EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

EUCAST recommendations for susceptibility testing of anaerobic bacteria

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Ulrik Stenz Justesen, Odense University Hospital, DK

EUCAST seminar 9 December 2021

Agenda

- Introduction (Gunnar Kahlmeter)
- MIC testing using agar dilution (Trefor Morris and Sarah Copsey Mawer)
- Disk diffusion methodology (Erika Matuschek)
- Testing anaerobicity (Ulrik Stenz Justesen)
- Quality control (Jenny Åhman)
- Discussion

Disk diffusion of anaerobic bacteria – a joint project …… organised by EUCAST

- The EUCAST Development Laboratory, Växjö, Sweden (Gunnar Kahlmeter, Erika Matuschek, Jenny Åhman)
- UKARU – UK Anaerobe Reference Unit, Public Health, Wales (Trefor Morris and Sarah Copsey-Mawer)
- Clinical Microbiology, Odense, Denmark (Ulrik Stenz Justesen)
- Colleagues in many European laboratories taking part in field tests 1 and 2.

Currently used methods for AST of anaerobic bacteria

- Agar dilution (EUCAST all anaerobic species; CLSI for most species)
- Broth microdilution (CLSI for Bacteroides fragilis; EUCAST none)
- Gradient tests (for routine susceptibility testing of anaerobes)*

- Disk diffusion methods have been largely abandoned (CLSI, BSAC, SRGA, and others).

Many/Most routine laboratories either

- perform gradient tests
- decline susceptibility testing of anaerobes
- on request refer anaerobic bacteria to reference laboratories for AST

*As part of the current evaluation of reference method and standardised disk diffusion on well defined collections of anaerobic bacteria, an evaluation of gradient tests using the same organisms is underway.

Clinical Infectious Diseases

IDSA Infectious Diseases Society of America

hivma hiv medicine association

Volume 59, Issue 5
1 September 2014

EDITOR'S CHOICE

Antimicrobial Resistance and Susceptibility Testing of Anaerobic Bacteria FREE

Audrey N. Schuetz

Clinical Infectious Diseases, Volume 59, Issue 5, 1 September 2014, Pages 698–705,
https://doi.org/10.1093/cid/ciu395
Published: 27 May 2014 **Article history** ▾

Quote:

Disk Diffusion

Disk diffusion (Kirby-Bauer) tests should not be performed for anaerobic bacteria for the purpose of obtaining susceptibility results, as the results are inaccurate and do not correlate with the agar dilution method [46]. Special-potency antibiotic disks of vancomycin (5 µg), kanamycin (1000 µg), and colistin (10 µg) are occasionally used in the laboratory as an aid in preliminary identification of some anaerobes based on patterns of resistance [53]. However, due to the high antibiotic concentrations in these disks, these disks are not intended for use in determining antibiotic susceptibility for therapeutic purposes.

Based on this rather discouraging advice, we decided to develop a ”EUCAST disk diffusion susceptibility test method”, based on a different medium than the classical Brucella Blood Agar (BBA) and our attention turned to FAA, which by then could be obtained from several manufacturers, a pre-requisite for EUCAST development.

We also decided to limit species, agents, inocula, atmospheres, incubation time etc to where we could show that the method ”would work”.

Extending recommendations to more agents, more species, longer incubation time etc are ”next-step projects”:

Plans for extending the panels of agents*

Antimicrobial disk	***Bacteroides* spp.**	***Prevotella spp.***	***F. necrophorum***	***C. perfringens***	***C. acnes***
Ampicillin/Amoxicillin		●	●	●	●
Amoxicillin-clavulanic acid	●	●	●	●	●
Ceftriaxone					●
Ertapenem	●	●	●	●	●
Imipenem	●	●	●	●	●
Linezolid				●	●
Chloramphenicol	●	●	●	●	●

*Part of this project will also be to evaluate whether some agents can be used to predict susceptibility and resistance to other agents.

Anaerobe MIC testing by Agar Dilution

Trefor Morris, Lead Scientist
Sarah Copsey-Mawer, Senior Scientist and AST Lead

UK Anaerobe Reference Unit, Public Health Wales, Cardiff, Wales

GIG CYMRU NHS WALES
Iechyd Cyhoeddus Cymru
Public Health Wales

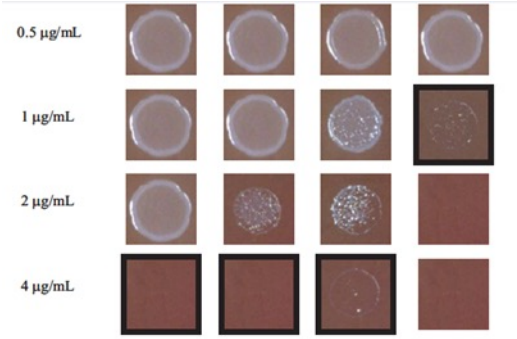
UK Anaerobe Reference Unit (UKARU)

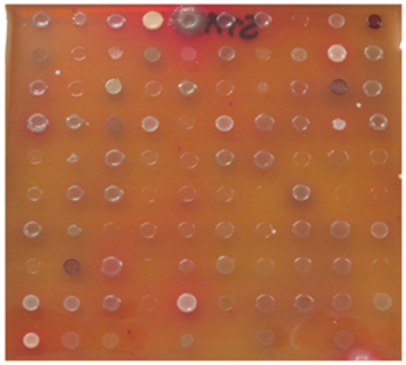
- Identification, susceptibility testing, AMR surveillance
- Clinical & technical advice
- *C. difficile* WGS surveillance
- Maintenance of clinical isolate collection
- Training and education – P&CMAn (16th & 17th June 2022)
- Research and development

ARU
UK ANAEROBE REFERENCE UNIT | UNED GYFEIRIO ANAEROBIG Y DU

Susceptibility Testing: UK ARU

- Weekly agar dilution since May 2016
- **Advantages**
  - CLSI Gold standard approved method
  - Used for monitoring trends over time
  - Identifying unusual/unexpected resistance
  - Data comparable across institutions
  - UKAS accredited – **ISO 15189**





- **Challenges**
  - Labour intensive to perform and interpret
  - Only suitable for large studies or reference laboratories

UKARU weekly Agar Dilution Panel

First line

- Penicillin
- Co-amoxiclav
- Ceftriaxone
- Piperacillin-tazobactam
- Meropenem
- Clindamycin
- Metronidazole
- Vancomycin
- Doxycycline*
- Rifampicin*

Extended panel (gradient strips)

- *C. acnes*/*P. acnes*
  - Levofloxacin
  - Teicoplanin
- MDR Gram negatives
  - Linezolid
  - Moxifloxacin
  - Tigecycline

- Others on request

Agar Dilution - practicalities

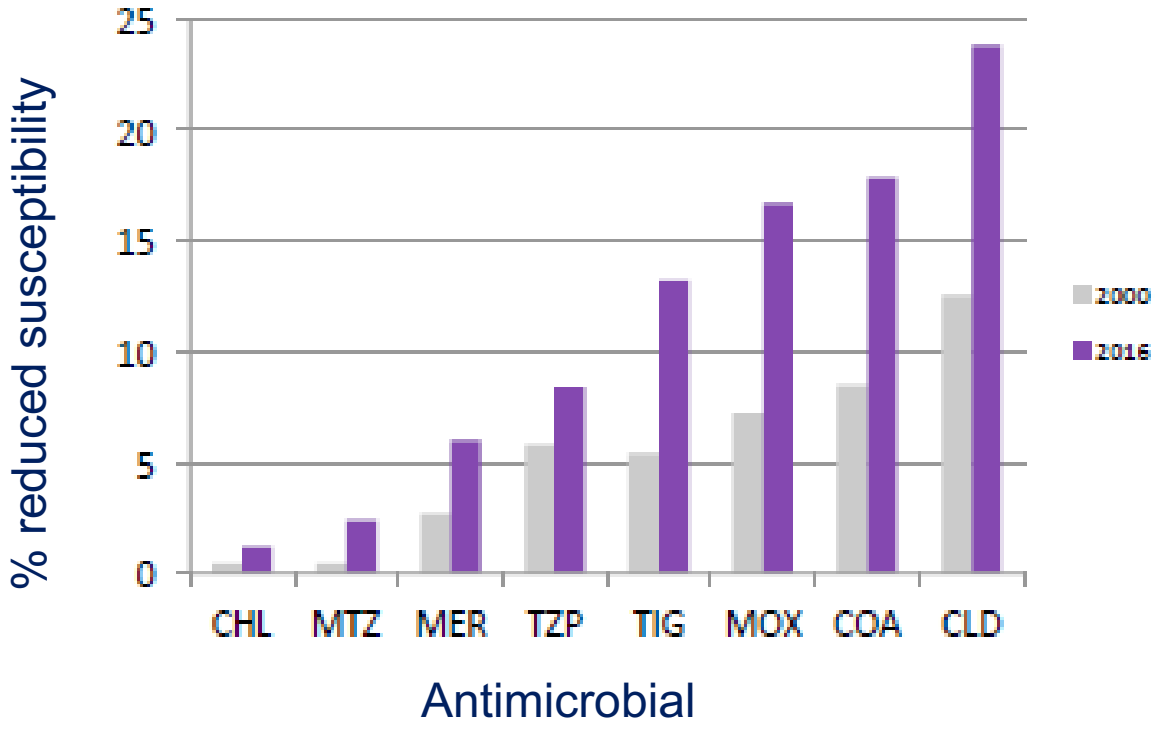
- Requires time and expertise to set up and deliver
- Consider the equipment needed
- Consider the space required
- ISO accreditation
- Additional value – clinical relevance, other developments?

