Report of the Methodology Working Group

January 2015

Methodology Group Members

- Stephen Jenkins
- Brandi Limbago
- Romney Humphries
- Sandra Richter
- Darcie Roe-Carpenter
- Katherine Sei
- Susan Sharp
- Ribhi Shawar
- John Turnidge
- Tracy Dooley

Distinguished Consultant

- Laura Koeth

Methodology ad hoc groups

- Direct AST
  Romney Humphries
- Anaerobe
  Darcie Roe-Carpenter
- Broth Microdilution
  Bill Brasso
- Polymyxins
  John Turnidge
- Tables 1 & 2 cleanup
  Mary York

- Molecular Results Reporting
  Cathy Petti & Thomas Kirn
- Alternate Disk Potency
- Intrinsic Resistance
  Barb Zimmer
- Atypical Staph
  Romney Humphries

General Updates

- ISO Standard for Mueller Hinton broth- for consideration in June 2015
  - Will replace M6 and M32
  - Approved by DIS and will be published in near future
- Alternate disk potency WG forming
- Testing of atypical Staphylococci – collection now exists
  - Are PBP2a / mecA testing all that could be used on these organisms?
  - Also concerns re. inoculum preparation for mucoid organisms (e.g. *P. aeruginosa*)
  - Important beyond CF patients (e.g. orthopedic implants)
- Unmet needs:
  - Evaluate impact of P-80 on AST results
    - In inoculum preparation
    - In drug preparation
    - In panel

Molecular Results Reporting WG

- Cathy Petti – co-chair
- Thomas Kirn – co-chair
- Paul Edelstein
- Yi Wei Tang
- Ferric Fang
- Neil Woodford
- Karen Carroll will join in June 2015

Ad Hoc Anaerobe Working Group

Darcie Roe-Carpenter - Chair
Audrey Schuetz
Joanne-Dzink-Fox
Nilda Jacobus
Hanna Wexler
Diane Citron
Steve Jenkins
Laura Koeth
Karen (Kitty) Anderson
Cindy Knapp
Meredith Hackel

Reporting to: Methods Working Group
Co-Chair Dr. Jenkins and Dr. Limbago

Report from Sunday Session

- MIC Epidemiology Cutoff Values (ECV) for *Propionibacterium acnes* – In M100-S25
  - Will pull together data for Clostridium sp. and vancomycin for ECV (intestinal and extraintestinal sources)
- Agar vs Broth – Still need data
  - will discuss at March conference call
- Drafting revisions to M11 document
  - review revisions at March Conference call and June meeting
  - keep gradient diffusion method in mind in mind during revisions
- Publication of antibiogram changes
  - Working on during next six months
- Intrinsic resistance table – revisions (next slide)

Appendix B – Intrinsic Resistance

B.5 Anaerobic Gram-Positive Bacilli

Organism \ Antimicrobial Agent	Vancomycin	Aminoglycosides
Clostridium species		R
Clostridium innocuum	R	R
~~Clostridium ramosum~~	~~R~~	~~R~~
~~Lactobacillus~~	~~R~~	

B.6 *Anaerobic Gram-Negative Bacilli*

Organism \ Antimicrobial Agent	Aminoglycosides	Penicillin	Ampicillin	Quinolones
Bacteroides species	R	R	R	
Fusobacterium canifelinum	R			R

Vote requested

WG voted to approve Table: 9/0/0

Future projects

- ECVs for other gram-positive species and vancomycin

Joint Polymyxins WG

MIC measurement for Polymyxins

- Bind to plastics and other laboratory materials
  - Electrostatic interaction (polymyxins are polycationic in solution)
  - Concentration dependent – lower binding at higher concentrations
- Mixtures of two major components (A & B, B1 & B2)
  - Ratios can vary – available evidence suggests similar potencies
- **Q1:** Are we agreed that we can ignore this variation?

MIC measurement for Polymyxins

- Reference method:
  - BMD in Mueller-Hinton with no polysorbate-80
    - P-80 acts synergistically with polymyxins, so "falsely" lowers the MICs
    - Reproducibility established in previously presented QC studies
  - For colistin, the test reagent is colistin sulphate (not methanesulfonate)

- **Q2:** Should we specify that the trays should be made of polystyrene?

Other Susceptibility Testing Methods

- Agar dilution – may be acceptable, needs further work
  - Gales et al., JCM 2001 (only 35 isolates)
- Disk diffusion – poor correlation
  - Gales et al., JCM 2001
  - Van der Heijden et al., ACMA 2007
- Gradient diffusion – poor correlation
  - Van der Heijden et al., ACMA 2007
- **Q4:** Can we confirm that BMD is the only currently acceptable method?
- If yes, need to remove disk breakpoints from M100

Clear All

P. aeruginosa: Colistin ECV/ECOFF

Step 1. Population Data | *P. aeruginosa* | Colistin

MIC	Log_2MIC	Raw Count	Cum. Count	Fitted
0.001	-10		0	0.0
0.002	-9		0	0.0
0.004	-8		0	0.0
0.008	-7		0	0.0
0.016	-6		0	0.0
0.03	-5	1	1	0.0
0.06	-4	5	6	0.1
0.125	-3	18	24	5.1
0.25	-2	99	123	127.1
0.5	-1	917	1040	892.7
1	0	1786	2826	1823.7
2	1	1160	3986	1097.9
4	2	131	4117	193.0
8	3	29	4146	9.6
16	4	46	4192	0.1
32	5	6	4198	0.0
64	6	1	4199	0.0
128	7	12	4211	0.0
256	8		4211	
512	9		4211	
1024	10		4211	

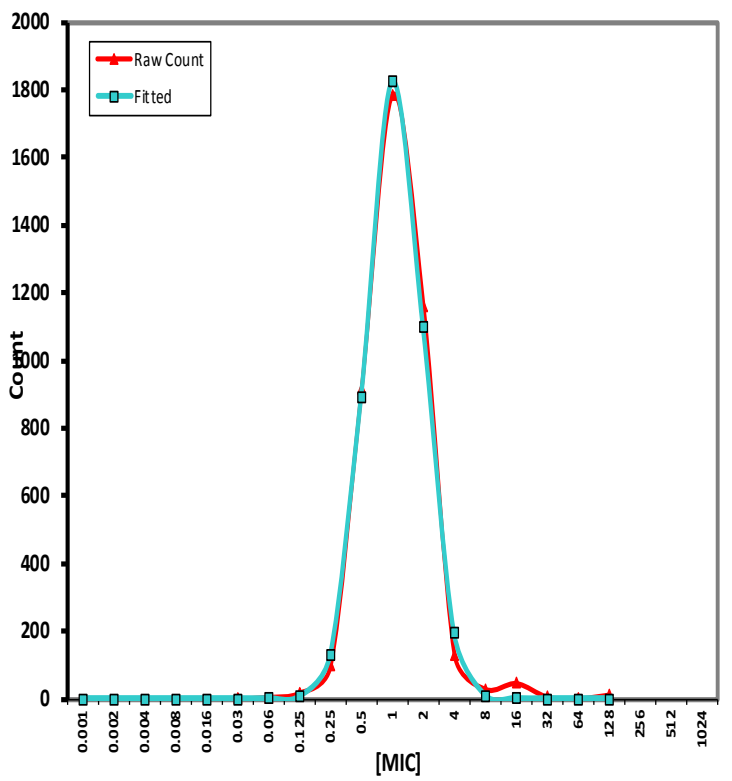
▲ Enter/paste data in this column

Modal MIC	1	
Log_2MIC Mode	0	
Max Log_2MIC	7	
Selected Log2 Mean	-0.42	=0.75
Selected Log2 SD	0.855	

