMENT Health Pathology

Members of the *Staphylococcus intermedius* group

Staphylococcus intermedius

Staphylococcus pseudintermedius

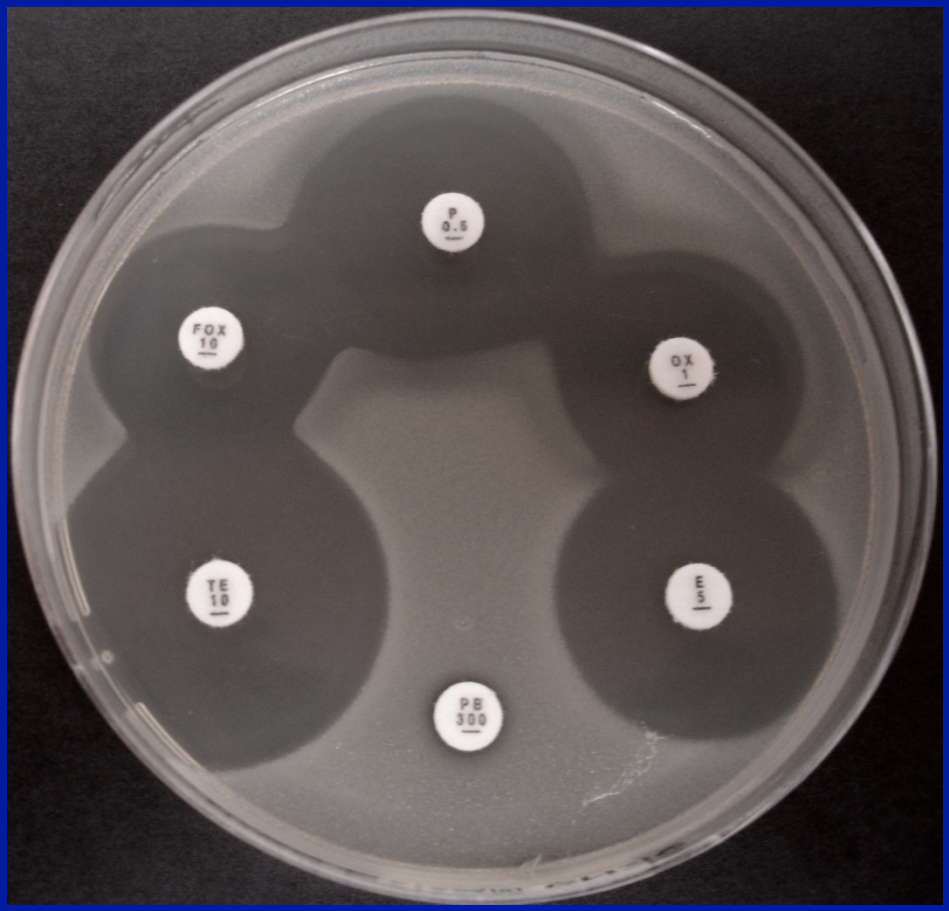
Staphylococcus delphini

(only molecular testing can conclusively differentiate between members of SIG)

Laboratory Identification

Biochemical Test	Staphylococcus aureus	SIG
Coagulase	+	+
DNase	+	+
Clumping Factor	+	Species Dependent
Polymxin B	0mm	Small zone

Should we use Cefoxitin 10µg or Oxacillin 1µg as the surrogate disc for reporting Methicillin susceptibility?