Agar Dilution - evolution - FAA

- FAA commonly used in UK as primary isolation media (+ preliminary promising results in internal AST studies)
- Developed by Lab M – specifically for cultivation of all anaerobes, with growth factors included
- FAA first suggested in 1990s but never adopted, proposed to EUCAST more recently
- Integration into ISO accredited service in UKARU in early 2022

Impact of integrating AD:
1) UK *Bacteroides* species 2000 vs 2016

Overall increase in % with reduced susceptibility



CHL: chloramphenicol

MTZ: metronidazole

MER: meropenem

TZP: pip-tazobactam

MOX: moxifloxacin

TIG: tigecycline

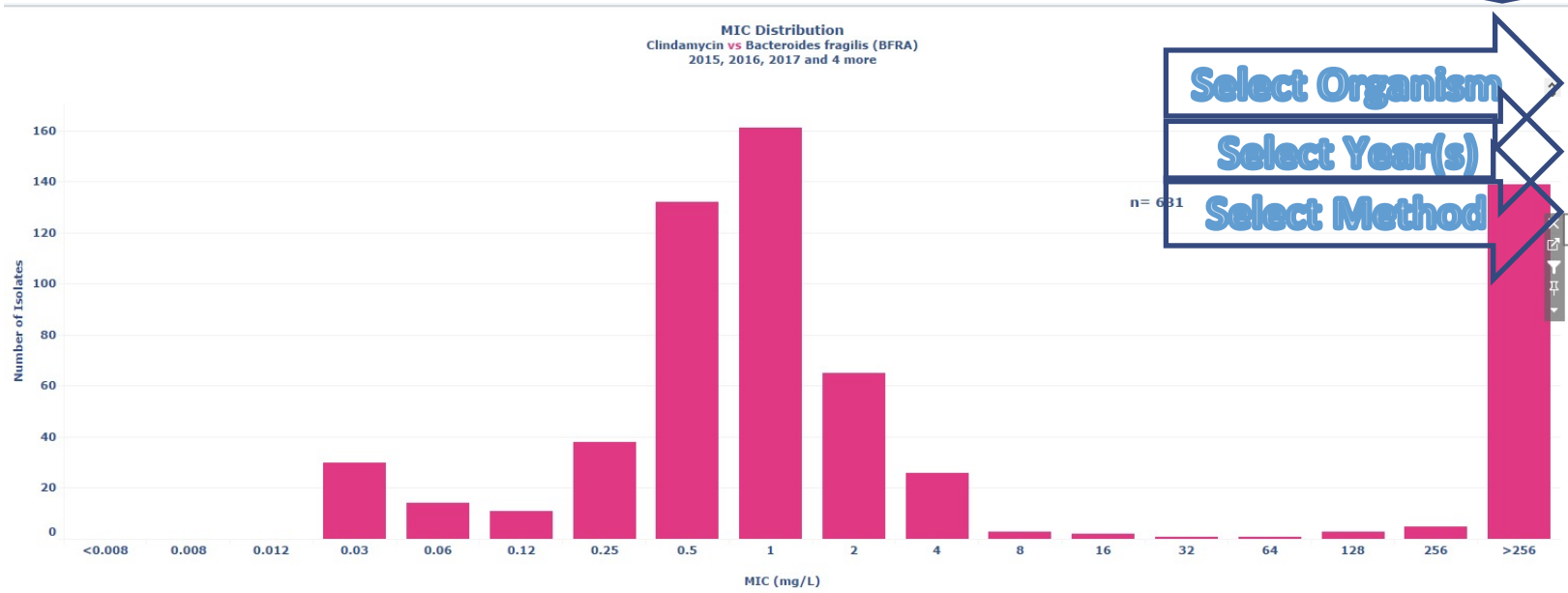
COA: co-amoxiclav

CLD: clindamycin

2) Building the ARUMIC database

- Aim
  - To provide accessible cumulative MIC data
  - Database continuously updated via Data-store and Tableau
- Benefits
  - Improved understanding of both common and rare species
  - Increased confidence in empiric antimicrobial prescribing
  - May improve antimicrobial stewardship
  - Feed into development of species-specific breakpoints for major genera of anaerobic bacteria (via EUCAST and others)

ARUMIC structure



Organism	Total	<0.008	0.008	0.012	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Bacteroides fragilis (BFRA)	631 (100.00%)				30 (4.75%)	14 (2.22%)	11 (1.74%)	38 (6.02%)	132 (20.92%)	161 (25.52%)	65 (10.30%)	26 (4.12%)	3 (0.48%)	2 (0.32%)	1 (0.16%)	1 (0.16%)	3 (0.48%)	5 (0.79%)	139 (22.03%)

Cumulative Real Time Results

Acknowledgements

UKARU team

Harriet Hughes	Clinical Lead UKARU
Michael Perry	Deputy Lead Clinical Scientist
Sarah Copsey-Mawer	Senior Biomedical Scientist/Quality and AST Lead
Selina Scotford	Biomedical Scientist
Beth Anderson	Biomedical Scientist
Liam Haglington	Associate Practitioner
Carol Davis	Medical Laboratory Assistant

Public Health Wales

Robin Howe	Professional Lead Consultant
Mandy Wootton	BSAC & SACU
Trevor Ede	Informatics

Diolch!

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EUCAST disk diffusion methodology for rapidly growing anaerobic bacteria

Erika Matuschek

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Species and antimicrobial agents

- 5 commonly isolated anaerobic bacteria
  - *Bacteroides* spp.
  - *Prevotella* spp.
  - *Fusobacterium necrophorum*
  - *Clostridium perfringens*
  - *Cutibacterium acnes*
- Clinically relevant antimicrobial agents
  - Benzylpenicillin 1 unit
  - Piperacillin-tazobactam 30-6 µg
  - Meropenem 10 µg
  - Vancomycin 5 µg
  - Clindamycin 2 µg
  - Metronidazole 5 µg

The development of a disk diffusion method for anaerobes

1. Investigation of parameters affecting the result*
2. MIC-zone diameter correlations for the five species (agar dilution MICs as reference)
   - Media from different manufacturers (in house and commercial), different systems for generating an anaerobic environment
3. Field trial at 16 laboratories
   - Phase 1: Disk diffusion of a common collection of 35 isolates with known MICs
   - Phase 2: Disk diffusion of local isolates

* Bavelaar et al. Clin Microbiol Infect. 2021 Nov;27(11):1695.e1-1695.e6.

Disk diffusion methodology

- **Medium:** Fastidious Anaerobe Agar (FAA) with 5% mechanically defibrinated horse blood
  - Agar depth 4.0 ± 0.5 mm
  - Plates must be dried prior to inoculation
- **Inoculum:** McF 1.0 (overnight culture from non-selective media)
- **Incubation:** Anaerobic environment
  35-37°C for 16-20 h

FAA media

- The humidity of the FAA plates affects reading.
  - Excess humidity may result in fuzzy zone edges, swarming and/or haze within zones.
- In-house prepared plates are usually less humid and zones are easier to read.
- Commercial FAA plates should be dried in two steps
  - Room temperature overnight, followed by 15 minutes at 35°C without lids

Streaking of plates and application of disks

- For *Bacteroides* spp., remove excess fluid by turning the swab against the inside of the tube to avoid over-inoculation.
- Spread the inoculum evenly over the entire agar surface, ensuring that there are no gaps between streaks.
  - This is particularly important for *Cutibacterium acnes*, which grows with small colonies and poor contrast to the FAA media.
- Limit the number of disks on each plate to allow good growth and to avoid overlapping of zones.

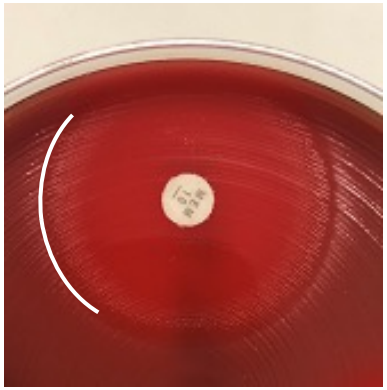
Incubation atmosphere and time

- The anaerobic atmosphere (irrespective of how it is created) should be checked regularly.
  - Note that the quality of the anaerobic atmosphere might change over time (especially workstations)
- Breakpoints and QC criteria are only valid for 16-20 h incubation.
  - Prolonged incubation is not allowed since this affect zone sizes significantly.

Reading of zones
General instructions

- Read FAA plates from the front with the lid removed illuminated with reflected light.
- Hold the plate about 30 cm from the eye at a 45-degree angle to the work bench when reading.
- Measure zone diameters with a calliper or ruler at the point of complete inhibition as judged by the naked eye.

Reading of zones
Specific instructions



- Ignore any faint haze within the inhibition zone and read the most obvious zone. **Tilt the plate towards you to better define the obvious zone edge.**
- Ignore haemolysis and swarming and read inhibition of growth.
- Isolated colonies within the inhibition zone should be taken into account. **For clindamycin, it is particularly important to examine zones carefully for colonies growing within the zone.**

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Reading guide

EUCAST disk diffusion for selected rapidly growing anaerobic bacteria on Fastidious Anaerobe Agar (FAA)

Version 1.0
September 2021

Bacteroides spp.


Piperacillin-tazobactam

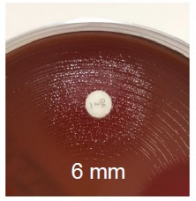

Piperacillin-tazobactam


Piperacillin-tazobactam


Meropenem


Meropenem


Meropenem



Clindamycin


Metronidazole

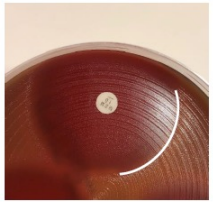
Fusobacterium necrophorum


Benzylpenicillin


Benzylpenicillin

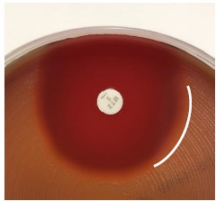

Piperacillin-tazobactam


Piperacillin-tazobactam


Meropenem


Clindamycin


Metronidazole

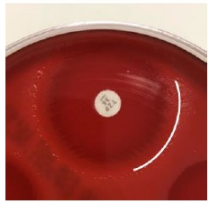

Metronidazole

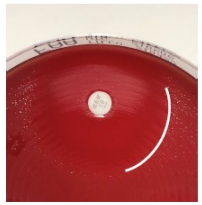
Clostridium perfringens

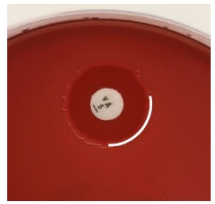

Benzylpenicillin


Benzylpenicillin

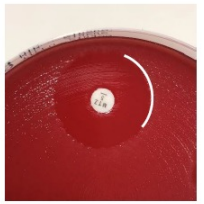

Piperacillin-tazobactam


Piperacillin-tazobactam


Meropenem


Vancomycin


Clindamycin


Metronidazole

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www.eucast.org

search term

AST of bacteria

... Disk diffusion methodology

EUCAST Disk Diffusion Test Methodology

The EUCAST disk diffusion test is based on MH media and disks of a good quality. It is calibrated to EUCAST clinical breakpoints using broth microdilution for MIC determination. Updates are published regularly.

See also EUCAST instruction videos.

