Equivalence Work Group Meeting Minutes

- January 8, 2020 Meeting Minutes; 2 pages
- February 12, 2020 Meeting Minutes; 2 pages
- February 26, 2020 Meeting Minutes; 14 pages
- March 11,2020 Meeting Minutes; 7 pages
- April 8, 2020 Meeting Minutes; 15 pages
- April 22, 2020 Meeting Minutes; 15 pages

Equivalence Work Group Teleconference Final Minutes

January 8, 2020; 1:00-2:00pm

Attending:

Steve Tomasino, EPA BEAD	Tony Rizzardi, Ecolab	Olivia Arends, Stepan
Rhonda Jones, EWG/SRC	John Hilgren, Ecolab	Milady Brutofsky, Lonza
Marc Carpenter, EPA BEAD	Becky Lien, SRC	Tim Curtis, Mason/Pilot
Pat Quinn, EWG	Karen Ramm, Microchem	Denise Burnside, SRC
Rebecca Pines, EPA BEAD	Dave Jones, Lonza	Dave Kang, Microbac
Rick Shimshock, ALG	Diane Falbo, SCJ	Sharon Hayden, Lonza
Matt Sathe, ALG		

Action Items (Due Date reflects actions completed as of 1/8/20):

No.	Due Date	Person	Description
1	Ongoing	Labs	Notify BEAD of any supply/equipment issues or back orders
2	Ongoing	Labs	When ordering carriers under new specification, update group with cull rate and quality of new batches
3	Ongoing	Labs	Perform Proficiency Test (PT) using two NaOCl concentrations on 3 test dates. Send raw data worksheets to BEAD after test date 1.
4	1-8-20	M. Carpenter	Send a general documentation trends list to all work group members including items discussed 11/13 (e.g. pH RT solutions, pH range, desiccant quantity)
5	1-8-20	R. Jones	Send a template to all Labs on information needed on new Chlorine Concentrate; and contact RB and Clorox to update them on the meeting.
6	1-10-20	Labs	Send email to BEAD using the template to confirm new Chlorine Concentrate testing details
7	1-15-20	S. Tomasino	Determine final soil recipe and mucin animal source for 8 Factor studies
8	1-15-20	BEAD	Add instructions to cool media/reagents to RT before pH measurement to SOP.
9	1-15-20	BEAD	Prepare Final Version of redlined SOP, 8-Factor Pseudomonas study protocol and related worksheets
10	1-15-20	S. Tomasino	Prepare task order/scheduling for MSU analysis of 8-Factor Pseudomonas data in Feb/Mar 2020.
11	1-31-20	BEAD, Lonza	Initiate 8-Factor Pseudomonas study
12	2-28-20	BEAD	Replicate Ecolab UDM work on Quat Concentrate (have not been cleared by EPA)

Meeting Summary:

- **Proficiency Testing/Readiness Update**
  - Review scorecard in the 12/18 minutes
    - Several labs are deemed proficient: several exhibiting low recovery for Pseudomonas with the low chlorine solution.
    - All paperwork is correctly completed, and no deviations were noted.
    - Media should be pH once cooled.
    - BEAD plans to hold troubleshooting meetings with individual labs
  - BEAD has shipped a single sample of reagent grade sodium hypochlorite with instruction sheet on preparation and titration.
    - Confirmed receipt: ALB, Microchem, Lonza, Ecolab
    - Review Prep Sheets sent 1/7:

- 1mL sodium hypochlorite concentrate + 306mL of OECD HW – should fall in 135-165ppm; BEAD gets near 150ppm every time.
- For titration, we are requiring all labs to conduct HACH titration the same using 10mL of above + 40mL DI = 50mL – use this for titration and multiply with 0.5 to get final ppm (Hach Manual page 90)
- Use 0.113 HACH cartridge, contact BEAD if not available.
- If you get a different value for titration, proceed to efficacy test and do not adjust the solution.
- If necessary, after testing, we will have a Chemistry Work Group to investigate/address.
- Send BEAD an email on when testing will be scheduled, confirm arrival of Concentrate, confirm use of 0.113 cartridge, and receipt of revised paperwork 1/10/20.
- New data sheets focus on 150ppm hypochlorite and controls only
- After 1st test date, send BEAD data before proceeding to 2nd test date.
- J. Hilgren asks if we are in the vertical slope of kill where outcome is more variable, and the performance criteria is set too narrowly causing the problem.
- Labs do not need to titrate the Concentrate as BEAD has conducted the analysis.

▪ Carrier Dry Time
- BEAD usually dries to the mid-point of the range 30-60mins range. Most collaborators dry for 30mins. Open to discussing a narrower range as necessary (parking lot issue). Concern is raised about changing this variable in addition to those above while balancing the need to have uniformly dry carriers. No change was made but labs are to assure dryness. It was noted the last Collaborative used 60mins. In the past, BEAD modified the range to accommodate testing of viruses.