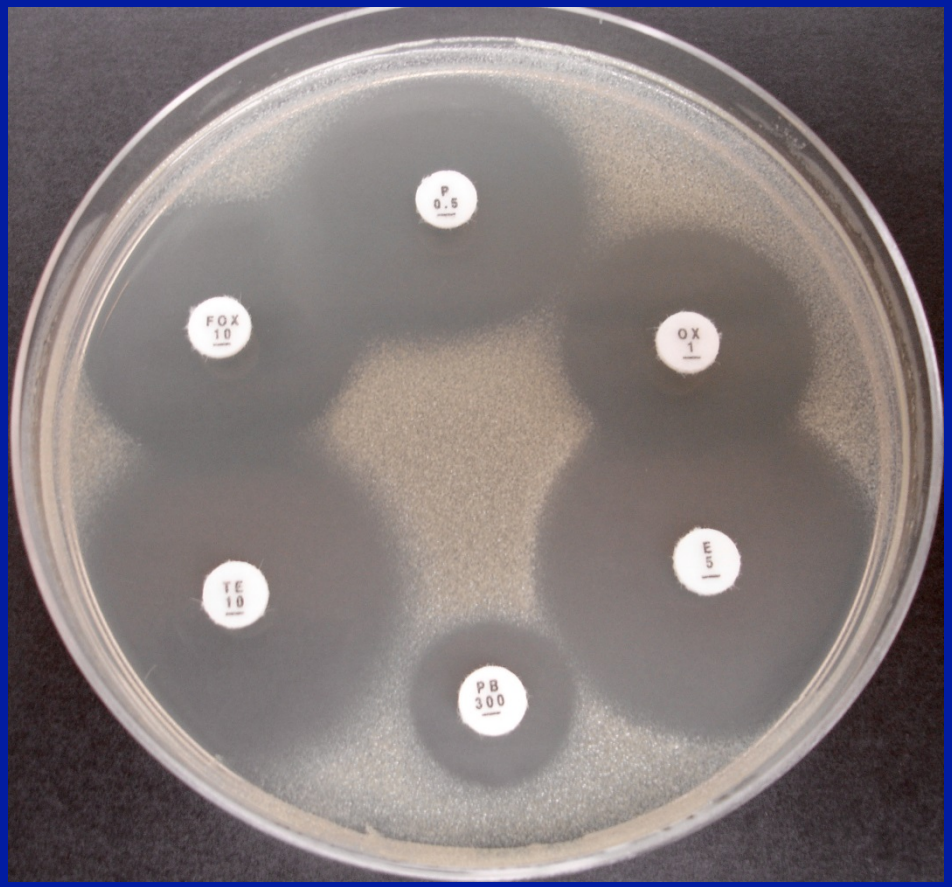


Staphylococcus aureus ACM 5190,

mec A negative

SIG testing vs Oxacillin/Cefoxitin/ *mec A* gene

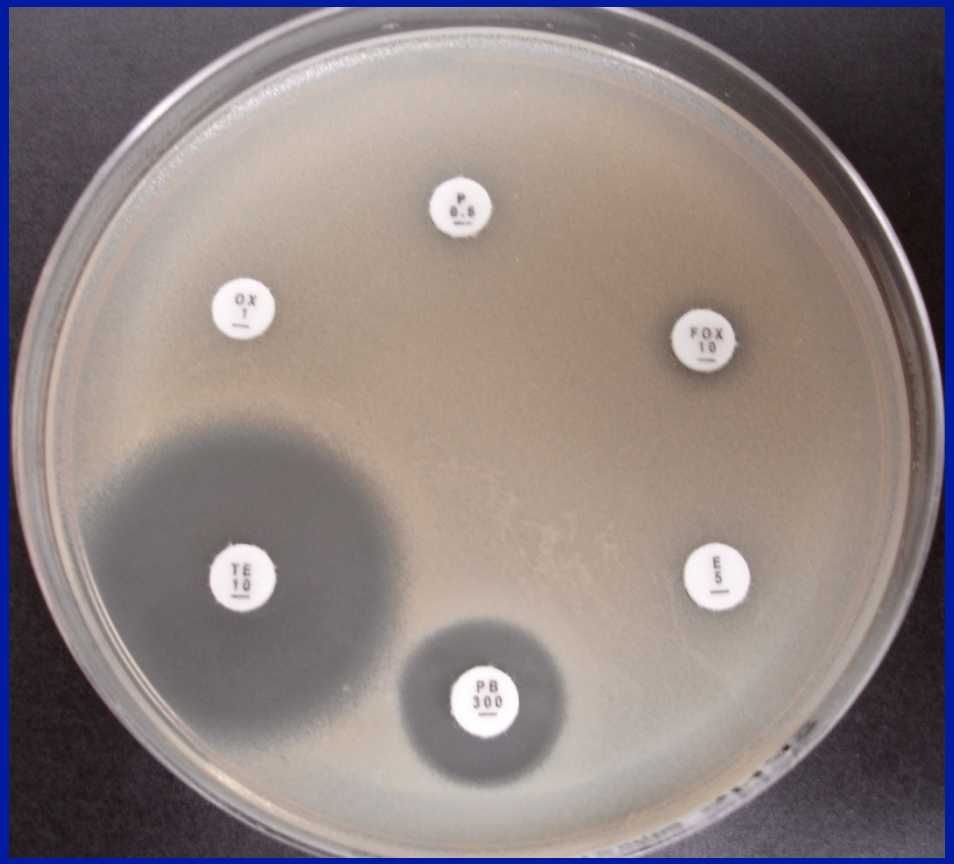
No. of Isolates n = 79	Oxacillin 1µg	Cefoxitin 10µg	Mec A
48	S	S	Negative
4	R	R	Positive
27	R	S	Positive