Selected CO_{WT} Values		%>
CO_{WT} 95.0%	2	5.3%
CO_{WT} 97.5%	4	2.2%
CO_{WT} 99.0%	4	2.2%
CO_{WT} 99.9%	8	1.5%

Clear All

REVIEW AREA



Clear All

E. cloacae: Colistin ECV/ECOFF

Step 1. Population Data | *E. cloacae* | Colistin

MIC	Log_2MIC	Raw Count	Cum. Count	Fitted
0.001	-10		0	0.0
0.002	-9		0	0.0
0.004	-8		0	0.0
0.008	-7		0	0.0
0.016	-6		0	0.0
0.03	-5		0	0.0
0.06	-4	30	30	2.0
0.125	-3	19	49	35.0
0.25	-2	170	219	193.9
0.5	-1	366	585	343.0
1	0	172	757	195.2
2	1	54	811	35.5
4	2	17	828	2.0
8	3	40	868	0.0
16	4	80	948	0.0
32	5	23	971	0.0
64	6	2	973	0.0
128	7	21	994	0.0
256	8		994	0.0
512	9	2	996	0.0
1024	10		996	

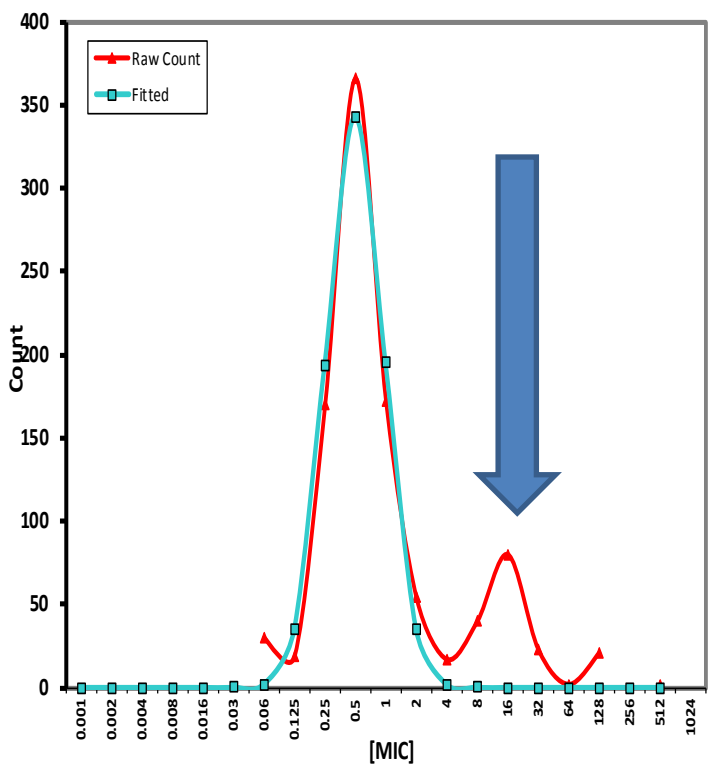
Enter/paste data in this column

Clear All

Modal MIC	0.5	
Log_2MIC Mode	-1	
Max Log_2MIC	9	
Selected Log2 Mean	-1.5	=0.35
Selected Log2 SD	0.891	

Selected CO_{WT} Values		%>
CO_{WT} 95.0%	1	24.0%
CO_{WT} 97.5%	2	18.6%
CO_{WT} 99.0%	2	18.6%
CO_{WT} 99.9%	4	16.9%

REVIEW AREA



Colistin ECVs/ECOFFs

Species	Calculated ECV (mg/L)	EUCAST 'eyeball' ECV (mg/L)
A. baumannii	2	2
E. aerogenes	1	2
E. cloacae	2	2
E. coli	1	2
K. oxytoca	2	2
K. pneumoniae	2	2
P. aeruginosa	4	4

Discussion: Could set ECV too close to wt distribution

Both vet group and fungal group chose calculated ECV

Q3: How should ECVs/ECOFFs be calculated?

For a vote

- **Q1:** Are we agreed that we can ignore component variation?

  WG Voted Yes 9; No 0; Abstain 1 (Pass)

- **Q2:** Should we specify that MIC trays should be made of polystyrene?
  - Should this be adopted beyond polymyxins?

  WG Voted Yes 8; No 0; Abstain 1 (Pass)

For a vote

- **Q3:** How should ECVs/ECOFFs be set? calculated vs eyeball
  - Both antifungal and veterinarian groups have voted to go with calculated approach with a value of 97.5%

    Yes: 4; No 1; Abstain 4 (no recommendation)
  - Whatever is decided, should method be adopted beyond polymyxins?
- **Q4:** Can we confirm that BMD is only reference method?
  - If yes, need to remove disk breakpoints from M100

    Yes 8; No 1; Abstain 1 (Pass)

Summary of Progress

- Susceptibility testing
  - Reference method confirmed for both agents
  - Comparability of agar dilution needs to be explored
- Animal model pharmacodynamics
  - Now established for colistin in mouse thigh and lung models
    - *P. aeruginosa* and *A. baumannii* only so far
    - Tentative pharmacodynamic cutoffs can be set once approach to MCS has been resolved
  - Insufficient information on polymyxin B so far
- Human clinical data
  - Many “noisy” single center clinical studies
  - Only one true PK/PD-focused study (multi-center NIAID funded) and clinical outcome data are still undergoing analysis, and only for colistin (methanesulfonate)

Table 1 Cleanup WG

- Mary York – Chair
- Dwight Hardy
- Tony Mazzulli
- Susan Munro – Secretary
- Barth Reller
- Tom Thomson
- Steve Jenkins

Considerations based on
- Sanford Guide
- Medical Letter
- IDSA guidelines

Table 1 guidelines

The recommendations for each organism group include agents of proven efficacy that show acceptable *in vitro* test performance. Considerations in the assignment of agents to specific test/report groups include clinical efficacy, prevalence of resistance, minimizing emergence of resistance, cost, FDA clinical indications for use, and current consensus recommendations for first-choice and alternative drugs

Proposed Changes to Table 1
Acinetobacter spp.