- **8-Factor Study Update – Pseudomonas**
  - Kick off meeting call is schedule 1/9/20 (BEAD, Lonza, SRC).
  - Protocol is drafted.
  - BEAD with confirm with A. Parker approach on 1/10/20 including J. Hilgren suggestions on analysis.
  - First test date is a mock 1 rep – 1 day dry run, then followed by 6 testing days.
  - BEAD will randomize 3000ppm and 2000ppm tests.
  - Dry Time:
    - Lonza dries typically 30-45mins.
    - BEAD dries at 45-50mins for the 20uL drops
    - BEAD will start mock assay on Tuesday
  - During next meeting, discuss how to present and interpret the data. Several industry members suggest we should develop how we will analyze Collaborative data and define what is the difference in LR that is needed to adopt a change or achieve success.

- **8-Factor Study Update – Staph:** Discuss on next call

Next call: January 22 at 10amEST

Equivalence Work Group Teleconference Final Minutes

February 12, 2020; 11:00-12:00pm

Attending:

Steve Tomasino, EPA BEAD	Tony Rizzardi, Ecolab	Olivia Arends, Stepan
Rhonda Jones, EWG/SRC	John Hilgren, Ecolab	Milady Brutofsky, Lonza
Marc Carpenter, EPA BEAD	Lisa Hellickson, Ecolab	Tim Curtis, Mason/Pilot
Pat Quinn, EWG	Bao Thach, Stepan	Sharon Hayden, Lonza
Kyle Smith, RB	Rick Shimshock, ALG	

Action Items (Due Date reflects actions completed as of 2/12/20):

No.	Due Date	Person	Description
1	Ongoing	Labs	Notify BEAD of any supply/equipment issues or back orders
2	Ongoing	Labs	When ordering carriers under new specification, update group with cull rate and quality of new batches
3	Ongoing	Labs	Perform Proficiency Test (PT) using two NaOCl concentrations on 3 test dates. Send raw data worksheets to BEAD after test date 1.
4	Done	S. Tomasino	Prepare task order/scheduling for MSU analysis of 8-Factor Pseudomonas data in Feb/Mar 2020.
5	Done	BEAD, Lonza	Initiate 8-Factor Pseudomonas study
6	2-28-20	BEAD	Replicate Ecolab UDM work on Quat Concentrate (completed and under EPA review)

Meeting Summary:

- **Report from EWG on the PT-readiness exercise**
  - BEAD held teleconferences with 2 labs to assist questions on the PT.
  - BEAD requests all labs continue to collect OECD data after meeting the PT criteria to maintain proficiency. .
  - BEAD confirmed the following labs have completed the PT: Lonza, BEAD, Clorox.
  - Microchem reports they will extend to end of Feb.
  - RB reports a new *Pseudomonas* culture has pushed back schedule until end of the week with the goal to complete all work by end of Feb.
  - ALG has conducted multiple iterations of testing and is planning more R&D.
  - Chemistry issues appear resolved by requirement of specific titration cartridge, single sample of NaOCl concentrate, and standardized dilution instructions.
  - When finished, BEAD will chart the NaOCl data though some of the 2000ppm data will be missing as the focus became the 150ppm. May have MSU evaluate the data but no firm commitment at this time. The Steering Committee will have to review and decide on MSU and docket posting of this information.

- **8 Factor *Pseudomonas* Update**
  - Conducted by BEAD and Lonza
  - BEAD started in fall 2019 figuring out procedural steps to accommodate changes to inoculum volume and soil by performing different culture dilutions for the different inocula volumes. BEAD also confirmed the recovery dilutions and plating scheme. The full dry run used 12c/desiccator with 4 desiccators in total. BEAD finished last rep 2/11,

a of total 7 test days including the dry run. No repeats are needed at BEAD; Lonza has a few minor repeats. Data are being reviewed to assure raw data was transcribed into spreadsheets correctly, then it will be compiled for MSU analysis. Dr. Parker will do an outlier and quality check of data at the outset.

- Lonza shared there was trouble with dilution plating initially (e.g., plating the appropriate dilutions to achieve countable filters). They used 2 large desiccators and dried carriers for 45mins. The vacuum pump remains on during drying which should be added to SOP. The valve can be turned to hold desiccation, but Lonza found that drying takes longer if the pump is turned off.
- Next Steps
    - Once BEAD finishes peer review of data – goal to submit by March 1st.
    - MSU will perform ruggedness review – goal complete by April 1st week
    - EPA will review MSU deliverable/presentation
    - Propose a call at work group level for Dr. Parker to present
    - Work Group will make a recommendation to the Steering Committee on which modifications to adopt and if further data collection is needed
    - MSU contract ends in April which BEAD will need to address
- Media and soil change make a significant difference in outcome.
- Method looks very repeatable within each lab and control counts appear acceptable.

- **8 Factor – *S. aureus***
    - Clorox unavailable to attend meeting but taking the lead on data development.
    - BEAD is available to do work in parallel.
    - BEAD recommends dropping 20uL inoculum due to technical challenges with *Pseudomonas;* Lonza concurred with encountering technical problems with the 20µl treatment (i.e., test substance coverage).
    - Clorox plans to conduct scoping work. They plan to follow Ecolab path with *Pseudomonas* by doing UDM across a concentration range. Finalize study design during next call.
    - BEAD is planning to develop a new study protocol (multi-lab) in May to confirm the final modifications (e.g. SB, animal sera) using the revised SOP with testing on the quat and possibly additional actives.