Staphylococcus intermedius Group S/Ox, S/Fox, *mecA* negative

SIG testing vs Oxacillin/Cefoxitin

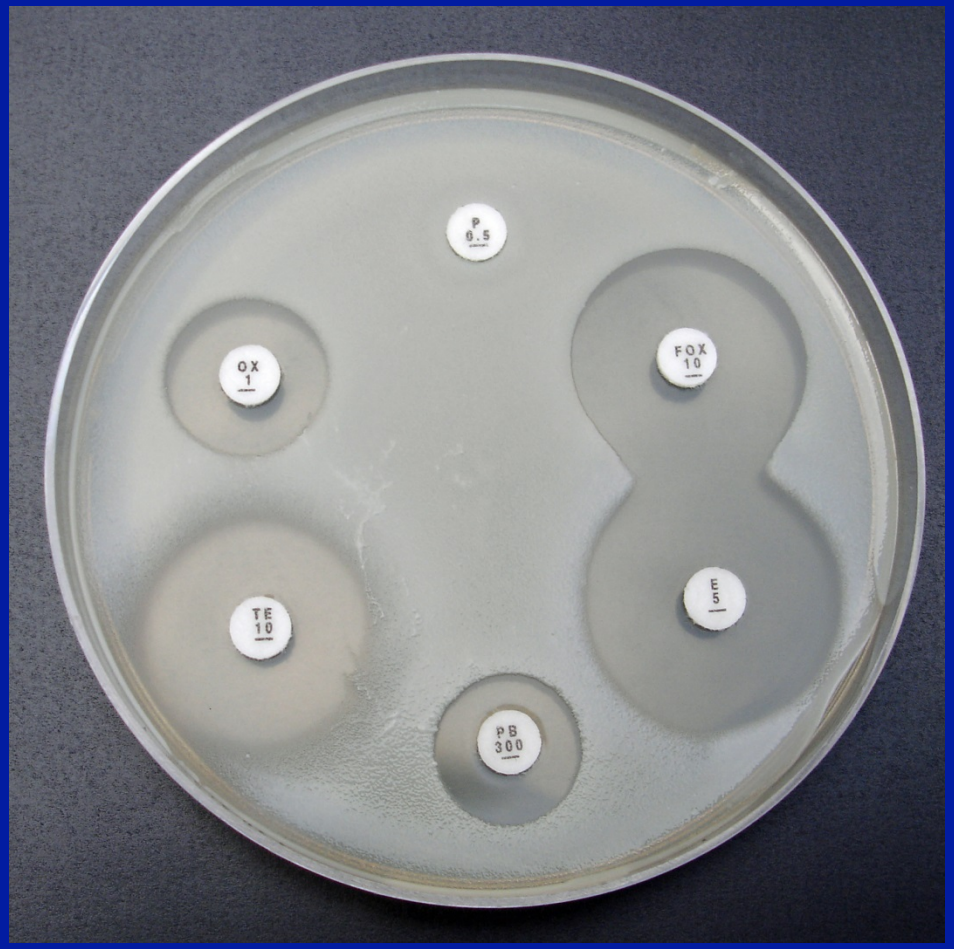
No. of Isolates n = 79	Oxacillin 1µg	Cefoxitin 10µg	Mec A
48	S	S	Negative
4	R	R	Positive
27	R	S	Positive



Staphylococcus intermedius Group R/Ox, R/Fox, *mecA* positive

SIG testing vs Oxacillin/Cefoxitin

No. of Isolates n = 79	Oxacillin 1µg	Cefoxitin 10µg	Mec A
48	S	S	Negative
4	R	R	Positive
27	R	S	Positive



Staphylococcus intermedius Group R/Ox, S/Fox, MecA positive

Conclusions

- Methicillin susceptible (*mec* A gene negative) SIG have a zone of inhibition to oxacillin 1µg discs >6mm and should be reported as susceptible to methicillin.
- Methicillin resistant (*mecA* gene positive) SIG have a zone of inhibition to oxacillin 1µg disc <6mm and should be reported as resistant to methicillin.
- When the identification of the isolate is not available at the time of susceptibility testing then both cefoxitin 10µg and oxacillin 1µg discs should be tested. If both have a zone >6mm then the isolate should be reported as susceptible to methicillin.

References

1. **Van Duijkeren,Eugeline *et al*.** 2011. Review on methicillin-resistant *Staphylococcus pseudintermedius*. Journal Antimicrob. Agents Chemother. 66**:2705-2714**.

2. **Bond, R. and Loeffler, A.** 2012. What's happened to *Staphylococcus intermedius*? Taxonomic revision and emergence of multi-drug resistance. Journal of Small Animal Practice doi:10.1111/j.vetmic.2011 .01.165.

3. **Devriese, L.A., *et al* .** 2005. *Staphylococcus pseudintermedius* sp.nov., a coagulase-positive species from animals. Int. J. Syst. Evol. Micobiol. 55, 1569-1573.

4. **Devriese, L.A., *et al* .** 2005. *Staphylococcus pseudintermedius* versus *Staphylococcus intermedius.* Vet. Microbiol. (2008), doi:10.1016/j.vetmic.2008.06.002.

5. **Van Hoovels, L *et al.* 2006**. First case of *Staphylococcus pseudintermedius* Infection in a Human. J.Clinical Microb. 44:4609-4612.

6. **Pottumarthy, S. *et al.* Clinical Isolates of *Staphylococcus intermedius* Masquerading as Methicillin-Resistant *Staphylococcus aureus*.** 2004. J Clinical Microb.Antimicrob . 42**:** 5881-5884