- Motions not entertained re. suggestion to move fluoroquinolones, gentamicin, tobramycin, and piperacillin-tazobactam from Group A to Group B
- Vote to remove ticarcillin-clavulanate from column entirely – approved (Yes 8; No 0; Abstain 2)
- Vote to remove cefotaxime and ceftriaxone from column entirely – approved (Yes 5; No 2; Abstain 2)
- Vote to change Title of Column to *Acinetobacter baumannii* complex
  - Move other *Acinetobacter* spp. to non-fermenters section
- Yes 7; No 0; Abstain 2 (Pass)

Proposed Changes to Table 1
Burholderia cepacia

- Move levofloxacin from Group B to Group A
- Vote: Yes 8; No 0; Abstain 2 (Approved)

- Move ticarcillin-clavulanic acid from Group B to Group C
- Vote: Yes 2; No 5; Abstain 2 (Failed)

Proposed Changes to Table 1 *Stenotrophomonas maltophilia*

- Move chloramphenicol from Group B to C
- Vote: Yes 7; No 0; Abstain 3 (Approved)

- Move levofloxacin from Group B to Group A
- Vote: Yes 2; No 3; Abstain 5 (Fail)

Other Proposed changes to Table 1

Motion to remove footnote from the bottom of Table 1

* MIC testing only; disk diffusion test unreliable

Vote: Yes 4; no 3; Abstain 3 (Failed)

Motion to remove ofloxacin from entire Table 1

Vote: Yes 8; No 0; Abstain 0 (Passed)

Other Proposed changes to Table 1

Motion to remove telithromycin from entire Table 1

Vote: Yes 7; No 0; Abstain 2 (Passed)

Recommendation from ad hoc WG re. the following comment was not entertained:

Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both. **Doxycycline and minocycline are not routinely reported on organisms isolated from the urinary tract because of low urine concentrations.**

Report from the Broth Microdilution Ad Hoc Working Group

CLSI AST Subcommittee Meeting

Ft. Lauderdale, FL

January, 2015

Broth Microdilution Ad Hoc WG Members

- Halsey Boyd
- Scott Killian
- Susan Kircher
- Cindy Knapp
- Laura Koeth
- Bob Rennie
- Katherine Sei
- Ribhi Shawar
- John Turnidge
- Michael Ullery
- Bill Brasso – Chair

Reporting to: Methodology WG

- Steve Jenkins
- Brandi Limbago

BMD Approach

Track 1- Defining the Main Sources of Variability in MIC Testing

BMD Survey Results & Recommendations

Track 2 - Dealing with Variability in MIC Testing – What to do with it?

STATS Team update

Clinical Laboratory Survey

- **Survey designed by the BMD Ad Hoc WG to help identify specific areas of variation when using BMD**

✓ **Finalize the survey & identify willing participants – Summer ’14**

✓ **Distribute to participants and collect completed surveys – Fall ’14**

❖ **Compile data, present to Methodology WG – Jan. ’15**

- **Participants were requested from various laboratories that routinely use the manual Reference AST (broth microdilution) method**
  - **14 Laboratories completed the survey**
    - **3 outside the U.S.**
- **Participants were asked, “Unless specified, the questions will pertain to testing routine, non-fastidious bacterial isolates.”**
- If “n” is >14, included multiple answers from one or more sites.

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Broth Microdilution Ad Hoc Working Group

NOTE: Summary slides NOT in Agenda.

Summary

Section	Question	Subject	Variability in Survey Responses	Recommendation
I. Inoculum Preparation	1	Liquid to prep inoculum	Moderate	
	2	Prep method	Low	
	3	Standardizing inoculum	**High**	??
	4	Colony counts on inoculum	Moderate	
	5	Plated media used to prep	Low	
II. Test Isolates	1	Storage of isolates	Low	
	2	# transfers before AST?	Moderate	Need clarification
III. Incubator/Incubation	1	Incubator - average temp	Moderate	Need clarification
	2	Humidity	Moderate	Pans of water; plastic bins, etc.
	3	Time of incub. for plated media	Moderate	Need to tighten??
IV. BMD panels/trays	1	Commercially or in-house prep	Moderate	OK
	2	Use M-07 for stock prep	Low	
	3	Storage temp for panels/trays	Low	
	4	Vol of media in wells	Low	
	5	Duration of thawing on bench	**High**	No current recommendation
V. Inoc of BMD panels	1	Time to transfer inoculum to panel	Low	
	2	Method to inoc panels	Moderate	
	3	Perform purity checks	Moderate	Need clarification
	4	Time to transfer panels to incubator	**High**	Need clarification

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Broth Microdilution Ad Hoc Working Group

Summary

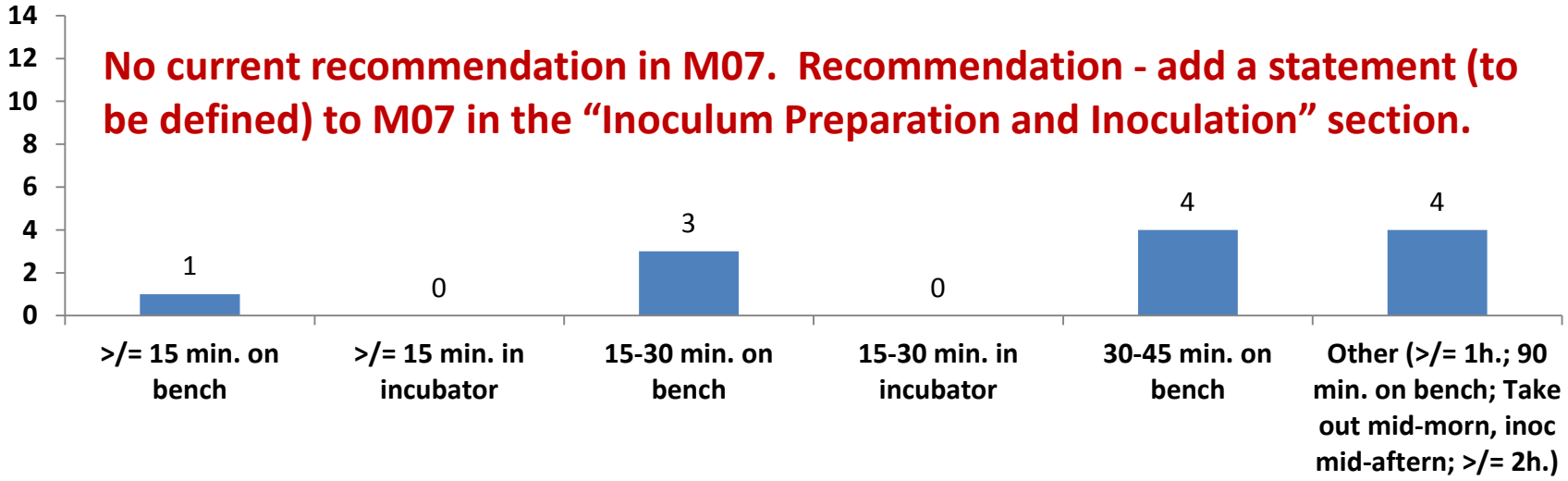
NOTE: Summary slides NOT in Agenda.