Disk diffusion - Manual v 9.0 (1 January, 2021)

Disk diffusion - Slide show v 9.0 (1 January, 2021)

Disk diffusion - Reading guide v 8.0 (1 January, 2021)

EUCAST disk diffusion of anaerobic bacteria is under development 2021. Reviewed clinical breakpoints and disk diffusion correlates will be published with breakpoint table v 12.0 (1 January, 2022). The method will be valid for 5 species (*Bacteroides* spp, *Prevotella* spp, *Fusobacterium necrophorum, Clostridium perfringens* and *Cutibacterium acnes*) and for anaerobic incubation for 16 - 20h (extended incubation not allowed). For anyone who wants to prepare and practice, EUCAST already now publish the methodology, reading guide and QC criteria.

- Disk diffusion and QC criteria for anaerobic bacteria - Manual v 1.0 (20 September, 2021)
- Disk diffusion of anaerobic bacteria - Reading guide v 1.0 (20 September, 2021)

For translations to other languages - see → Translations.

For previous versions of documents - see → Previous versions.

Anaerobic bacteria

For species not listed below, see EUCAST Guidance Document on how to test and interpret results when there are no breakpoints

Expert Rules and Intrinsic Resistance Tables

MIC determination (agar dilution)
Medium: Fastidious Anaerobe Agar (FAA)
Inoculum: 10^5 CFU/spot
Incubation: Anaerobic environment, 35-37ºC, 48h
Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent where a noticeable difference is seen in visible growth between the test and control plate.
Quality control: *Bacteroides fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124.
Clostridium perfringens DSM 25589 with a metronidazole 5 µg disk to monitor the anaerobic atmosphere.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Fastidious Anaerobe Agar (FAA). The plates should be dried prior to inoculation (at 20-25°C overnight or at 35°C, with the lid removed, for 15 min).
Inoculum: McFarland 1.0
Incubation: Anaerobic environment, 35-37ºC, 18±2h
Reading: Unless otherwise stated, read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light. See pictures below and the EUCAST Reading Guide for disk diffusion of anaerobic bacteria for further information.
Quality control: *Bacteroides fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124.
Clostridium perfringens DSM 25589 with a metronidazole 5 µg disk to monitor the anaerobic atmosphere.

Bacteroides spp.

Antimicrobial agent	MIC breakpoints (mg/L)			Disk content	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU	(µg)	S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Piperacillin-tazobactam	8[1]	8[1]		30-6	20	20		**1.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Piperacillin-tazobactam, *B. thetaiotaomicron*	IE	IE			IE	IE		**2/A.** The meropenem zone diameter breakpoint will detect all *cfiA* gene mediated carbapenem resistance in *Bacteroides fragilis*. Some isolates with an MIC of 1 mg/L may harbour the *cfiA* gene.
Meropenem	1[2]	1[2]		10	28[A]	28[A]		**3/B.** For information on how to use breakpoints in brackets, see https://www.eucast.org/eucastguidancedocuments/.
Clindamycin	(4)[3]	(4)[3]		2	(10)[B,C]	(10)[B,C]		**C.** Examine zones carefully for colonies within zones. Colonies should be taken into account when reading.
Metronidazole	4	4		5	25	25		

Prevotella spp.

Antimicrobial agent	MIC breakpoints (mg/L)			Disk content	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU	(µg)	S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.5	0.5		1 unit	20	20		**1.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Piperacillin-tazobactam	0.5[1]	0.5[1]		30-6	26	26		**A.** Examine zones carefully for colonies within zones. Colonies should be taken into account when reading.
Meropenem	0.25	0.25		10	34	34		
Clindamycin	0.25	0.25		2	31[A]	31[A]		
Metronidazole	4	4		5	22	22		

Fusobacterium necrophorum

Antimicrobial agent	MIC breakpoints (mg/L)			Disk content	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU	(µg)	S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.06	0.06		1 unit	25	25		**1.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Piperacillin-tazobactam	0.5[1]	0.5[1]		30-6	32	32		**A.** Examine zones carefully for colonies within zones in which case the organism should be reported resistant to clindamycin.
Meropenem	0.03	0.03		10	35	35		
Clindamycin	0.25	0.25		2	30[A]	30[A]		
Metronidazole	0.5	0.5		5	30	30		

Clostridium perfringens

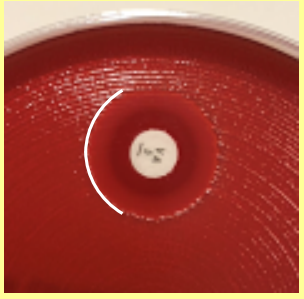
Antimicrobial agent	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.5	0.5		1 unit	15	15		1. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Piperacillin-tazobactam	0.5[1]	0.5[1]		30-6	24	24		
Meropenem	0.125	0.125		10	25	25		A. Examine zones carefully for colonies within zones. Colonies should be taken into account when reading.
Vancomycin	2	2		5	12	12		
Clindamycin	0.25	0.25		2	19[A]	19[A]		
Metronidazole	4	4		5	16	16		

Cutibacterium acnes

Antimicrobial agent	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.06	0.06		1 unit	24	24		1. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Piperacillin-tazobactam	0.25[1]	0.25[1]		30-6	27	27		
Meropenem	0.125	0.125		10	28	28		A. Examine zones carefully for colonies within zones. Colonies should be taken into account when reading.
Vancomycin	2	2		5	22	22		
Clindamycin	0.25	0.25		2	26[A]	26[A]		

Clostridioides difficile

Antimicrobial agent	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints.
Vancomycin	2[1]	2[1]			IP	IP		1 The breakpoints are based on epidemiological cut-off values (ECOFFs) and apply to oral treatment of *C. difficile* infections. There are no conclusive clinical data regarding the relation between MICs and outcomes.
Fidaxomicin	IE[2]	IE[2]			IE	IE		2. Fidaxomicin breakpoints and ECOFF have not been set because the available data show major variation in MIC distributions between studies.
Metronidazole	2[1]	2[1]			IP	IP		



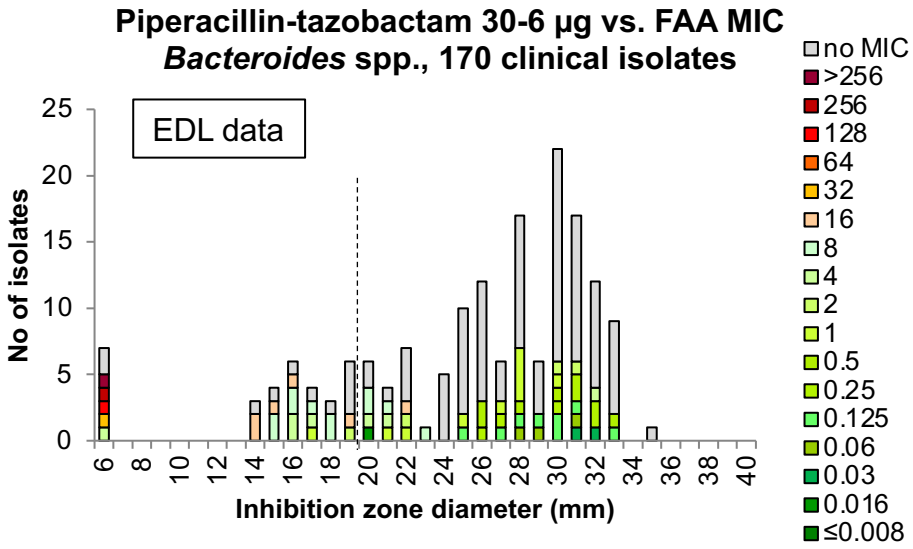
Examples of inhibition zones for anaerobic bacteria.

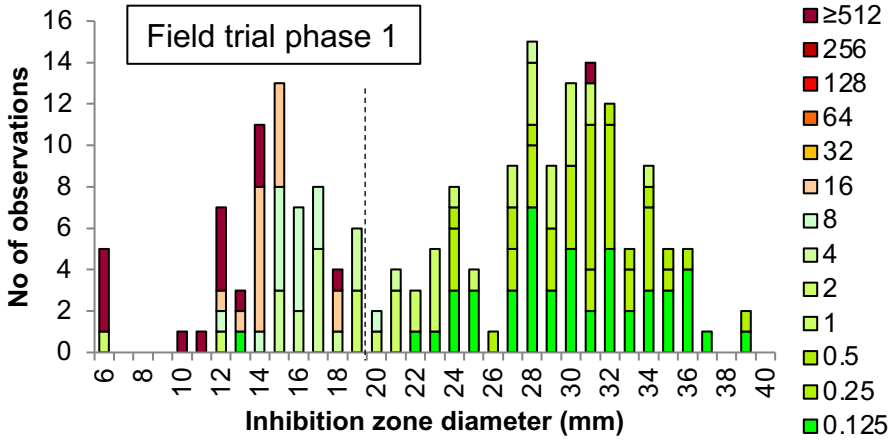
a) If haze within the zone occurs, read the most obvious zone edge. Tilt the plate towards you to better define the obvious zone edge.

b) Isolated colonies within the inhibition zone should be taken into account. For clindamycin, it is particularly important to examine zones carefully for colonies growing within the zone.

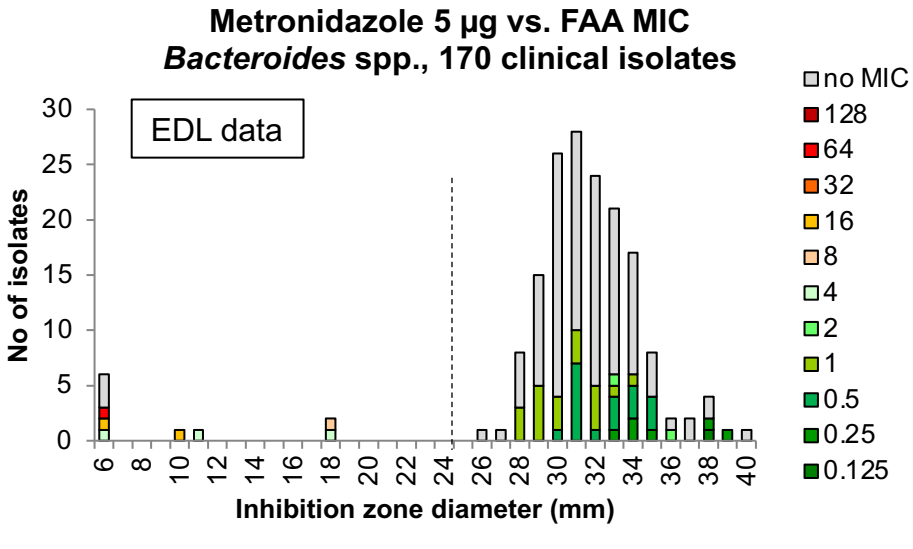
c) Ignore haemolysis when reading zones.