The CDS method…

….not just about measuring zone sizes

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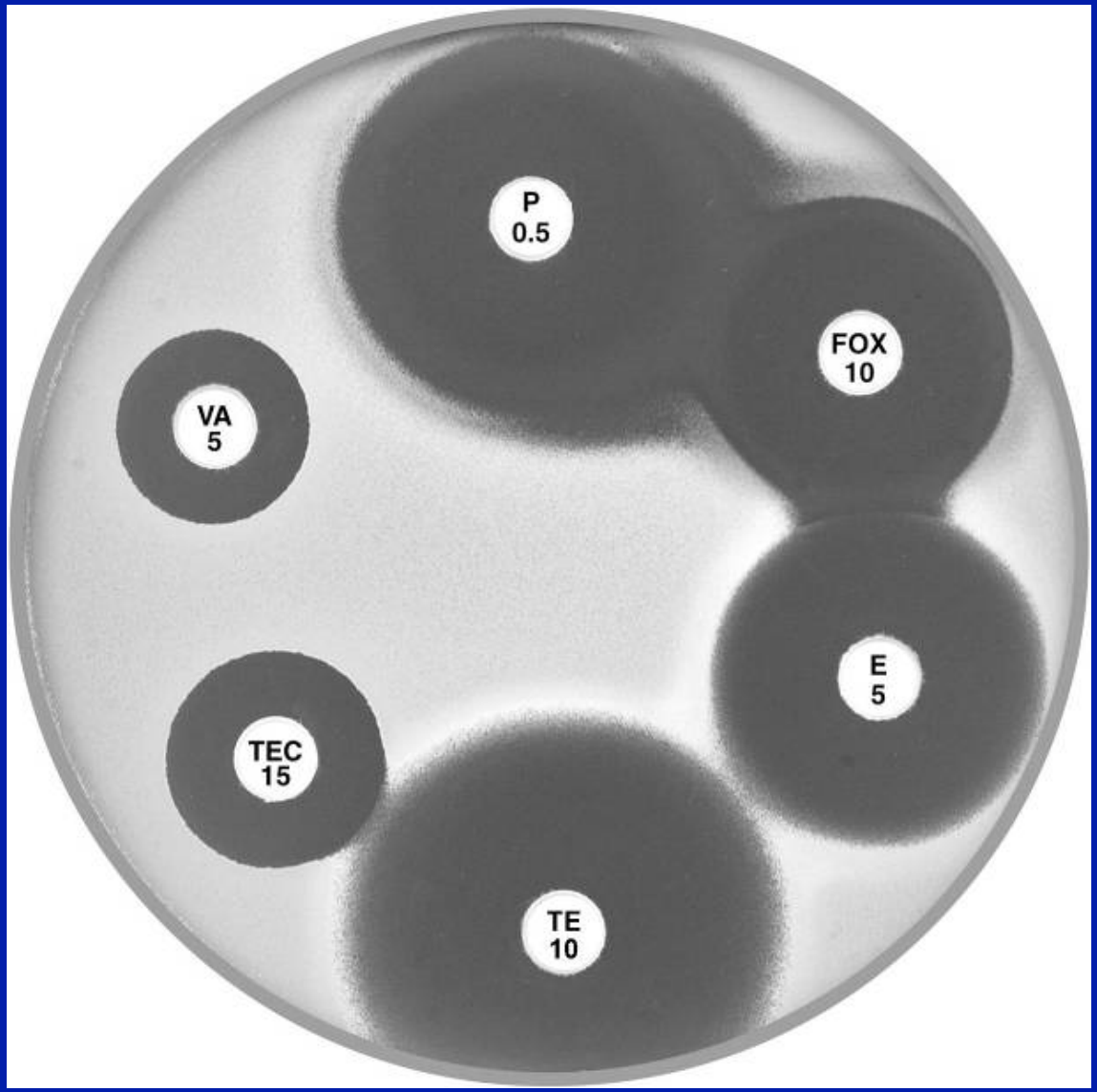
SEALS South