Section	Question	Subject	Variability in Survey Responses	Recommendation
VI. Stacking panels/trays	1	Do you stack?	Low	
	2	How high?	**High**	Need clarification
	3	How do you prevent drying out?	**High**	Need to tighten??
	4	Method used in #3 to prevent	**High**	Need to tighten??
	5	Duration of BMD panel incubation	Moderate	OK
VII. QC	1	How often do you test QC strains	Moderate	
	2	Handling QC failures	Low	
VIII. Reading / Interp	1	Standard used	Low	
	2	Use of viewing devices	**High**	Need clarification
	3	What to do when difficult to read	**High**	Need clarification
	4	Reading trailing	**High**	
	5	Reading skips	**High**	Need clarification

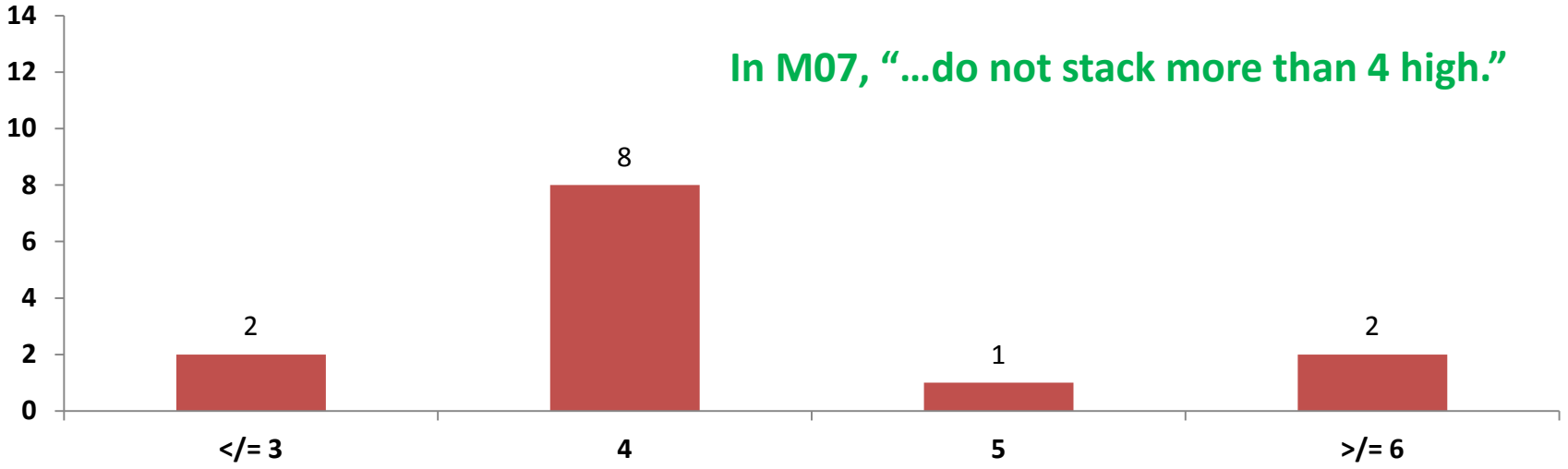
Will show two examples of these survey questions/answers where a clarification, or a need to ensure compliance with the M07 recommendations may be warranted.

Broth Microdilution Panels/Trays

How long do you allow your frozen broth microdilution trays to thaw?

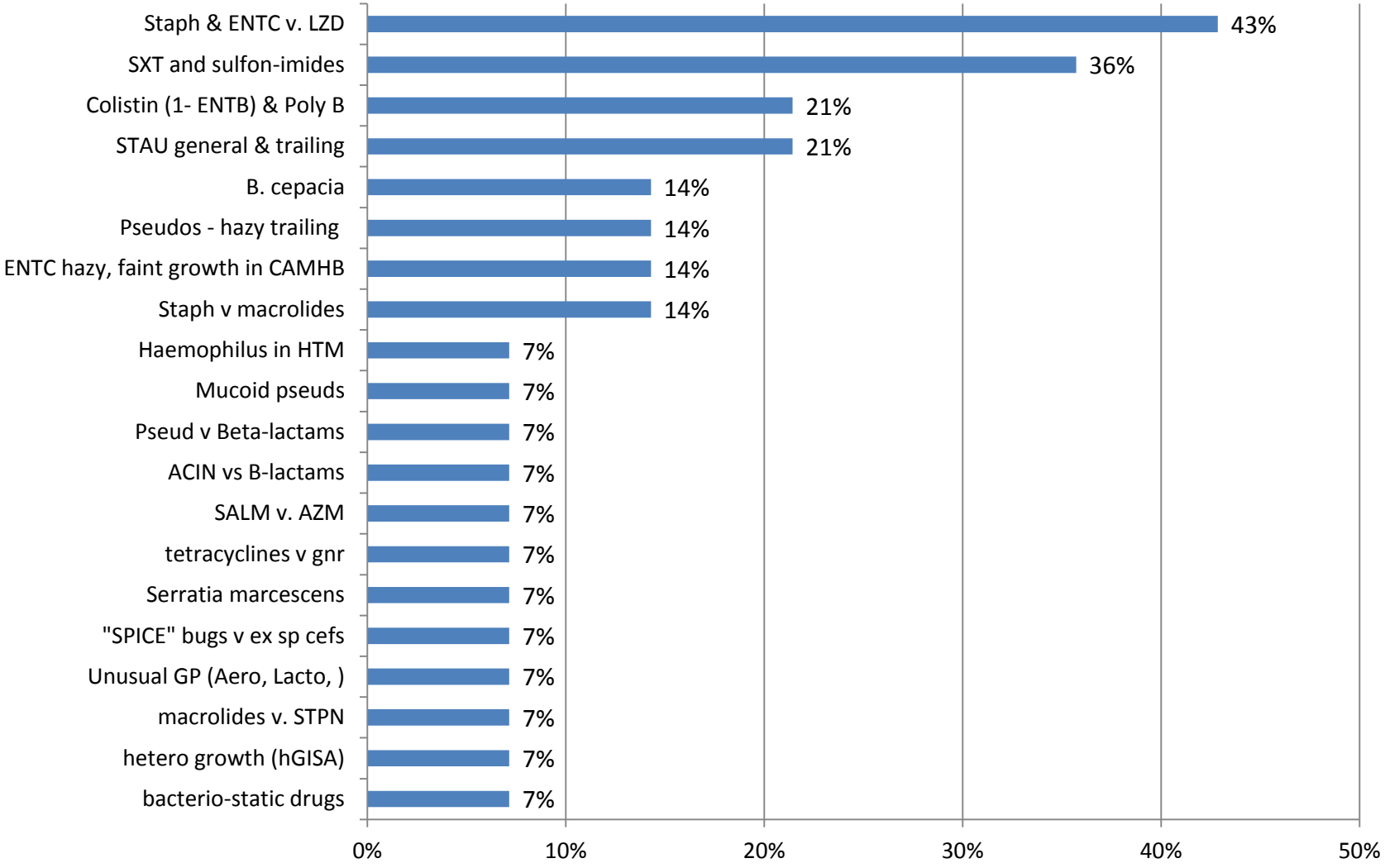


How many panels/trays do you normally stack together in the incubator?