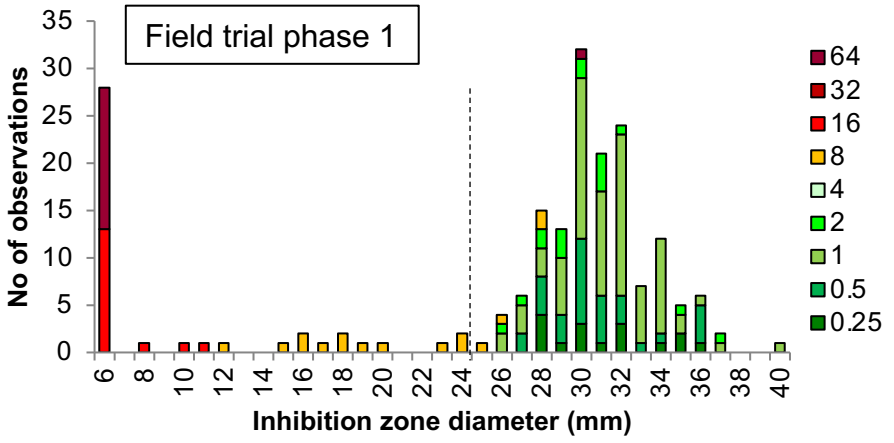
Bacteroides spp.
Piperacillin-tazobactam 30-6 µg