NSW GOVERNMENT Health Pathology

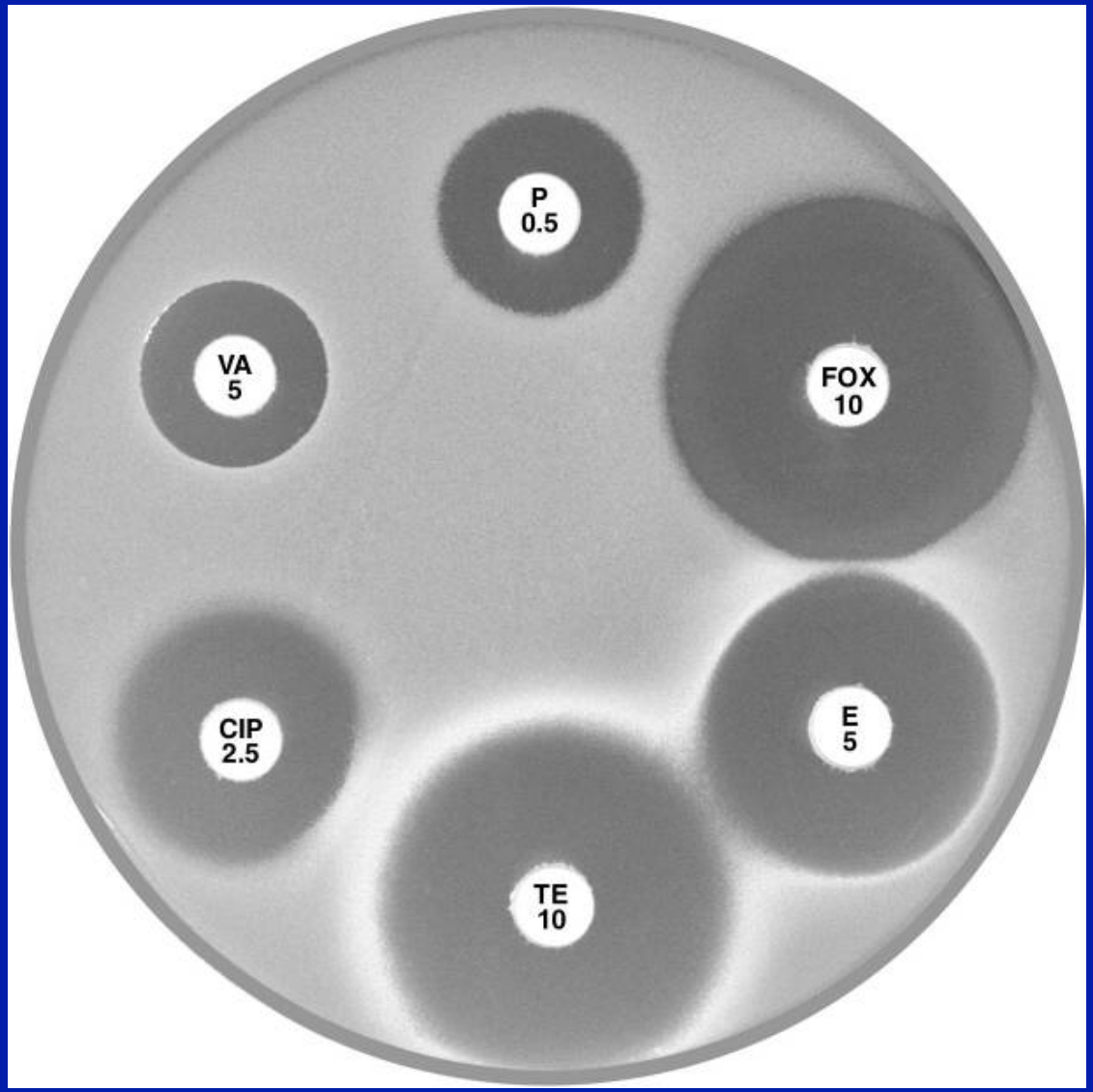
Reading CDS inhibitory zones

- Measure annular radius (CDS Manual 2.2.6)
  - Shortest distance from edge of disc to edge of confluent growth
  - From back of plate where possible
- Assess inhibitory zone morphology (CDS Manual 2.3.1)
  - Sharp edge:
    - β-lactamase positive *Staph. aureus* or *E. faecalis*
    - VSE
  - Hazy edge
    - VRE van B phenotype
    - Nitrofurantoin for *E. faecium*
  - Resistant mutants: hMRSA, MR-CNS, EESC coliforms
  - Disc approximation: ESBLs, inducible AmpC and ICR