Reading & Interpreting of Endpoints for Each Antibiotic in Panel/Tray

When reading broth microdilution panels, what organism/antibiotic combination(s) do you find the most difficult with regard to reading and interpreting endpoints?



Notes & Observations

1. What should you do if 3-5 isolated colonies are not available?
2. What should you do if colonies are “sticky”, and do not produce a homogeneous suspension?
3. What is the best way to prepare a purity plate – from the inoculum or the tray itself?
4. M07 says for a test to be considered valid, acceptable growth (=>2mm button or definite turbidity) must occur in the growth-control well.” Is this a good recommendation?

II. Track 2 - Dealing with Variability in MIC Testing

Planned Replicate Testing with Clinical Isolates

AST manufacturers replicate test clinical isolates (from 6-27 replicates) on reference panels as part of their product development.

This testing is done strictly per the CLSI guidelines.

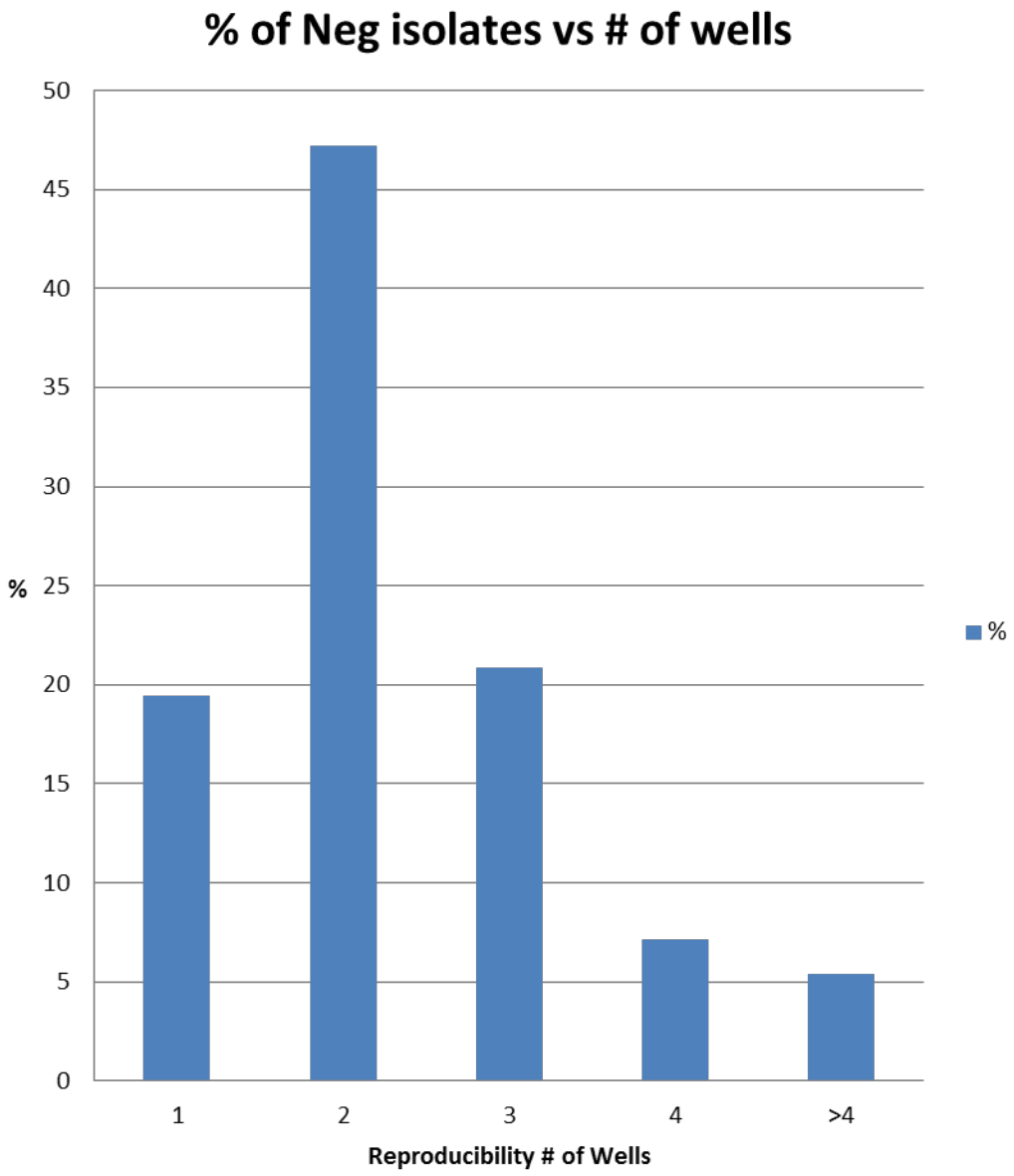
The team is in the process of reviewing the data, and plan to present at the June 2015 meeting.

Preliminary look…..

Preview with Gram Neg

Data set from BioMerieux:

- 123 Gram Neg Clinical Isolates tested with 20 different antibiotics.
- Only 19.45% had a singular MIC result.
- About a third of the results are spread across 3 or more MIC results—these isolates are variable in spite of strict test setup.