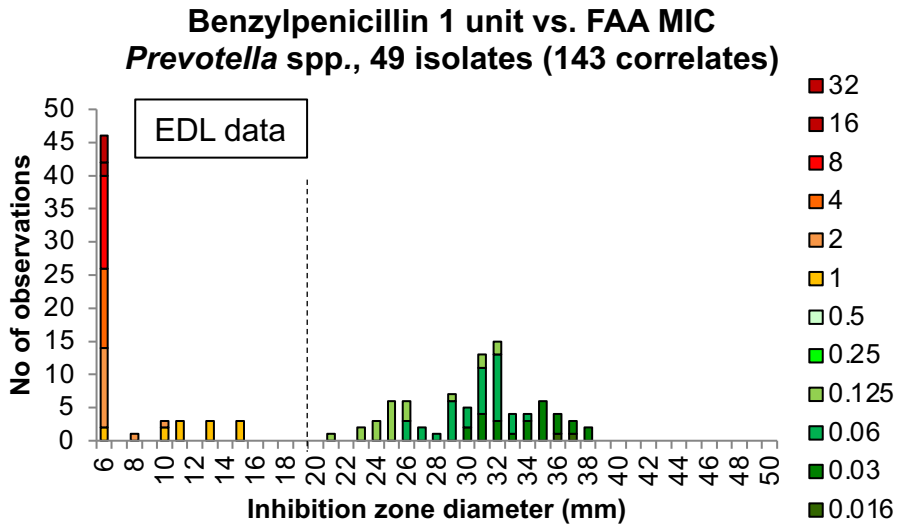


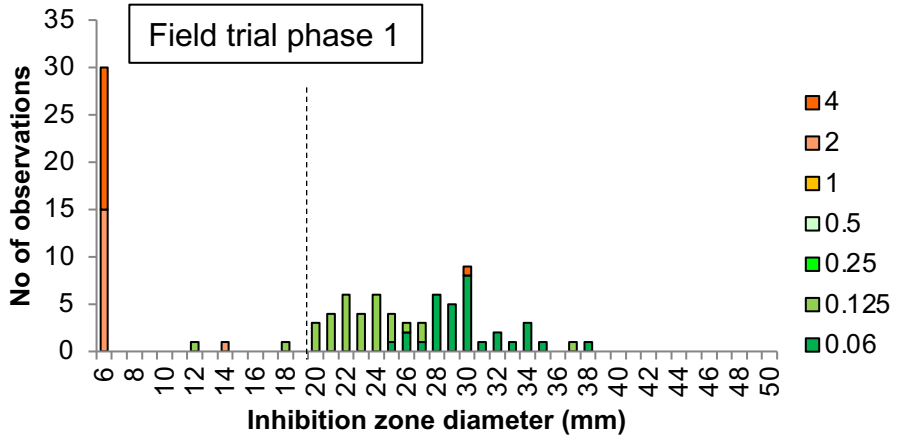
Bacteroides spp.
Metronidazole 5 µg



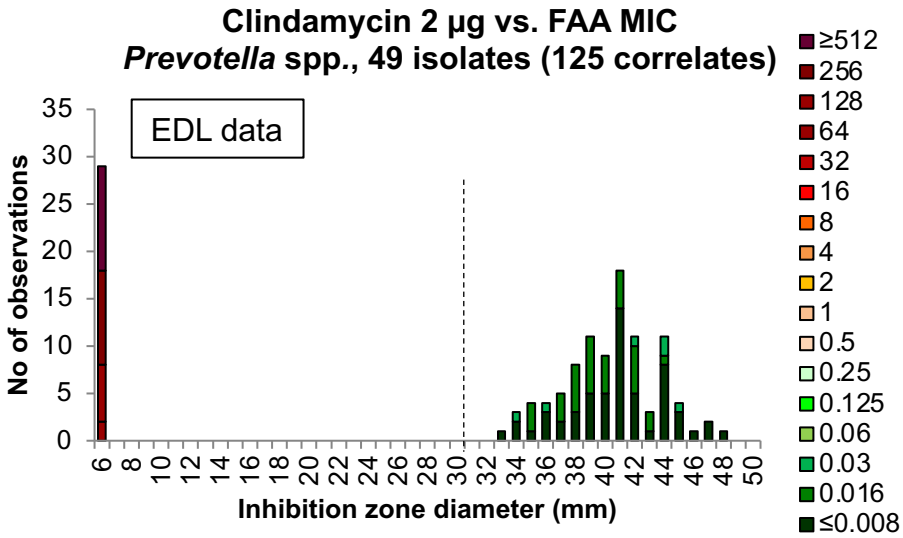


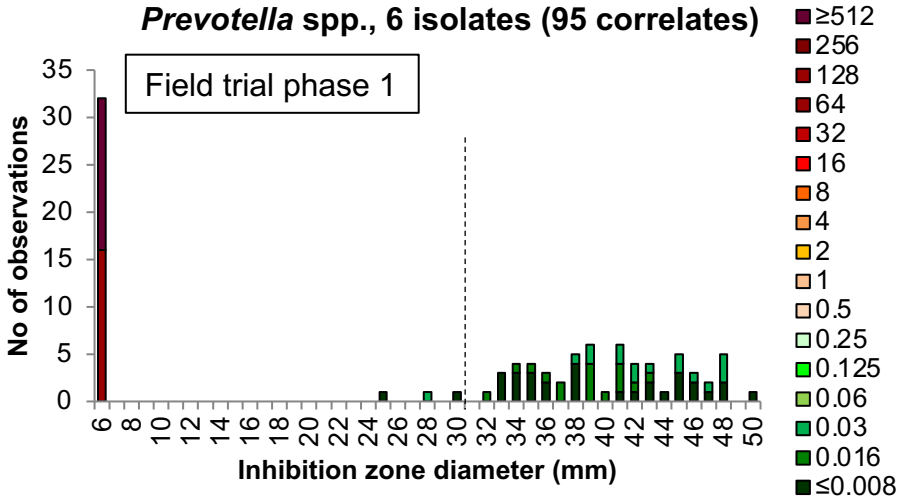
Prevotella spp.
Benzylpencillin 1 unit



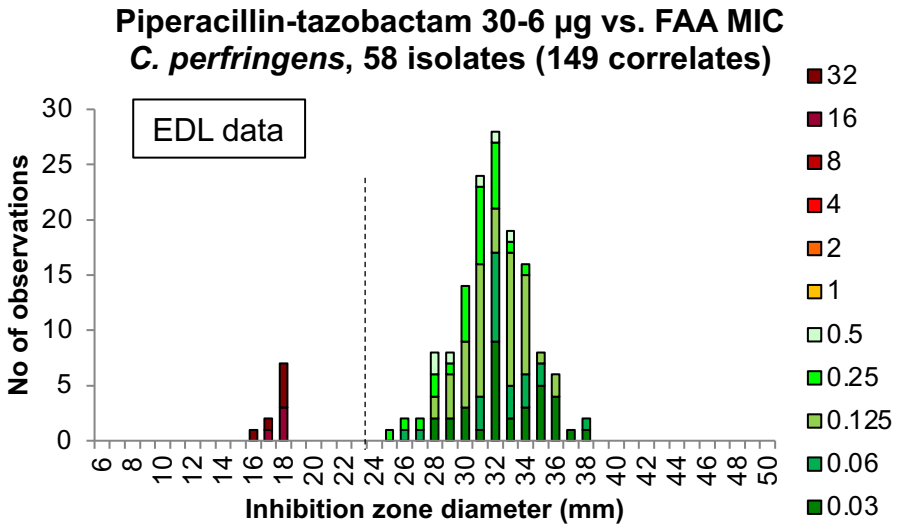


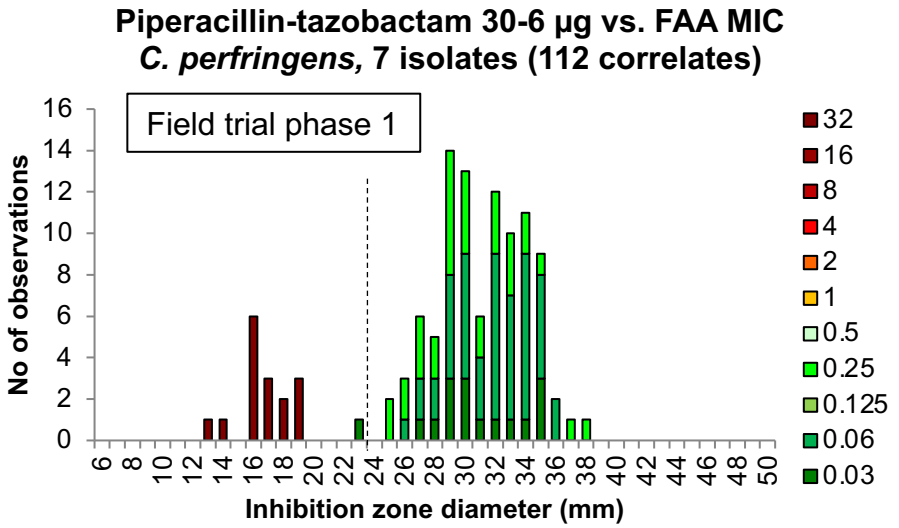
Prevotella spp.
Clindamycin 2 µg



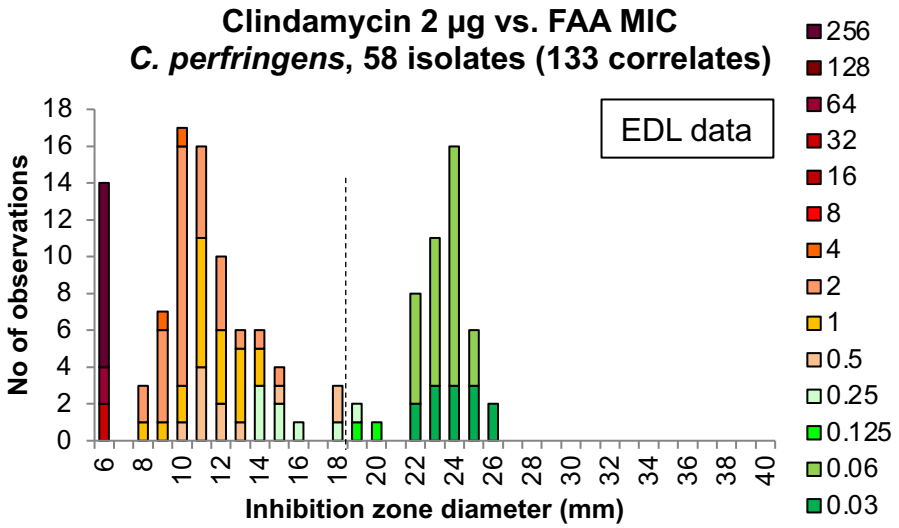


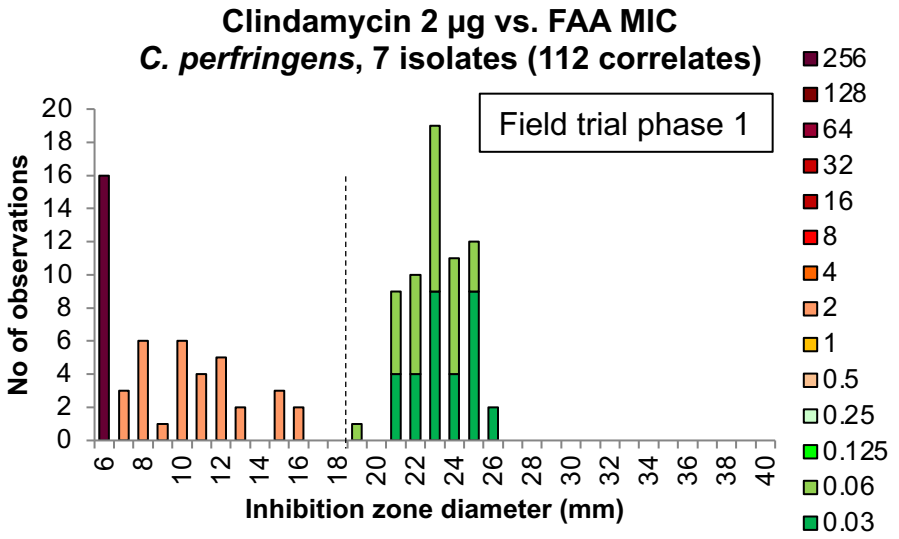
C. perfringens
Piperacillin-tazobactam 30-6 µg



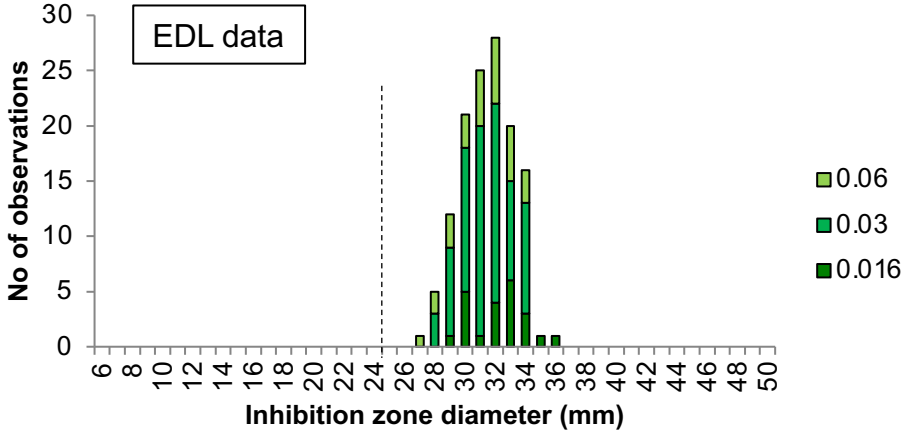


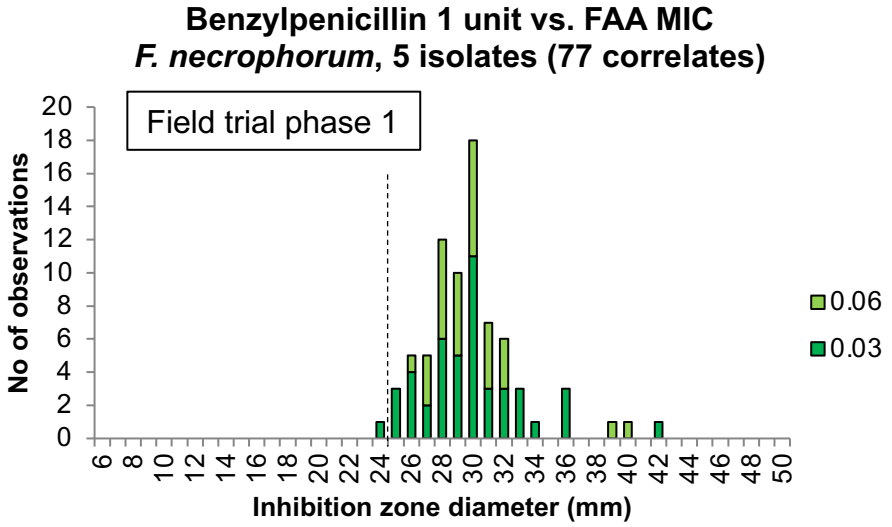
C. perfringens
Clindamycin 2 µg



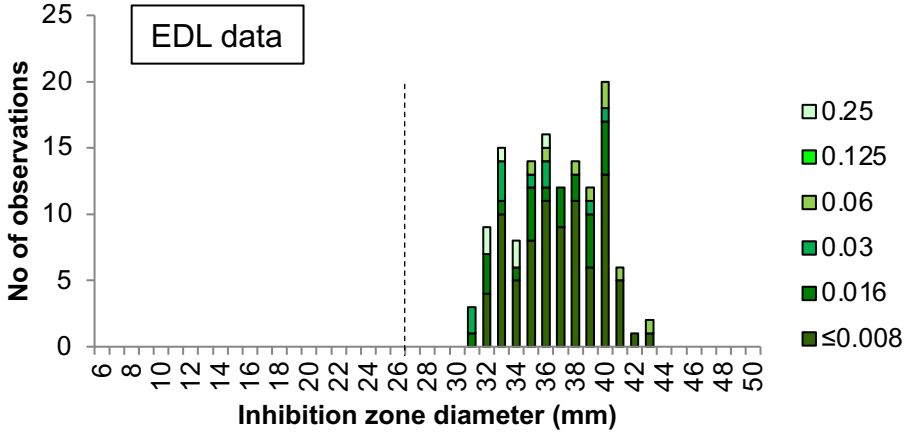


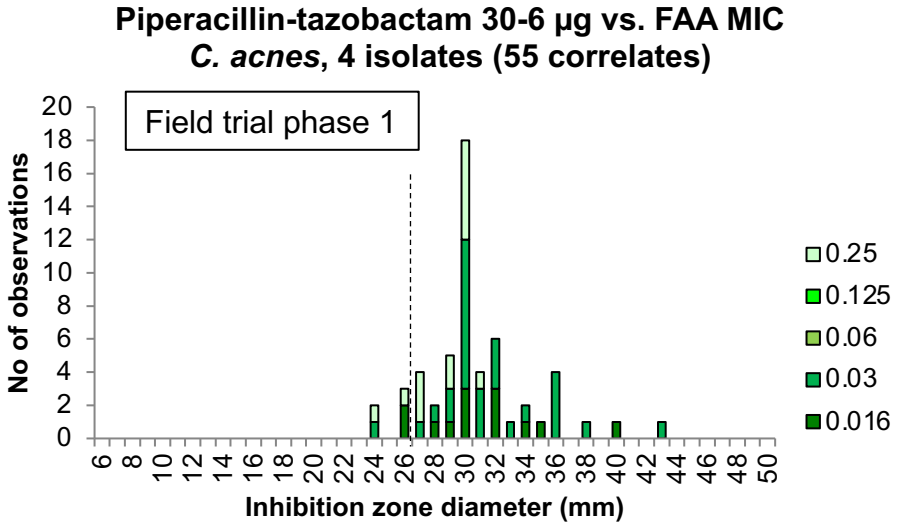
F. necrophorum
Benzylpenicillin 1 unit





C. acnes
Piperacillin-tazobactam 30-6 µg





Testing anaerobicity

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Anaerobicity?

- Anaerobicity – the state of being anaerobic (existing in the absence of free oxygen)
- Essential for the cultivation of anaerobic bacteria (the “susceptibility” of anaerobic bacteria to lethal oxygen (free oxygen radicals) is a continuum and the definition of anaerobic bacteria is not clear
- However, a common feature is that these bacteria grow significantly better in the absence of free oxygen
- In the case of antimicrobial susceptibility testing (AST) of anaerobic bacteria, standardized and constant anaerobicity is a prerequisite to do reproducible AST

Non-anaerobicity? Consequences

- Depending on the level of oxygen, the result will be from no growth of anaerobic bacteria, to poor growth, to reasonable growth etc.