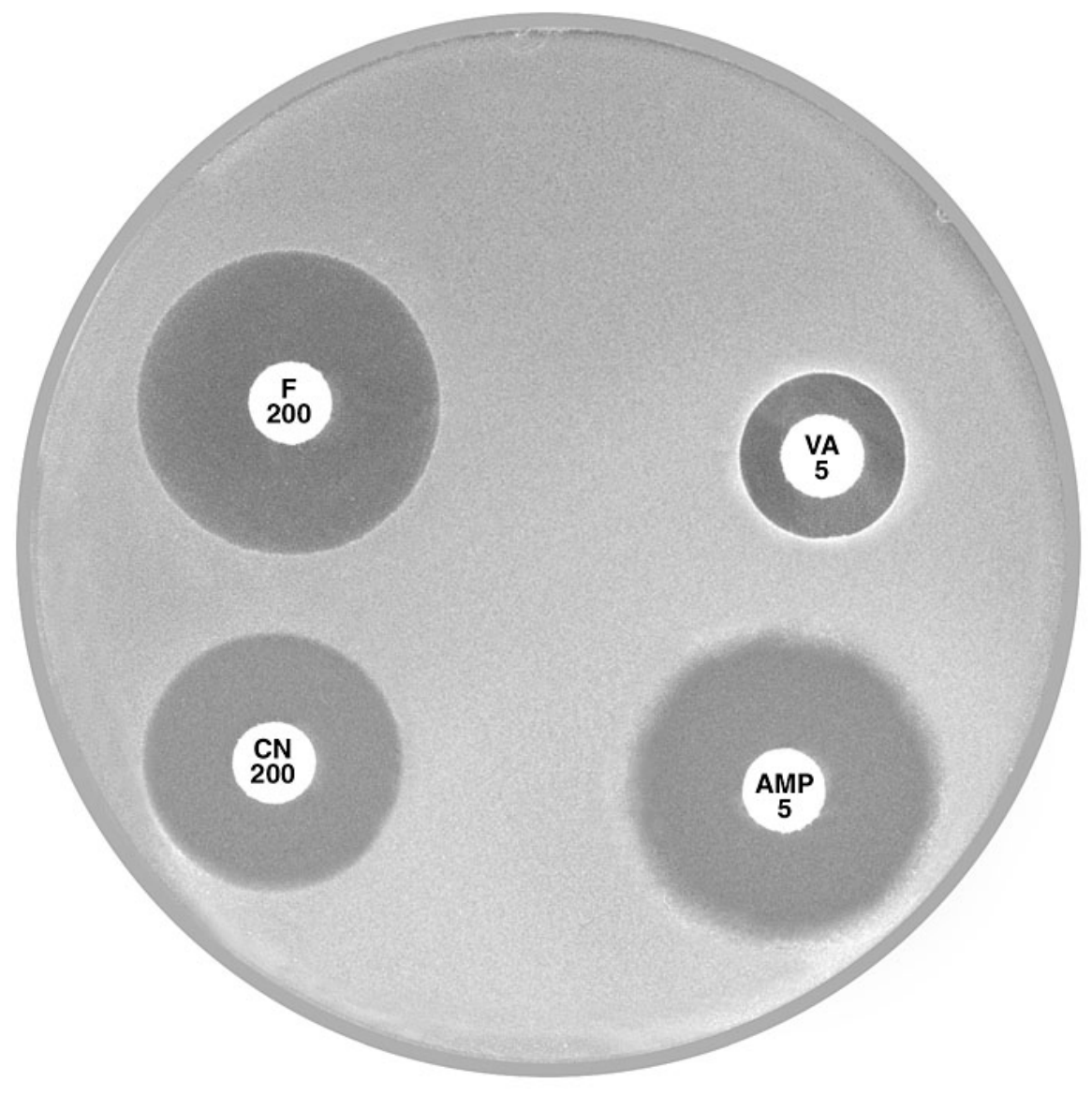
β-lactamase-negative *S. aureus*



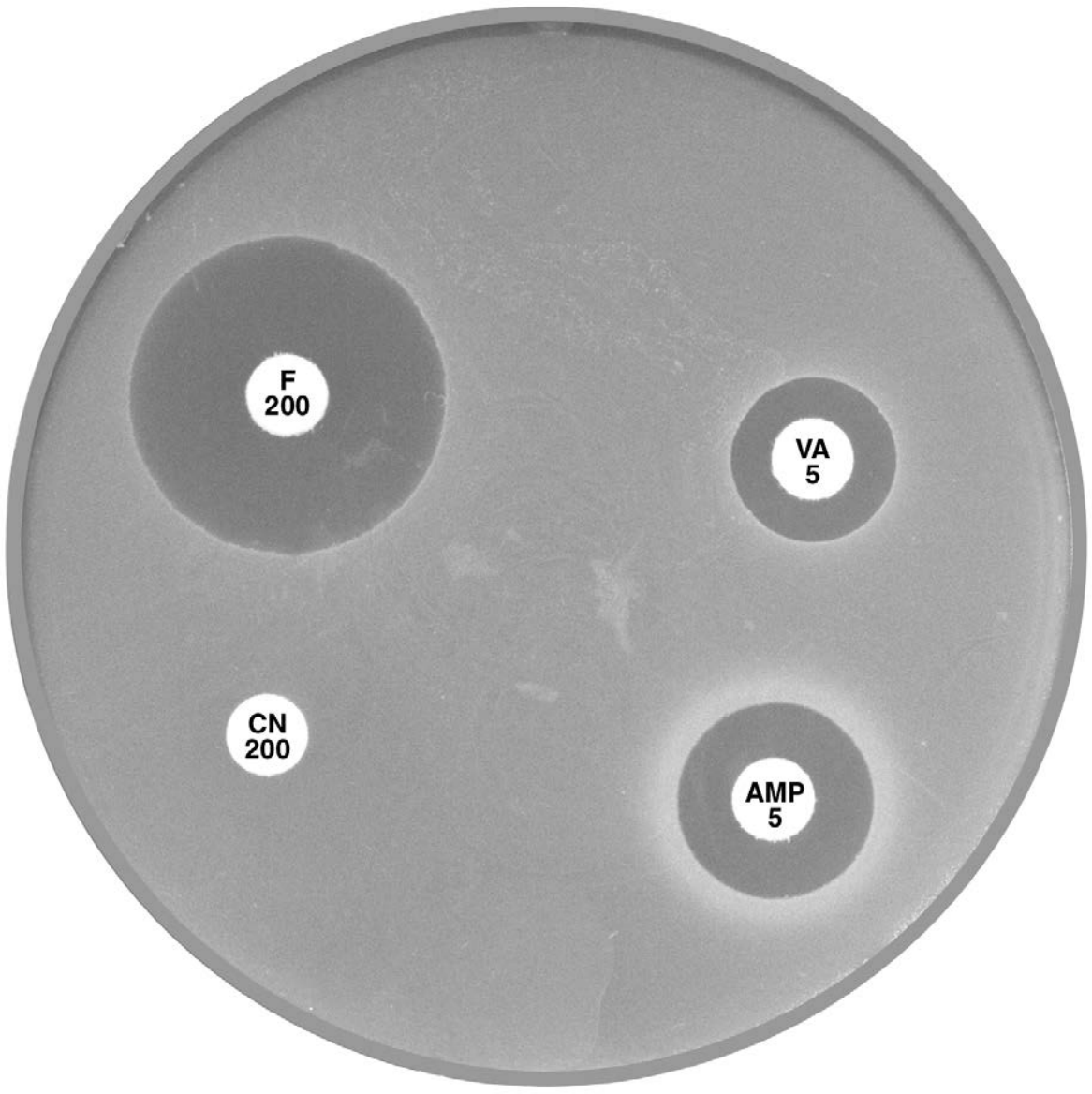