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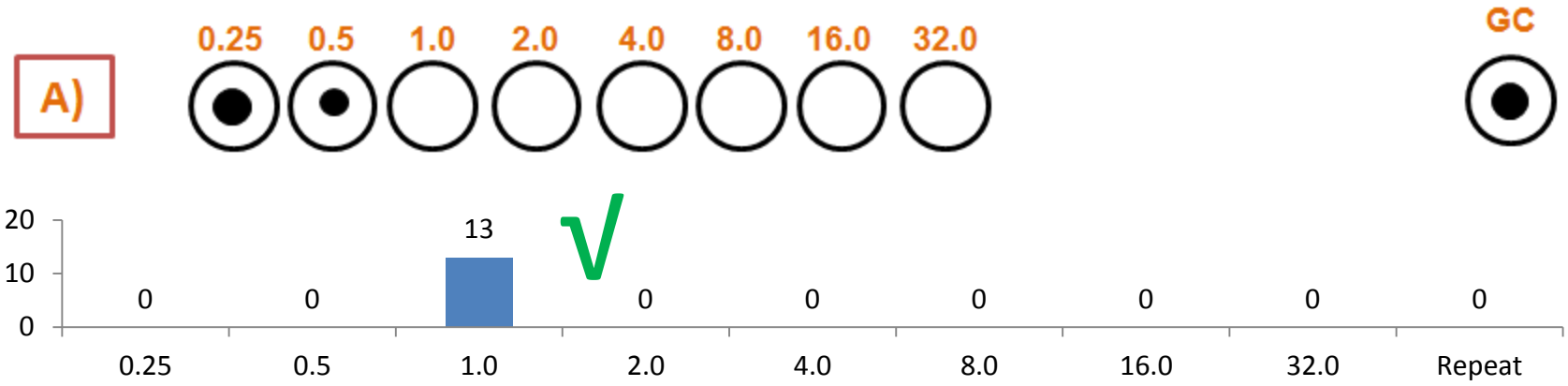
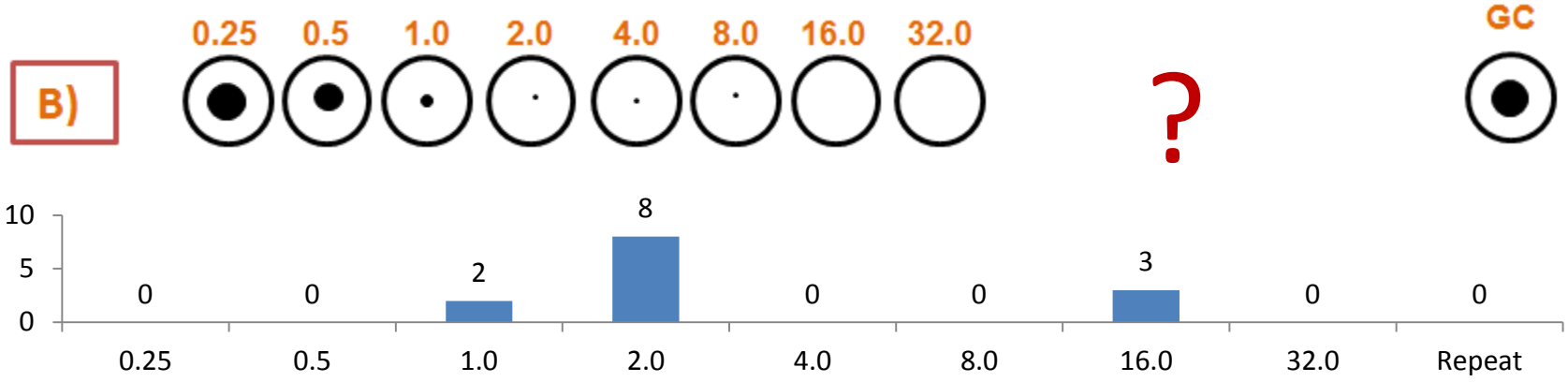
Back-up Slides

Broth Microdilution
Ad Hoc Working Group

Reading & Interpreting of Endpoints for Each Antibiotic in Panel/Tray

How do you interpret the endpoint if trailing is observed in a non-folate antagonist antibiotic? The 4 examples below, A-D, represent a typical broth microdilution panel with four (4) β-lactam class antibiotics, such as a cephalosporin, and the growth pattern from a member of the Enterobacteriaceae, such as a *K. pneumoniae*. A Growth Control (GC) well is on the right.