- All these cases will have huge effects on e.g. an antibiotic disk zone diameter

  - usually poor growth results in larger zones

  - in contrast, metronidazole is dependent on anaerobicity to be reduced to the active metabolite, i.e. zone becomes smaller (pseudo-resistance)

Testing anaerobicity? Redox indicators

Resazurin

White: No oxygen

Red: oxygen

Methylene blue

White: No oxygen

Blue: Oxygen

Pros and cons

Resazurin is very sensitive

Easy, inexpensive

Can switch between colour/no colour a few times

Only shows the present atmosphere and not ”over time”

Testing anaerobicity? Oxygen monitor

Oxygen Monitor

- accuracy: ±0.5% (0-25% range)

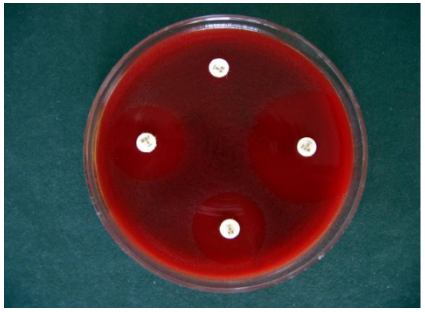
Pros and cons
Only shows the present atmosphere and not ”over time”

But an alarm can usually be included

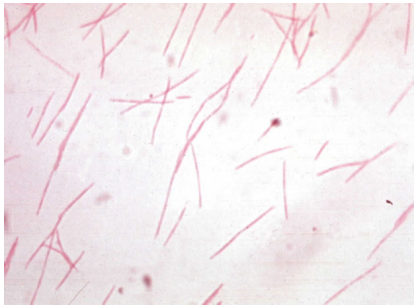
Accuracy can be a problem

Testing anaerobicity? Anaerobic growth indicators

Using strict anaerobic bacteria



Clostridium difficile
ATCC 700057



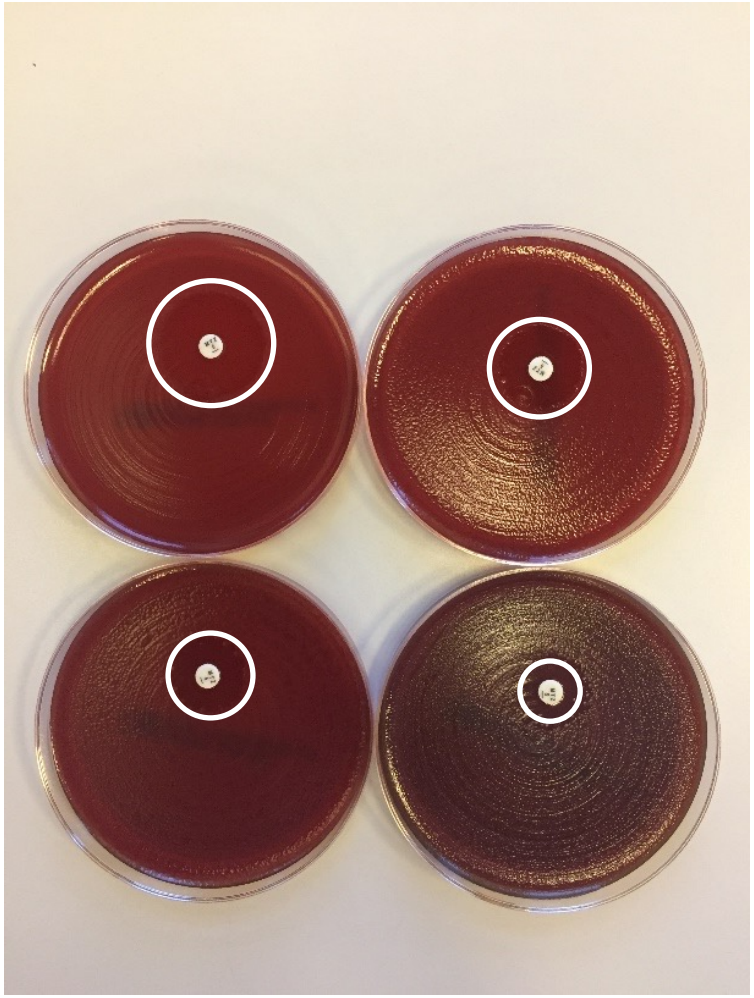
Fusobacterium nucleatum

Pros and cons
Strict anaerobic bacteria gives a good indication of the anaerobic atmosphere

However, no growth can be a result of no bacteria on the agar or oxygen, but the level is unknown

Testing anaerobicity? New principle

- DSM 25589 / CCUG 75076
- Aerotolerant *Clostridium perfringens*
- Will grow at 4% oxygen and even higher



McFarland 1 suspension. Top left: 0%, top right: 0.16%, bottom left: 1% and bottom right 2% of oxygen. The zone diameter is decreasing with increasing levels of oxygen

Testing anaerobicity? New principle

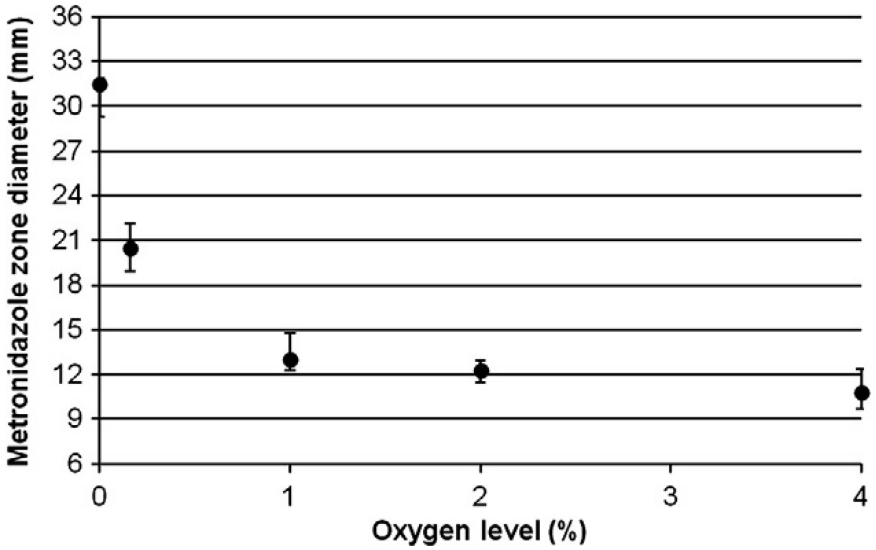


Fig. 2. Results from Part I of the study. The metronidazole zone diameter versus the oxygen level in the Anoxomat jar system. The median with range is shown (n = 12 at each oxygen level).

Justesen et al. Diagn Microbiol Infect Dis. 2013

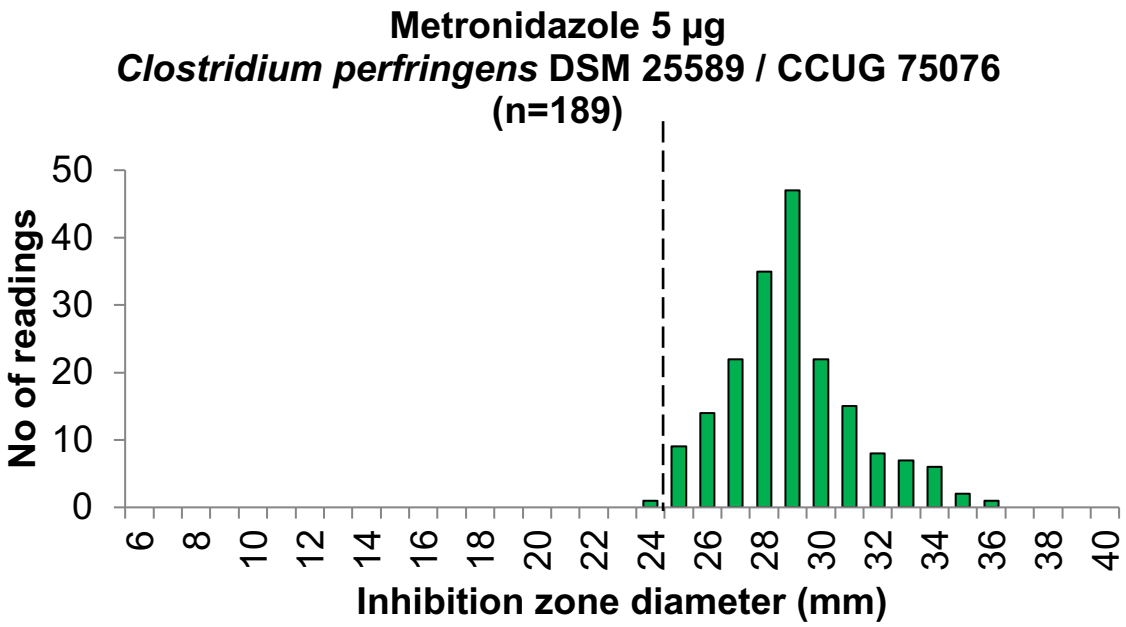
Pros and cons

Decrease in the metronidazole zone diameter from 30 to 22 mm at 0.16% of oxygen (extremely sensitive).

Confluent growth and a zone diameter above or equal to 25 mm indicates a sufficient anaerobic atmosphere over time

Cannot display the present atmosphere

Reproducibility? New principle



Readings over time from 16 different laboratories

Median: 29 mm

Target: ≥25 mm

Quality control

Jenny Åhman
EUCAST Development Laboratory

QC strains

- To monitor the anaerobic atmosphere:
  - *C. perfringens* DSM 25589 with metronidazole 5 µg
- To monitor test performance:
  - Gram negative : *B. fragilis* ATCC 25285
  - Gram positive: *C. perfringens* ATCC 13124
- Test frequency:
  - At each test occasion or at least 4 times per week.

Internal quality control

- Control of materials and equipment
  - Antimicrobial disks
  - Agar plates
  - Incubators
  - Densitometer

- Control of the testing procedure
  - Inoculum and inoculation
  - Incubation time and atmosphere
  - Reading of results

QC testing procedure

- Use an overnight culture of the QC strain
- Follow the same testing procedure as for clinical isolates.
- QC results should be read and evaluated before reporting AST results for clinical isolates.
- Evaluate results against criteria (target and ranges) in EUCAST QC Tables.