S. aureus with low β-lactamase activity



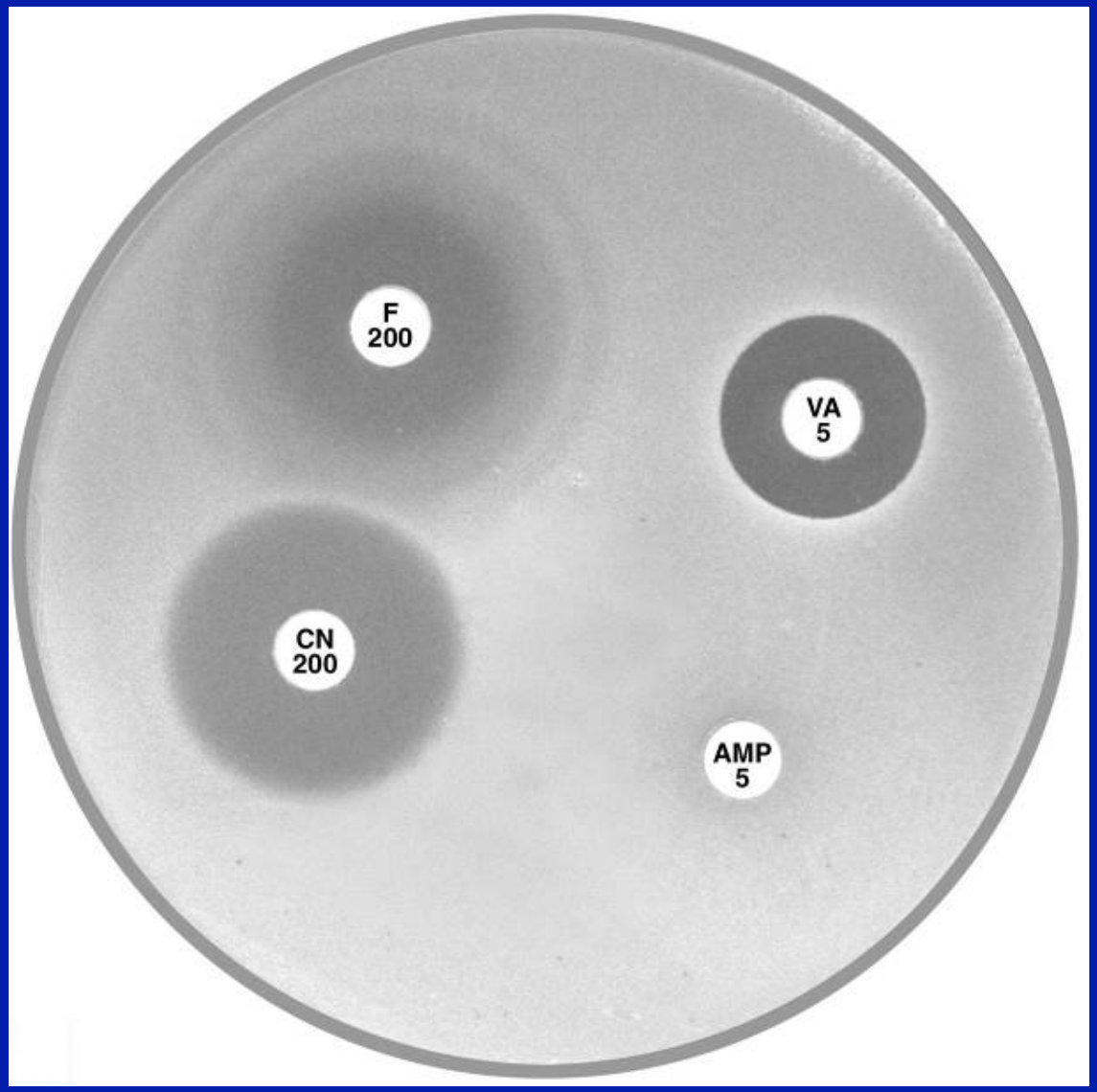
Ampicillin susceptible *E. faecalis* ACM 5184