- **Other technical questions**:
    - *Pseudomonas* pellicle management: It is important to assure harvest is consistent. BEAD recommends a single method for removal. BEAD and Lonza use different techniques. Work group prefers aspiration. Parking lot issue.
    - Desiccation Time – BEAD recommends standardization. Parking lot issue.

Next Meeting Agenda for Feb 26 @ 11amEST:

- Lonza Staph Data Presentation
- Clorox Staph Scoping Data Presentation, if available
- Discuss 8-Factor Staph Study Design
- Ecolab Data Presentation on Pseudomonas – Chlorine kill curve with OECD method

Equivalence Work Group Teleconference FINAL MINUTES

February 26, 2020; 11:00-12:15pm

Attending:

Steve Tomasino, EPA BEAD	Tony Rizzardi, Ecolab	Olivia Arends, Stepan
Rhonda Jones, EWG/SRC	John Hilgren, Ecolab	Milady Brutofsky, Lonza
Marc Carpenter, EPA BEAD	Karen Ramm, Microchem	Tim Curtis, Mason/Pilot
Pat Quinn, EWG	Dave Jones, Lonza	Denise Burnside, SRC
Rebecca Pines, EPA BEAD	Kristie Restrepo, Ecolab	Dave Kang, Microbac
Rick Shimshock, ALG	Bruce White, Ecolab	Sharon Hayden, Lonza
Matt Sathe, ALG	Kyle Smith, RB	Bao Thach, Stepan
Bill King, Clorox	Nipa Modi, Clorox	Mrudula Srikanth, Clorox

Action Items (Due Date reflects actions completed as of 2/12/20):

No.	Due Date	Person	Description
1	Ongoing	Labs	Notify BEAD of any supply/equipment issues or back orders
2	Ongoing	Labs	When ordering carriers under new specification, update group with cull rate and quality of new batches
3	Ongoing	Labs	Perform Proficiency Test (PT) using two NaOCl concentrations on 3 test dates. Send raw data worksheets to BEAD after test date 1.
4	2-28-20	BEAD	Replicate Ecolab MEC UDM work on Quat Concentrate with Pseudomonas (completed, and under review by EPA. Data consistent with Ecolab's findings.)
5	3-30-20	Labs	Replicate Ecolab MEC UDM work on Quat/Hypochlorite Concentrate with *Staph*. Lonza (preliminary data) and Clorox taking lead on data collection.
6	4-21-20	EWG	Present proposed testing plans to Steering Committee

Meeting Summary:

Lonza Presentation of *S. aureus* OECD Testing (M. Brutofsky) (attached)

- Lonza conducted testing with the revised OECD method with Quat and Hypochlorite Concentrates on *S. aureus* (ATCC 6538) using 3 carriers/product and control per combination.
    - Quat Concentrate results:
        - Showed Log Reduction (LR) increase of 2-3 with Synthetic Broth and 5% animal sera compared to TSB + OECD soil mixture.
        - This outcome aligns with their results on *P. aeruginosa*.
        - Lonza recommends consideration of 800, 1000, and 1500ppm for 8 Factor *Staph* study
    - Hypochlorite Concentrate results:
        - Showed LR increase of 4-5 with either media by using 5% animal sera over the OECD soil mixture.
        - The choice of the growth media seems to have little/no impact on LR for *S. aureus*.
    - Several Work Group members suggested that UDM Minimum Effective Concentration data was needed for *S. aureus* for reference purposes. BEAD will need to gain Steering Committee approval to conduct UDM testing.

- Work Group suggested it was useful to assure the changes proposed based on *P. aeruginosa* testing had not negatively impacted performance for *S. aureus*. BEAD suggests less data may be needed on *Staph* as we could rely on the *Pseudomonas* data if they trend together. This will be decided once data are generated.
- BEAD recommended the testing plan be presented to the Steering Committee for approval.

Ecolab Presentation on OECD - Pseudomonas Chlorine Kill Curve (K. Restrepo, Bruce White)(attached)