Tentative QC criteria

Bacteroides fragilis ATCC 25285

(NCTC 9343, DSM 2151, CCUG 4856T)

Antimicrobial agent	Disk content (µg)	Inhibition zone diameter (mm)	
		Target[1]	Range[1]
Piperacillin-tazobactam	30-6	***32***	***29-35***
Meropenem	10	***35-36***	***32-39***
Clindamycin	2	***26***	***23-29***
Metronidazole	5	***32-33***	***29-36***

Clostridium perfringens ATCC 13124

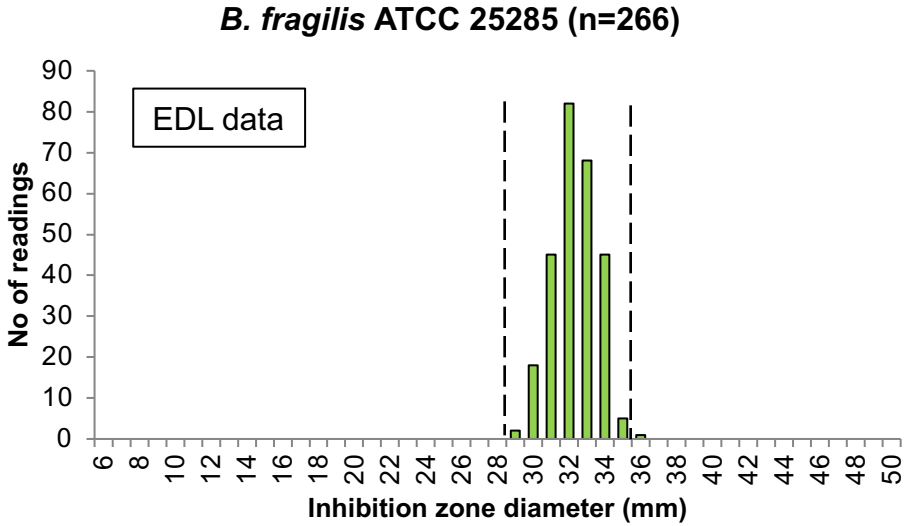
(NCTC 8237, CIP 103409, DSM 756, CCUG 1795T, CECT 376 T)

Antimicrobial agent	Disk content (µg)	Inhibition zone diameter (mm)	
		Target[1]	Range[1]
Benzylpenicillin	1 unit	**26**	**23-29**
Piperacillin-tazobactam	30-6	***33***	***30-36***
Meropenem	10	***37***	***34-40***
Vancomycin	5	***17***	***14-20***
Clindamycin	2	***23***	***20-26***
Metronidazole	5	***23***	***20-26***

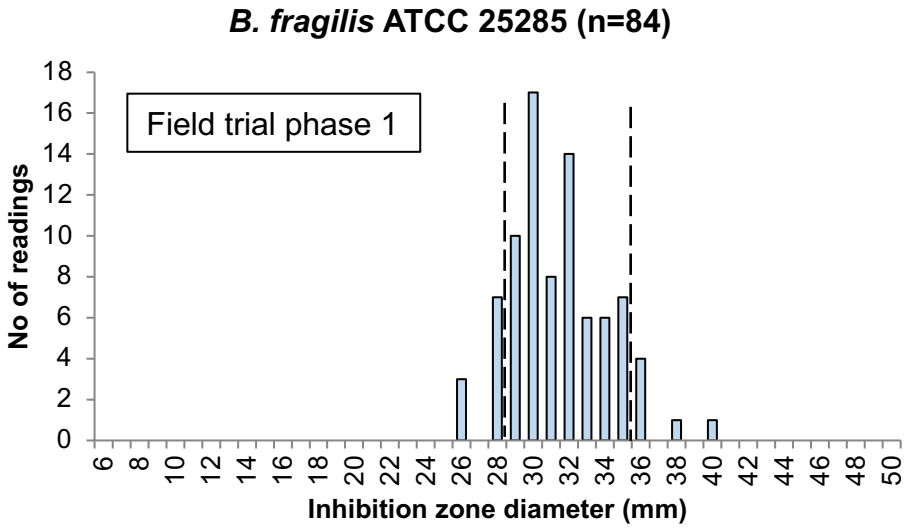
Range
Set to allow for random variation

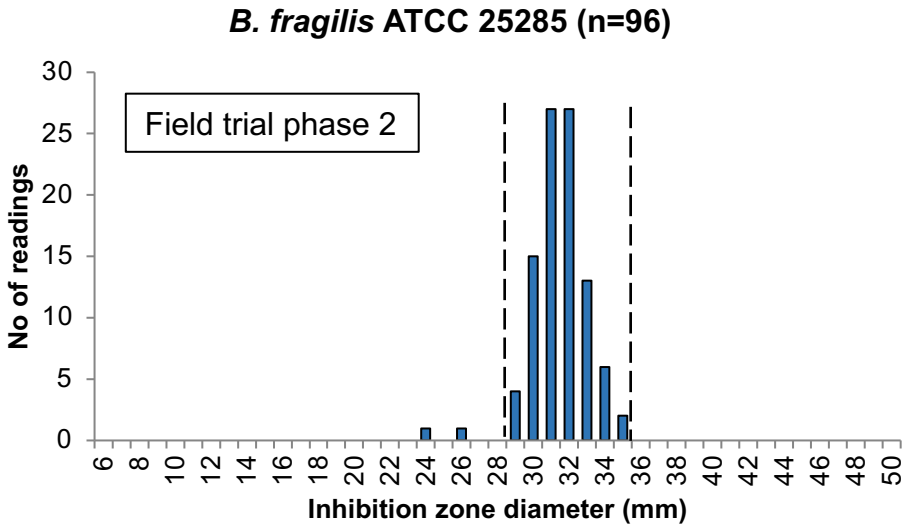
Target
Mean values from repeated measurements should optimally be on target ± 1 mm