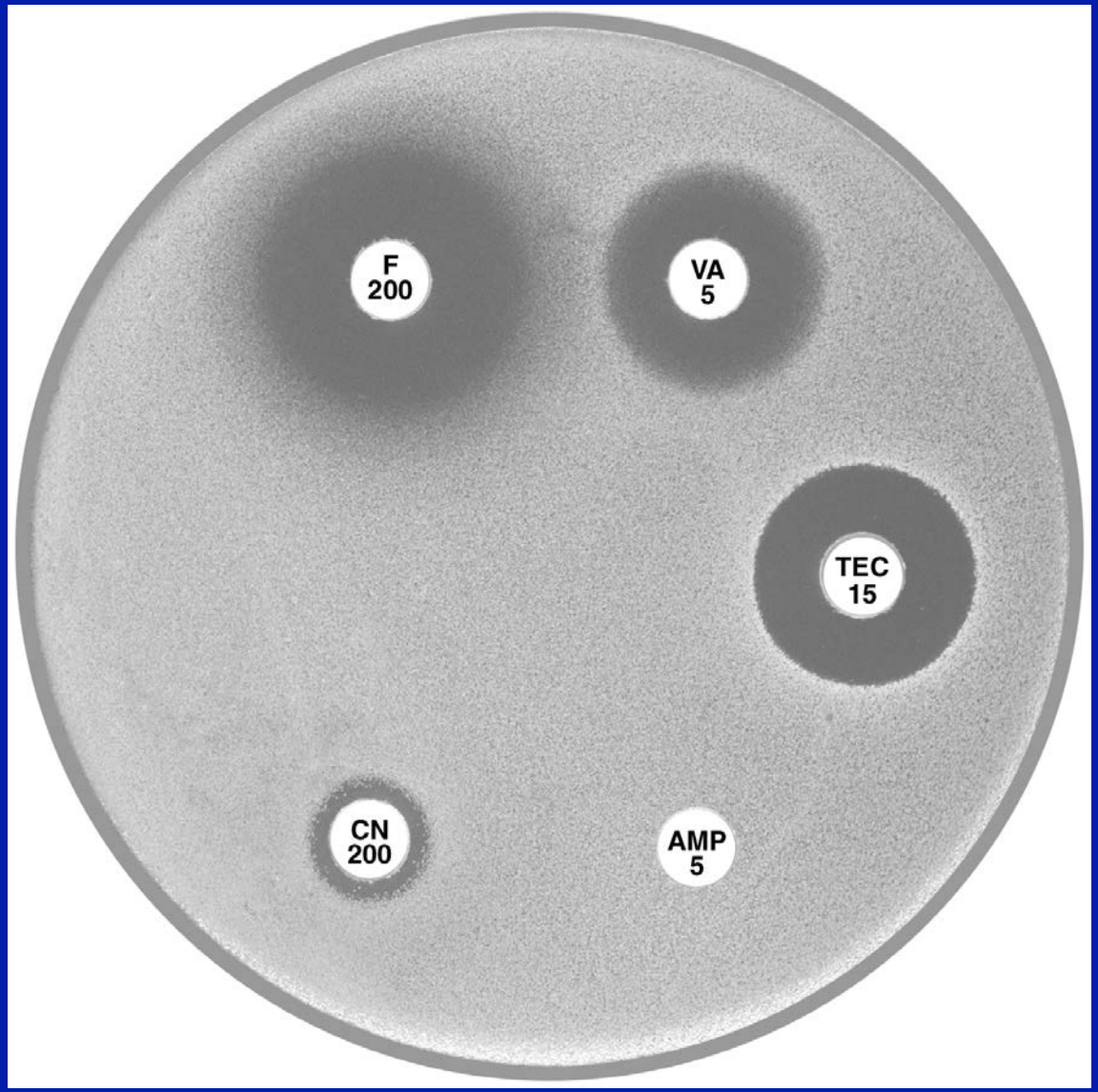
β-lactamase producing *E. faecalis*



Vancomycin susceptible *E. faecium*



Van B Vancomycin resistant *E. faecium*



CDS Inoculum

- 10^7 CFU/mL
- Simple preparation:
  - Stab single colony with straight wire
  - 3-5 colonies for small colonies (e.g. streptococci)
  - Mix in 2.5 mL sterile saline
- Flood agar plate, distribute by rocking, remove excess, dry 5-30 min.
- Confluent & uniform lawn of growth

- cf EUCAST & CLSI:
  - McFarland 0.5 turbidity standard (~1-2 x10^8 CFU/mL)
  - Spread over agar with cotton swab in 3 directions or use automatic plate rotator

EUCAST

S. aureus and benzylpenicillin

- Examine with transmitted light (plate held up to light).
  - Disk diffusion is more reliable than MIC for detection of penicillinase producers, provided the zone diameter is measured AND the zone edge closely inspected.

S. aureus with sharp zone edge and zone diameter ≥ 26 mm = Resistant

S. aureus with fuzzy zone edge and zone diameter ≥ 26 mm = Susceptible

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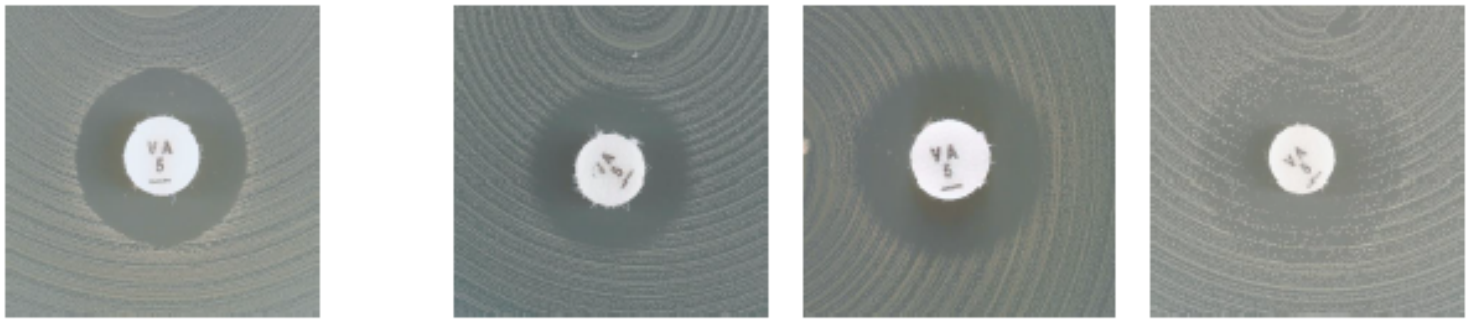
Source: EUCAST Disk Diffusion Reading guide (v. 4.0, 19 June, 2014)

EUCAST

Enterococci and vancomycin

- Examine with transmitted light (plate held up to light).
  - Fuzzy zone edges and colonies within zone indicate vancomycin resistance. If the zone diameter is ≥ 12 mm and the zone edge is fuzzy, investigate further.



E. faecalis
non-VRE

E. faecium
VRE

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Source: EUCAST Disk Diffusion Reading guide (v. 4.0, 19 June, 2014)

Summary

- The uniform lawn of growth produced by the CDS method is advantageous in assessment of the inhibitory zone edge
- The CDS method of flooding the agar plate with the inoculum is simple & assures the entire inoculum is applied to the plate