Reading & Interpreting of Endpoints for Each Antibiotic in Panel/Tray

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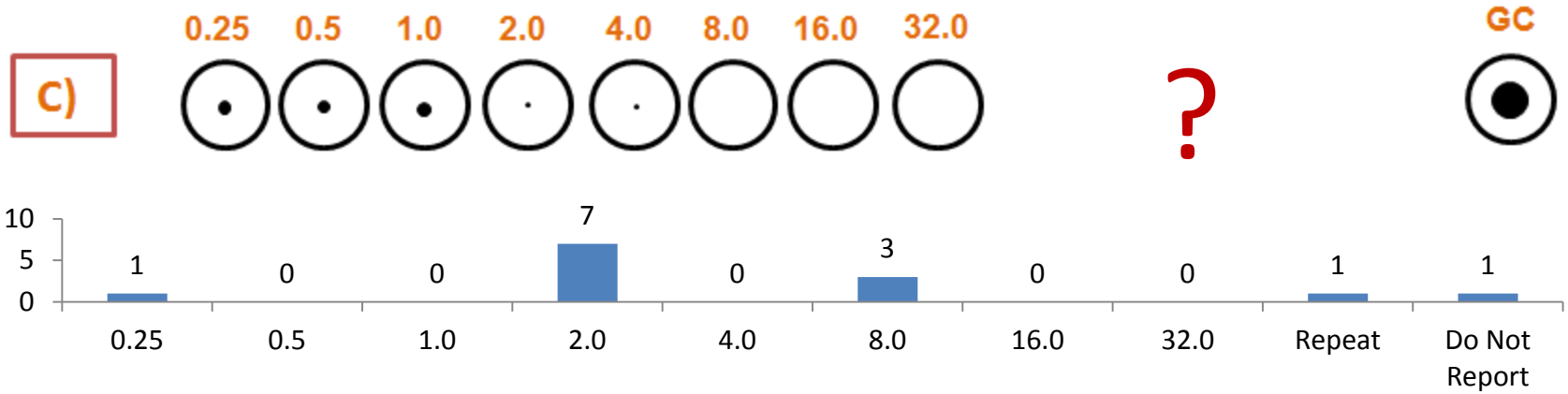
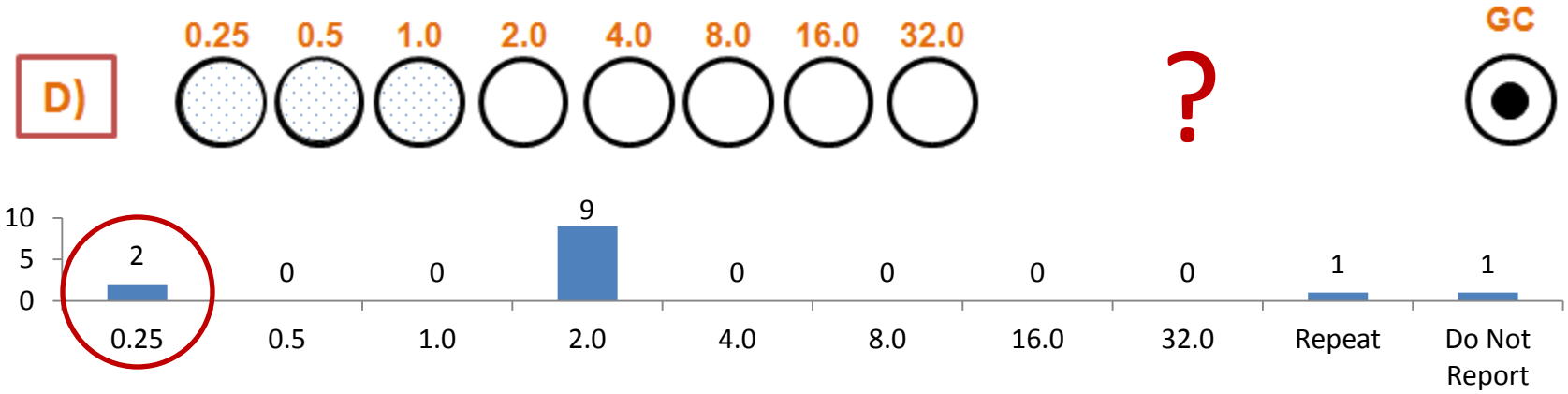
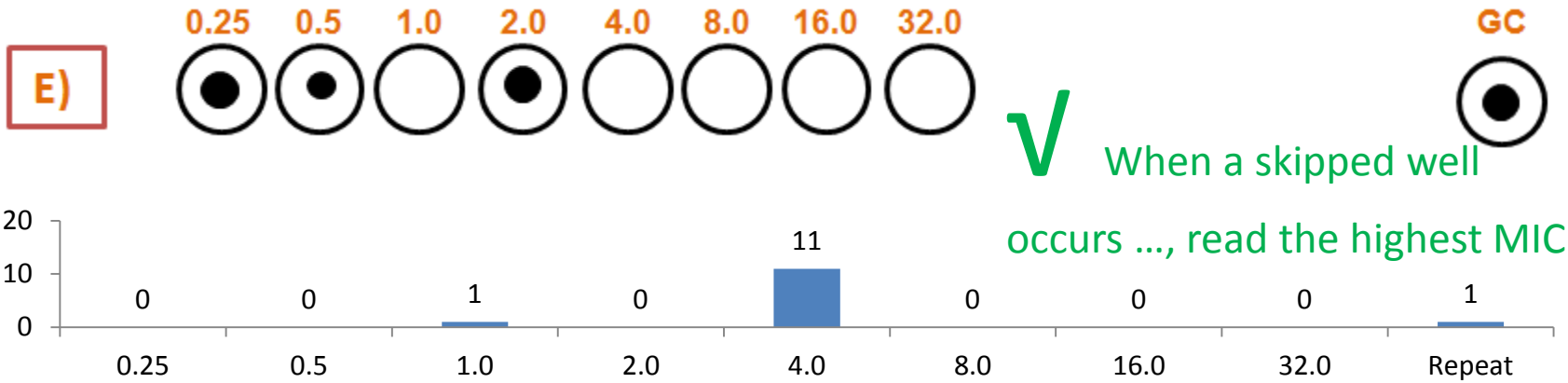


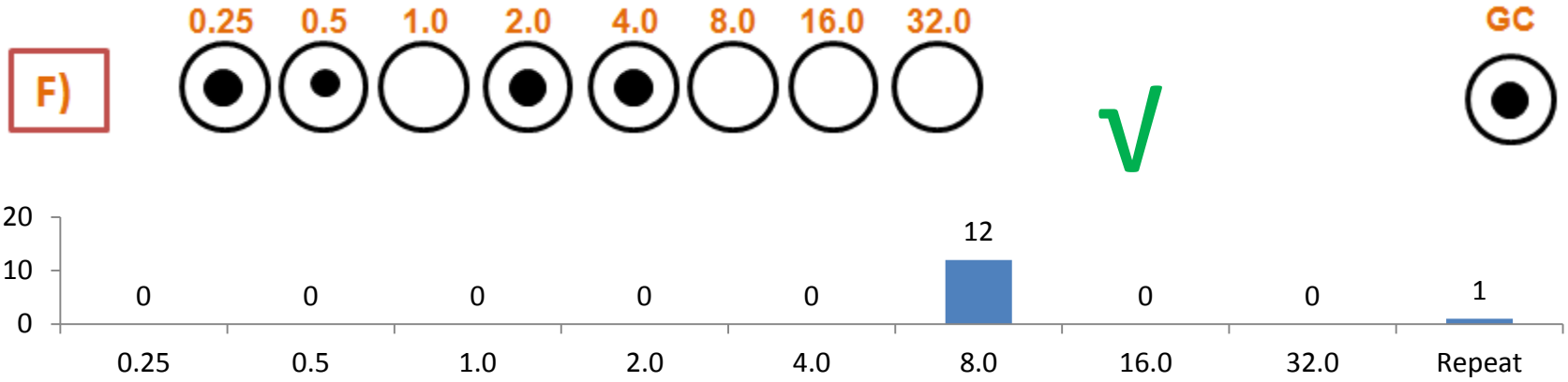


Reading & Interpreting of Endpoints for Each Antibiotic in Panel/Tray

How do you interpret growth in a dilution series that has a single skipped well, or multiple skipped wells? The 4 examples below, E-H, represent a typical broth microdilution panel with again, four (4) β-lactam class antibiotics, such as a cephalosporin, and the growth pattern from a member of the Enterobacteriaceae. A Growth Control (GC) well is on the right.



What if the drug was meropenem for either of these? "S", or "R"?