B. fragilis ATCC 25285
Piperacillin-tazobactam 30-6 µg

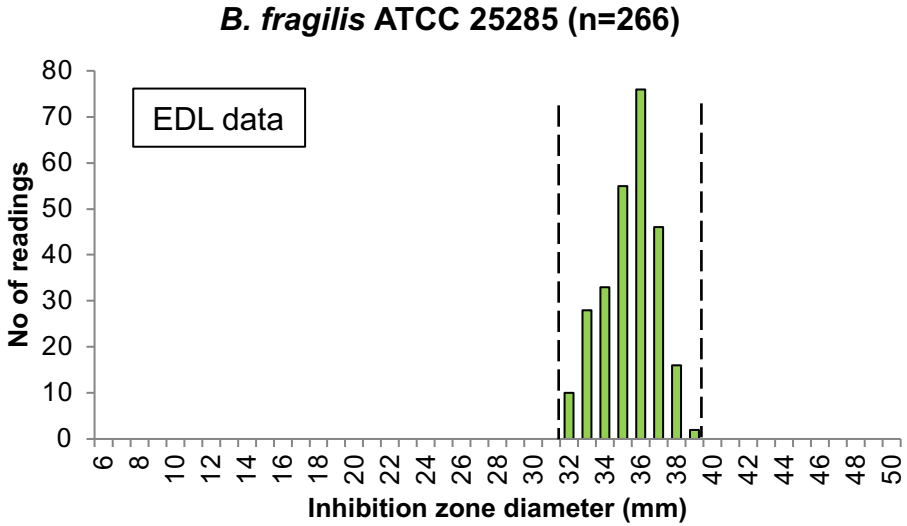


QC criteria:
Range 29-35 mm
Target 32 mm

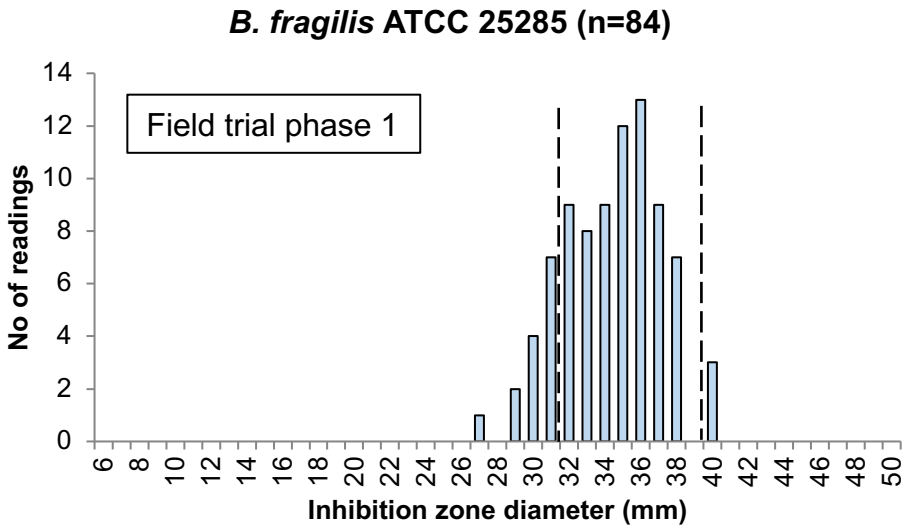


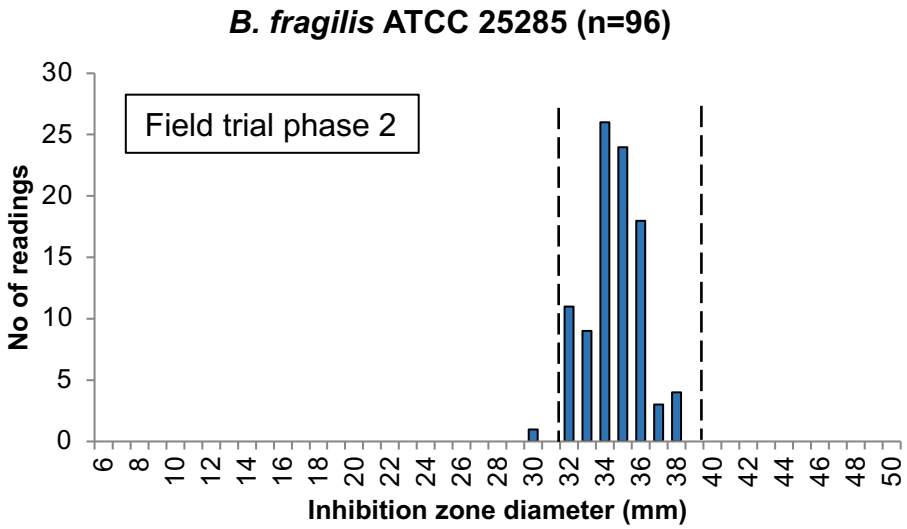


B. fragilis ATCC 25285
Meropenem 10 µg

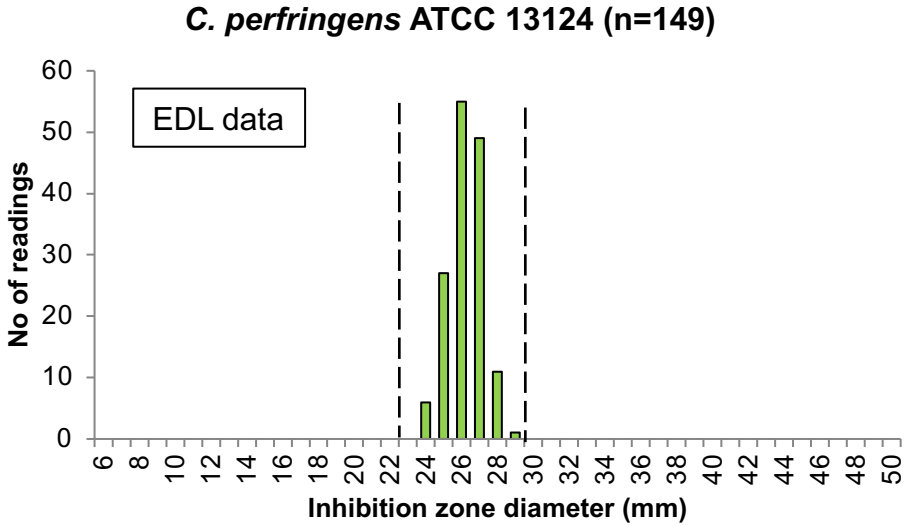


QC criteria:
Range 32-39 mm
Target 35-36 mm

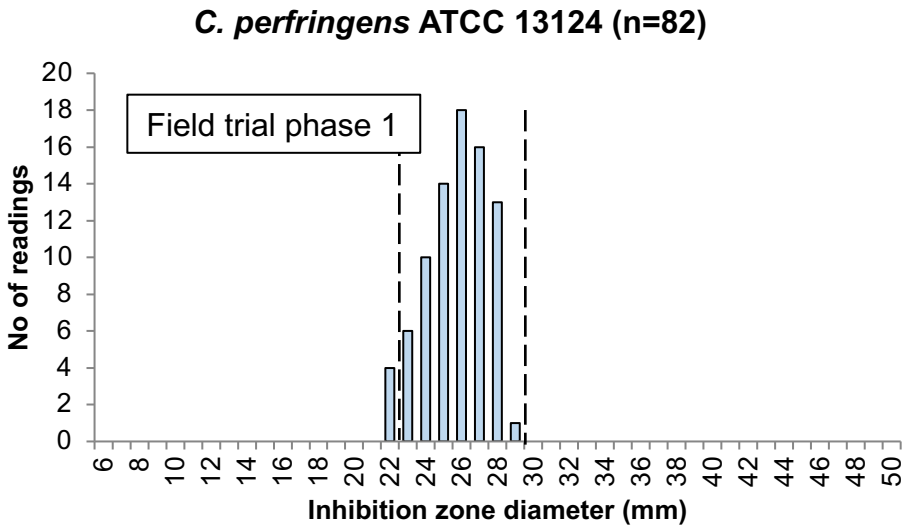


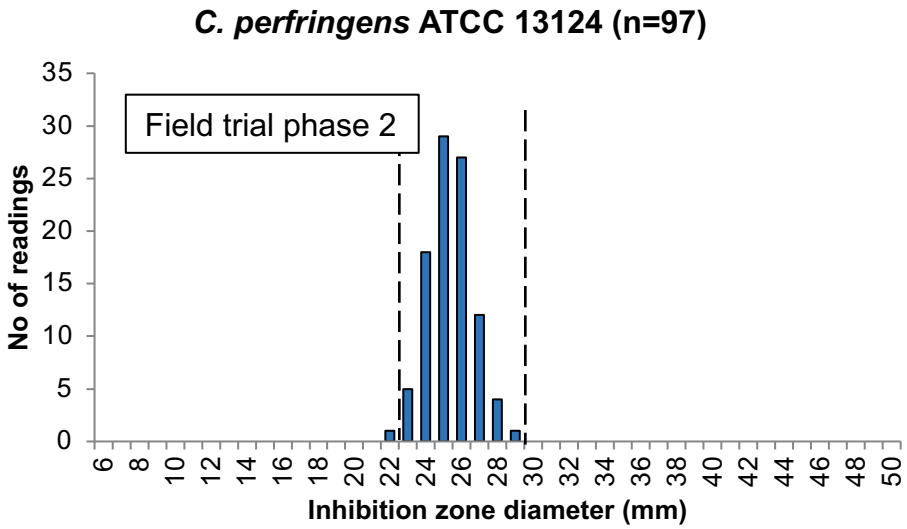


C. perfringens ATCC 13124
Benzylpenicillin 1 unit

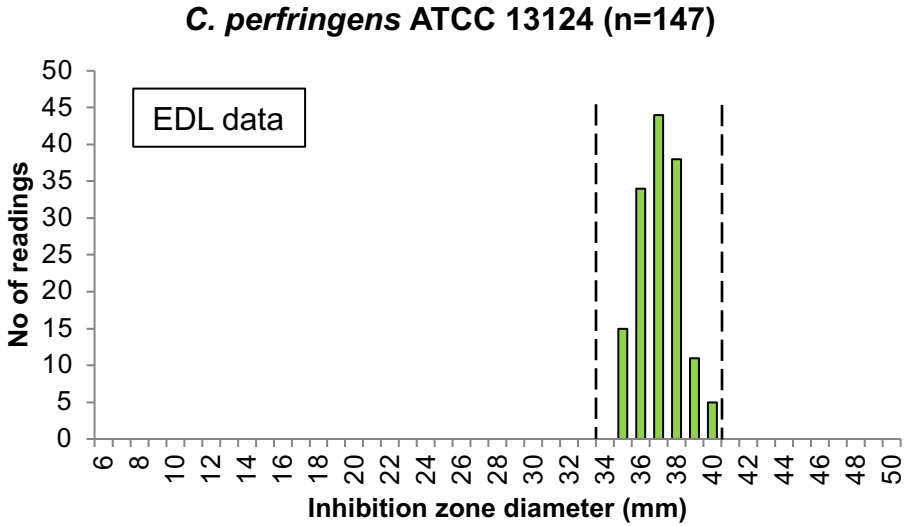


QC criteria:
Range 23-29 mm
Target 26 mm

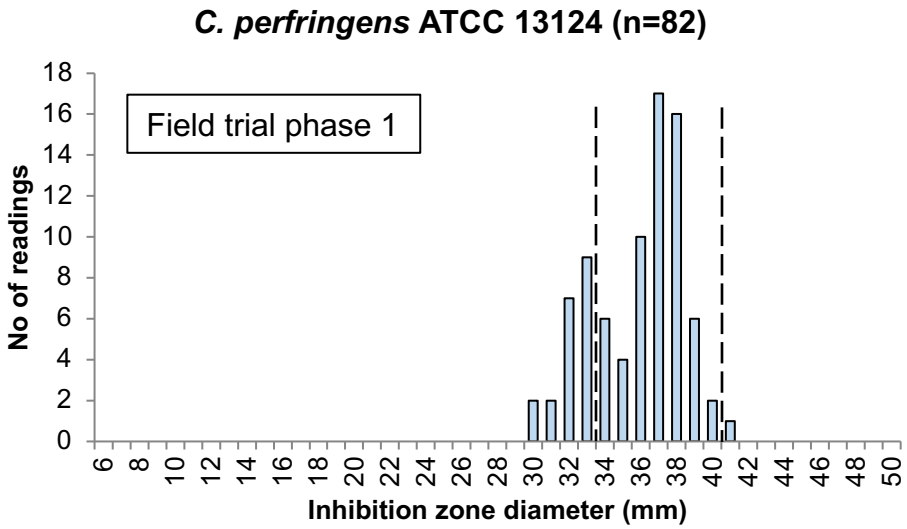


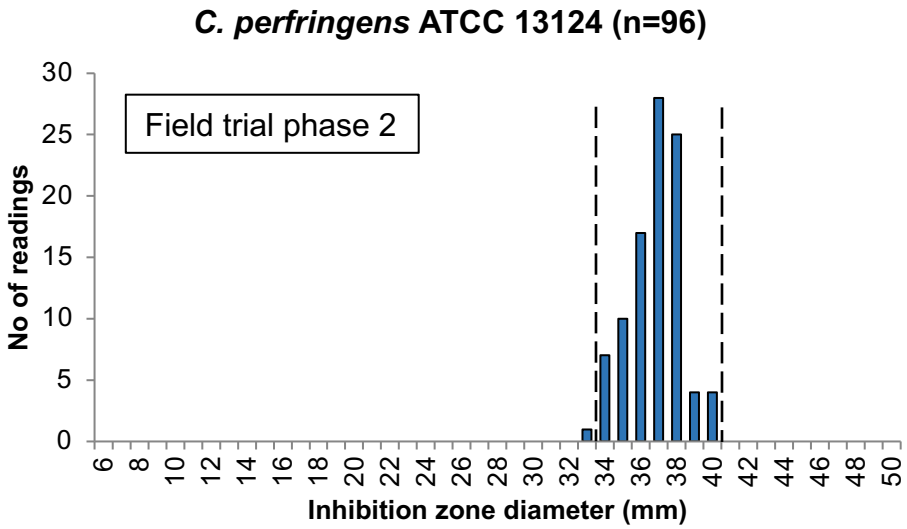


C. perfringens ATCC 13124
Meropenem 10 µg



QC criteria:
Range 34-40 mm
Target 37 mm





QC results within range

	Results within range (%)			
	B. fragilis ATCC 25285		*C. perfringens* ATCC 13124	
Antimicrobial agent	**Phase 1**	**Phase 2**	**Phase 1**	**Phase 2**
Benzylpenicillin			95	99
Piperacillin-tazobactam	81	98	83	97
Meropenem	80	99	74	99
Vancomycin			98	100
Clindamycin	87	100	93	100
Metronidazole	96	100	83	98
Total percentage	**86%**	**99%**	**88%**	**99%**
Total number of tests	n=336	n=384	n=490	n=579

OVERALL: 87% → 99%

Response to QC results out of range

- If QC results are out of range, troubleshoot!
- Investigate for possible sources of error:
  - Antimicrobial disks
  - FAA plates
  - Insufficient anaerobicity
  - Reading difficulties
  - QC strain
  - Not adhering to methodology

Introducing the method in the laboratory

- Anaerobic incubation
  - Workstation, gas-generating envelopes, Anoxomat
- FAA plates
  - In-house or commercial
  - No supplements (except horse blood), correct agar depth
- Training of staff
- Practice on the QC strains
- Use the Reading Guide

In summary

- The EUCAST disk diffusion method seems to provide accurate, robust and reproducible results for the 5 species and the agents for which it has been developed.
- The method will in 2022 be extended to include more agents (and eventually) more species.

- Previous attempts have been less successful. Why would EUCAST succeed?
  - EUCAST has been careful to set **species specific** clinical breakpoints which do not split wild type distributions – improves reproducibility.
  - The breakpoints and methods are valid only for defined and properly evaluated species.
  - EUCAST has introduced a strict and standardised method with robust and field tested QC criteria.
  - The method is based on a medium which can be obtained from several manufacturers and still retain limited variability and which supports good growth of targeted species in a short time (16-20h). The latter is beneficial in a disk diffusion test.