- Based on variability observed across the Proficiency Testing, Ecolab conducted testing to confirm the OECD hypochlorite kill curve for *P. aeruginosa*. The 150ppm solution is at a steep area of the kill curve which may contribute to variability among labs. The data was further analyzed at 95% CI to determine the performance prediction interval at 150ppm. The single lab (2 replicate) data suggested the performance range is 2.3-4.3 LR. The acceptance criterion was 0.1-3.5LR. A work group member suggested that the performance criteria applied to the Readiness exercise be re-evaluated based on the results of these experiments. Additional data sets from other labs can be added to the data for a more robust outcome.
- Work Group discussed how the PT acceptance criteria was established. The group recommended for future PT events to use a similar data collection and prediction interval design as proposed by Ecolab statisticians. The group discussed addition of all the lab data including the many staff at BEAD that participated. BEAD confirmed the historical reproducibility has been +/-1.2 logs though the controls are +/- 0.5 log and repeatability is+/- 0.8-1.0 log. The labs that are experiencing greater kill than the criteria continue to investigate and conduct additional studies. A work group member asked if there is no obvious reason for the stronger kill and the lab has repeated it, should we re-examine the acceptance criteria? R. Jones questioned if the labs that are performing below 2.3 LR should also be considered as not performing. Several members of the work group felt that perhaps the 150ppm was not an ideal concentration due to its location in a steep part of Ecolab's kill curve.
- BEAD pointed out that it is important to select concentrations that will uncover lab error and variability >1 log would not be acceptable in their lab. BEAD would investigate analyst-to-analyst variability, differences between techs, optimize recovery, time from dilution to use, etc. They plan to reduce troubleshooting exercise for labs achieving high LR results for low NaOCl concentration. BEAD proposed use of quat concentrate for PT to avoid contribution for instability of the chemistry, but the work group did not support this initiative at this time but identified it as a future project. Work group members stated the decay rate is much higher at lower concentrations of hypochlorite.
- Work Group feels it is critical to resolve this as it is important to enroll more labs for the future collaborative and other work (e.g. 8-Factor Staph, Product Survey). BEAD indicated that there are currently enough proficient labs to move forward with testing and that additional testing cannot be delayed.
- Ecolab shared that in the 2014 Collaborative at 200ppm, the results ranged from 1.1 – 4.4 LR which is similar to the presented data and the experience of many of the PT volunteer labs.

Clorox Staph Scoping Workplan: Hold for next meeting

Discuss 8-Factor Staph Study Design: Hold for next meeting

8-Factor Pseudomonas Update – BEAD is working to get data compiled and submitted to MSU for analysis. The data will not be released until after analysis. J. Hilgren requests the data be shared concurrently with the work group and/or with Bruce White (Ecolab Statistician) for evaluation. BEAD recommends the Work Group petition the Steering Committee for early data release.