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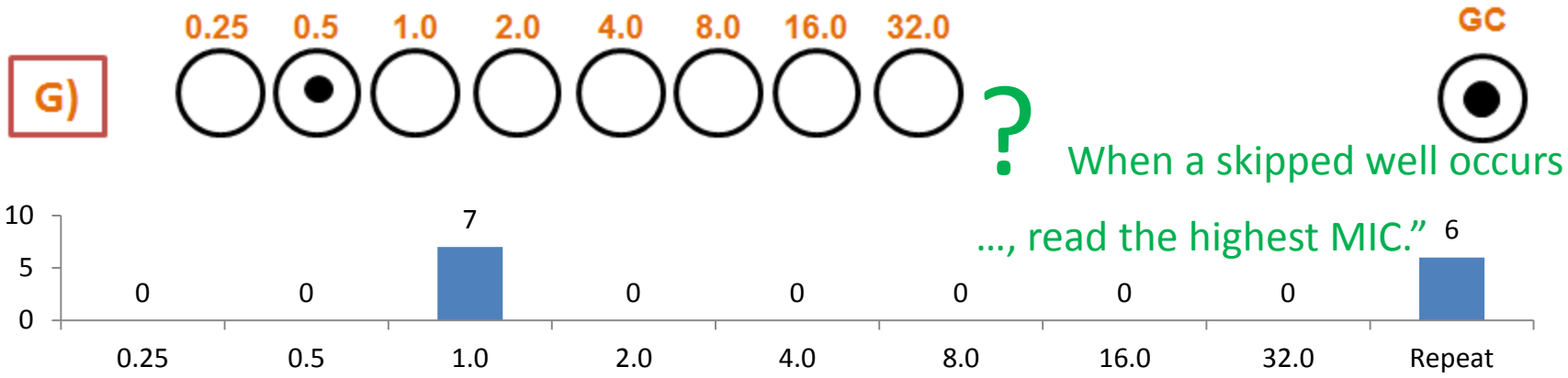
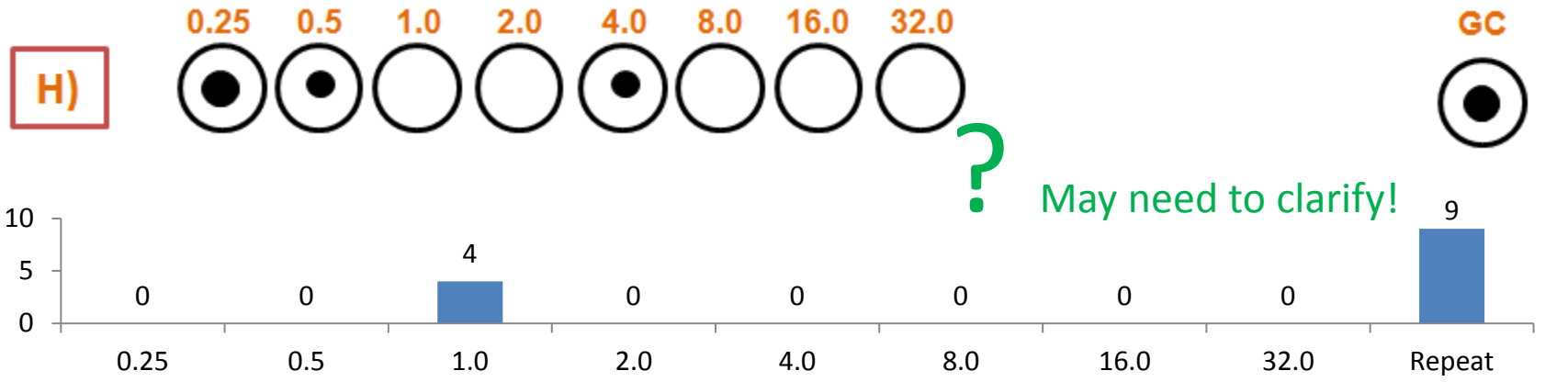




There will be Variance

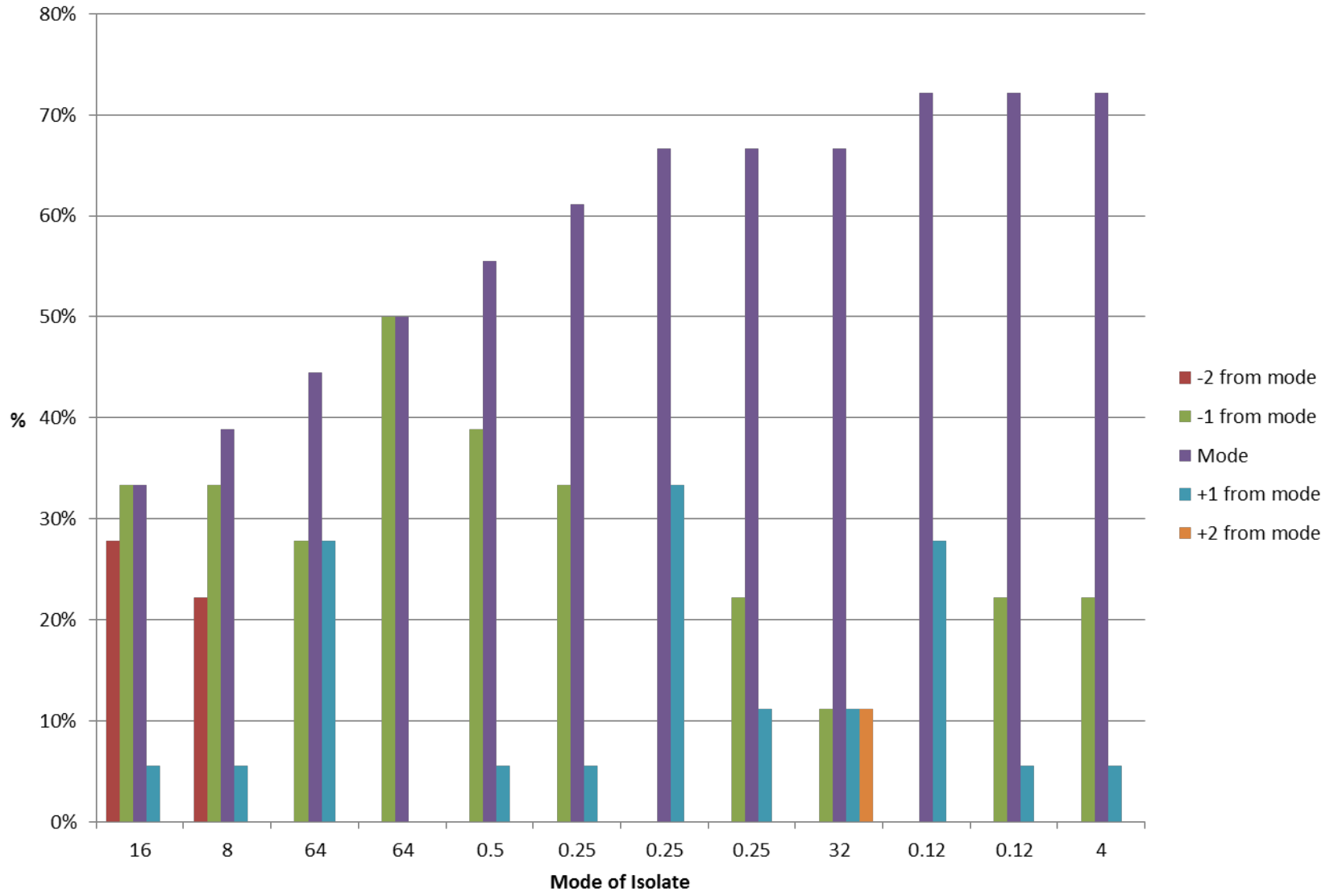
- If we better define the parameters for reference testing, there will still be some variance, as there is for any assay.
- We've seen replicate testing of the QC organisms, but what about clinical isolates?

Last June 2014

Testing with one species with one drug

E. coli Isolates, Replicate Testing, Cefotaxime

Distribution of MIC for each isolate around it's mode



Last June 2014