Next Call: March 11, 2020 @ 11amEST

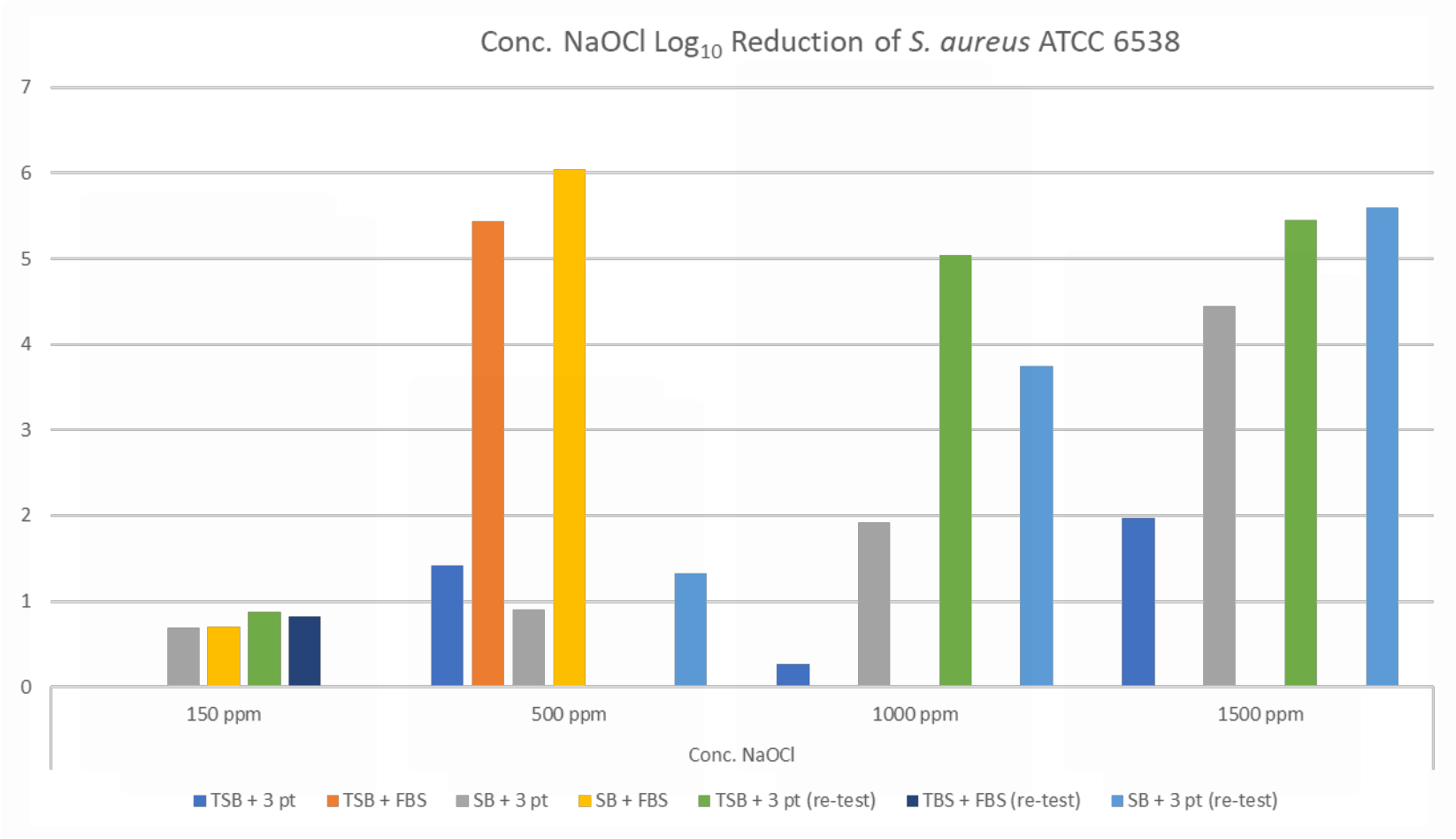
Lonza

EPA SOP MB-25-05 OECD NaOCl & Quat Concentrate *S. aureus* ATCC 6538

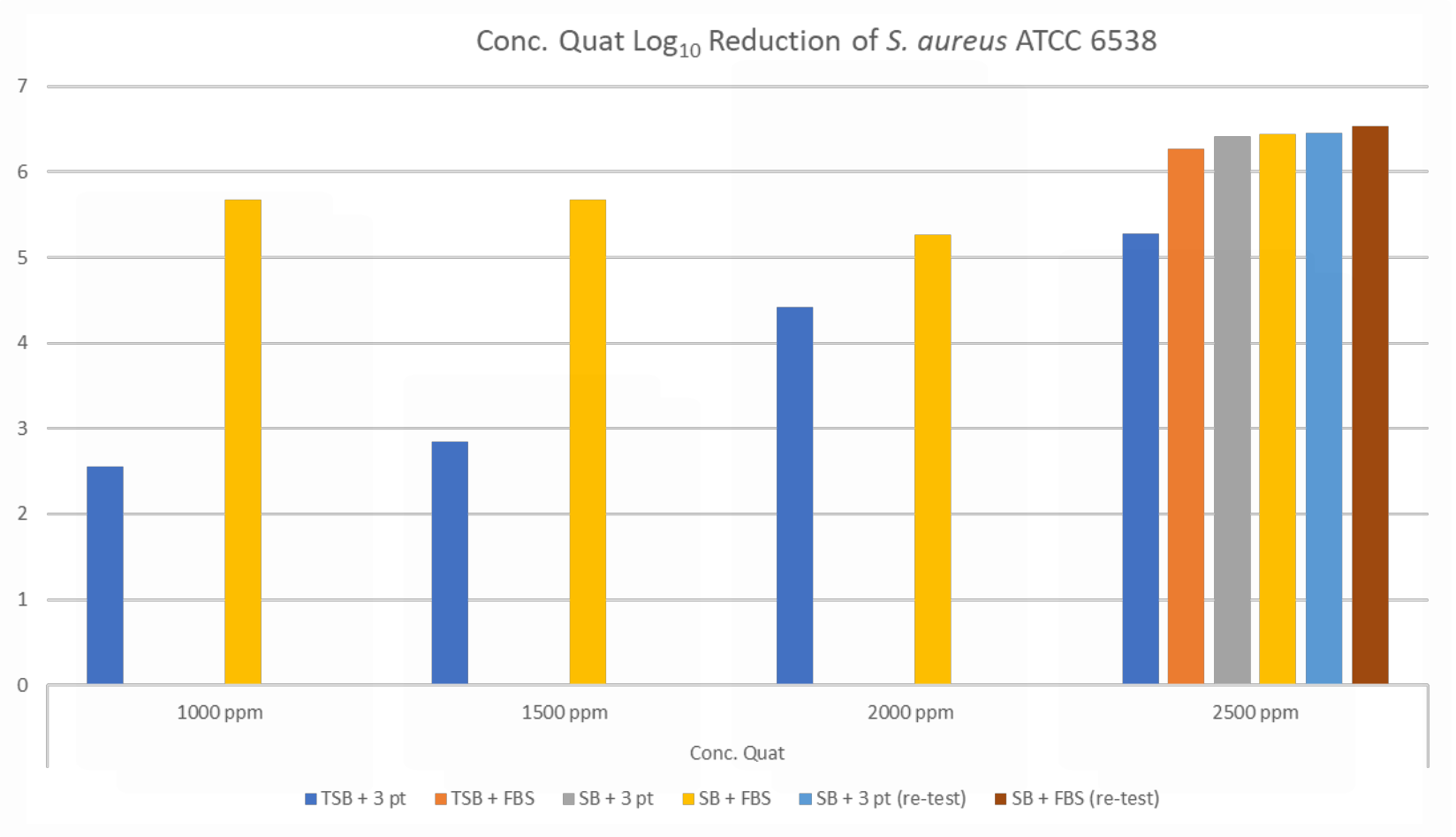
OECD NaOCl & Quat Concentrate

- Organism: *S. aureus* ATCC 6538
- Contact time: 5 minutes
- Soil:
  - OECD 3 parts soil: BSA, Mucin (Bovine), Yeast Extract
  - 5% FBS (Heat-inactivated)
- Growth Medium: TSB & SB
- Inoculum volume: 10 uL
- Dilution: OECD Hard Water 375 ppm (338-394 ppm)
- Data point represent single test dates / Re-test are labeled
- Procedure: Used latest EPA OECD Proficiency Testing for Efficacy/ Chemistry

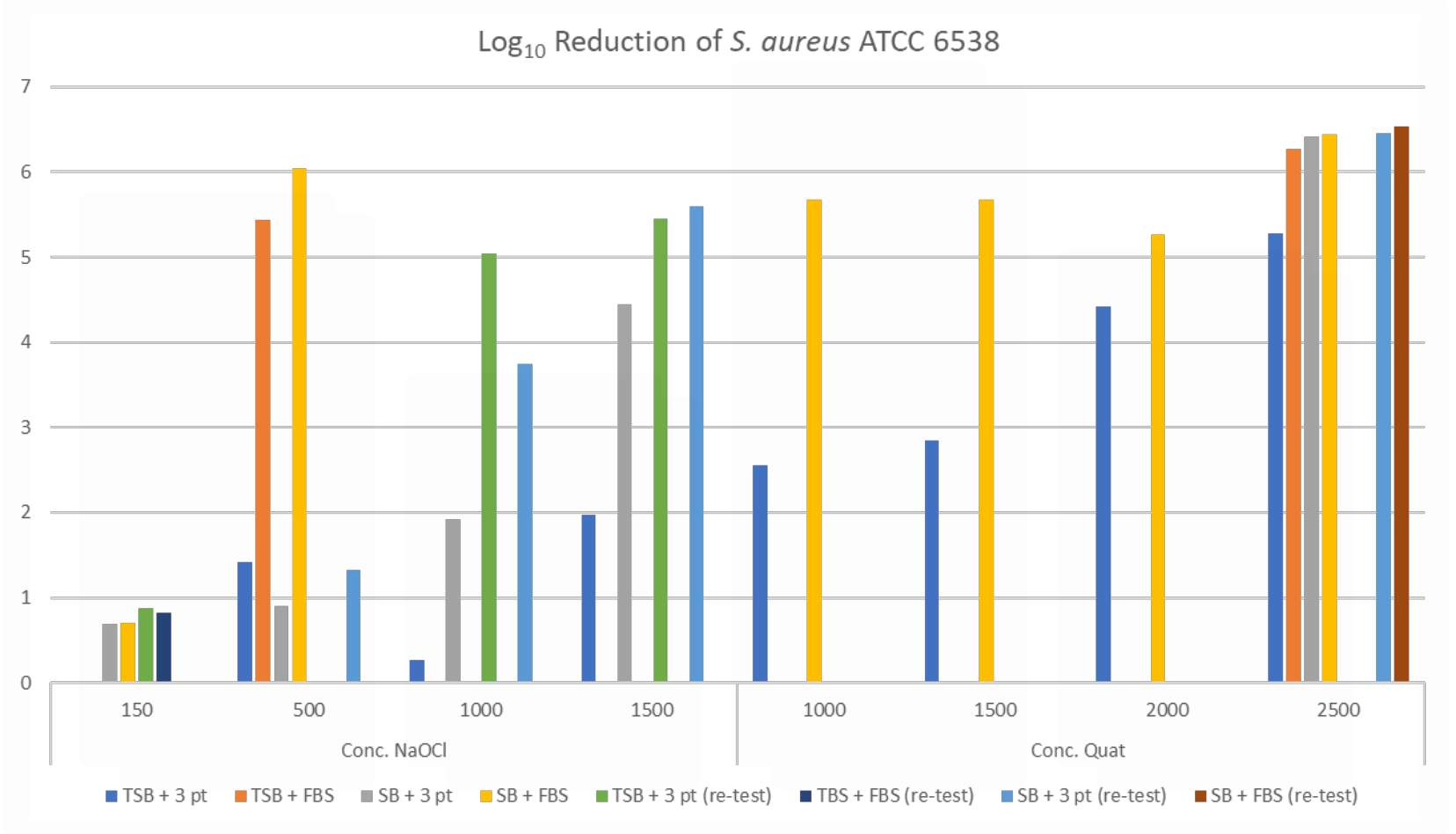
OECD NaOCl Concentrate



OECD Quat Concentrate



OECD NaOCl & Quat Concentrate



OECD NaOCl & Quat Concentrate

- Conclusion
  - Quat Conc.
    - SB + FBS restores performance to approx. 5.5 LR level
      - Increasing the overall LR by 2-3 logs as compared to TSB + OECD soil
      - Outcome aligns with the *P. aeruginosa* outcome
    - Outcomes at 2500ppm are difficult to compare due to complete kill
    - Suggests optimum concentrations for 4-Factor – *Staph* study would be 1000 possible around 800ppm?
  - NaOCl Conc.
    - Use of FBS in either media increases the LR by 4-5 logs as compared to use of OECD soil at 500ppm
    - Further testing could be completed to optimized NaOCl concentration

- Clorox planning work to run a 4-Factor study with 2 labs against *S. aureus*

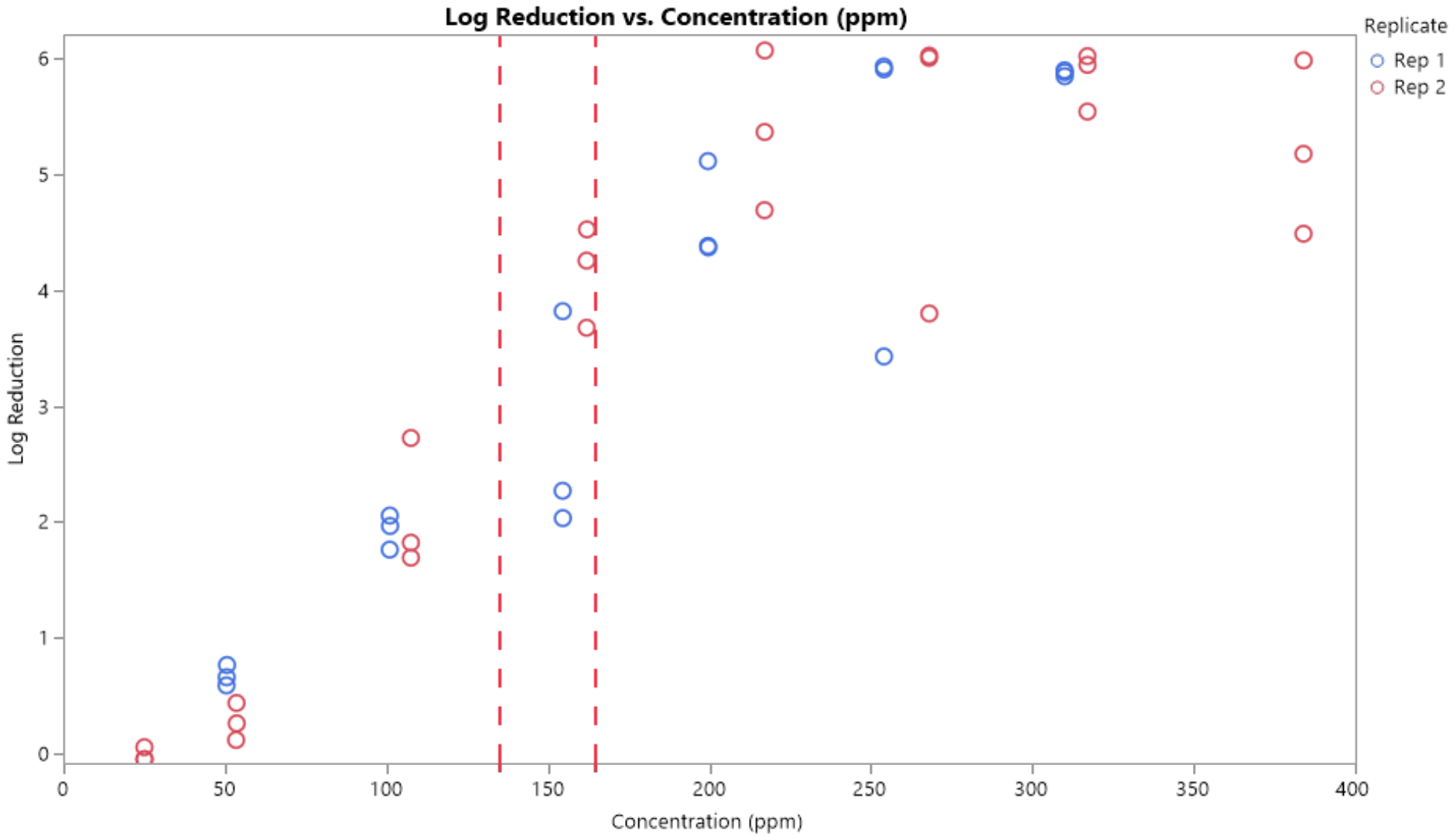
SODIUM HYPOCHLORITE KILL CURVE

Ecolab

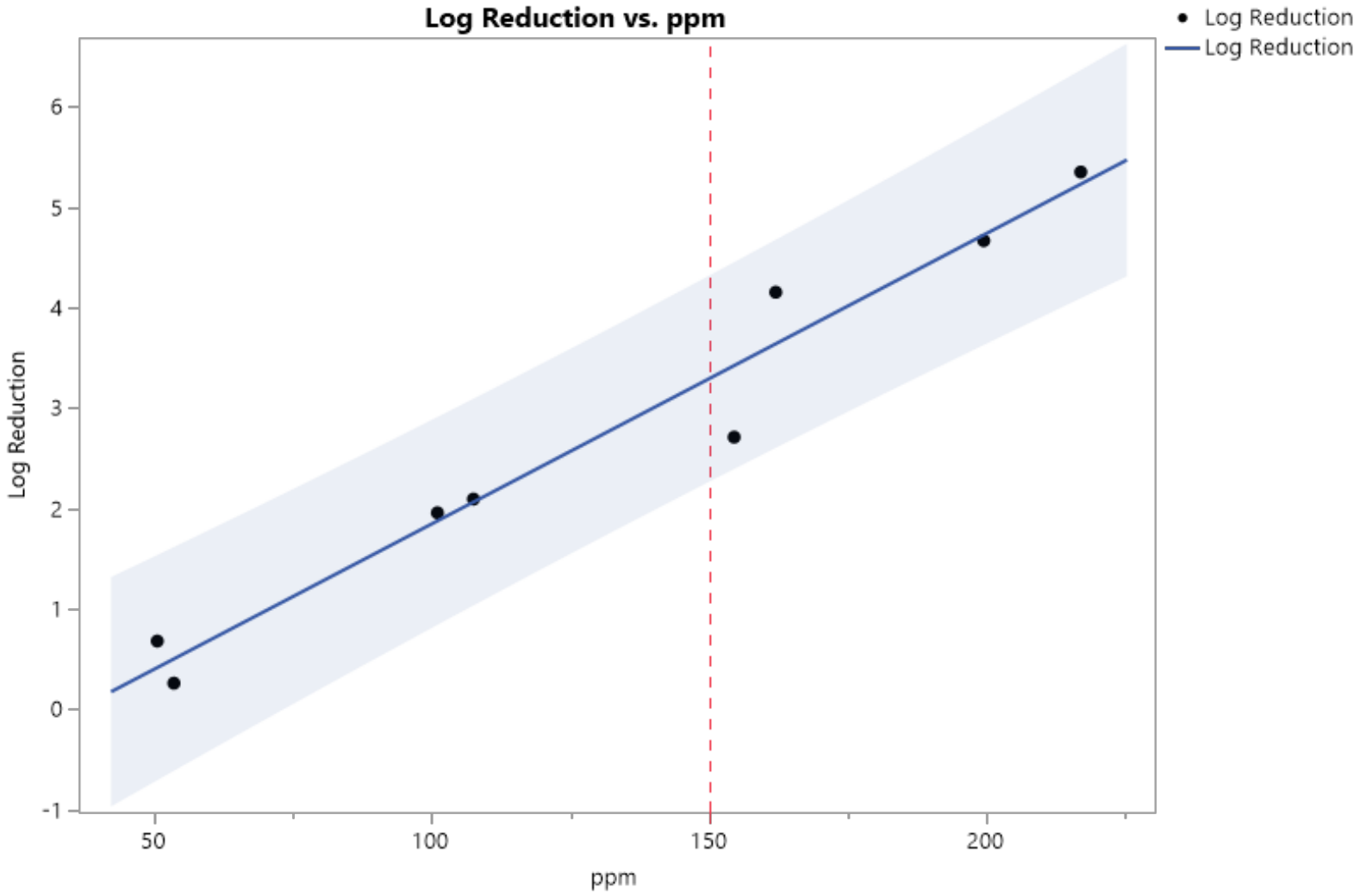
BACKGROUND

- Variability was observed in 150 ppm level of the Proficiency Testing for some of the collaborative study labs, including our own lab
- Hypothesis: The 150-ppm level is in the middle part of the curve which is leading to variability in test results.
- Performed two tests to assess the kill curve of sodium hypochlorite
- Test Conditions:
  - Target Concentrations: allowed ± 10% range around the target
    - 25 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm
  - Test substance diluent: standard hard water specified in OECD test method
  - Number of carriers tested on each test date: 3 carriers per target concentration, 3 control carriers
  - Carrier dry time: 45 minutes
  - Exposure time: 5 minutes
  - Included OECD 3-part soil load
  - 304SS Carriers

DATA


Log Reduction vs. Concentration (ppm)
Replicate
Rep 1
Rep 2
Log Reduction
0
1
2
3
4
5
6
0
50
100
150
200
250
300
350
400
Concentration (ppm)

PREDICTION INTERVAL



CONCLUSIONS

- Used prediction interval - 2.3 to 4.3 Log Reduction (preliminary values at 150 ppm)
- Can we add additional data?
- Decide on the final conclusion regarding proficiency after additional data analysis

Equivalence Work Group Teleconference Final Minutes

March 11, 2020; 11:00-12:00pm

Attending:

Steve Tomasino, EPA BEAD	Tony Rizzardi, Ecolab	Olivia Arends, Stepan
Susan Lawrence, EPA BEAD	John Hilgren, Ecolab	Bao Thach, Stepan
Marc Carpenter, EPA BEAD	Kristie Restrepo, Ecolab	Karen Ramm, Microchem
Rebecca Pines, EPA BEAD	Lisa Hellickson, Ecolab	Tim Curtis, Mason/Pilot
Pat Quinn, EWG	Dave Jones, Lonza	Denise Burnside, SRC
Rick Shimshock, ALG	Milady Brutofsky, Lonza	Bill King, Clorox
Matt Sathe, ALG	Kyle Smith, RB	Mrudula Srikanth, Clorox

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4	2-28-20	BEAD	Replicate Ecolab MEC UDM work on Quat Concentrate with Pseudomonas (complete and under review, consistent results)
5	3-30-20	Ecolab	Replicate Ecolab MEC UDM work on Quat/Hypochlorite Concentrate with *Staph.*
6	4-8-20	Work Group	Final Selection of 8-Factor Staph testing concentrations
7	4-8-20	BEAD	Seek EPA management approval to release raw data/summary of 8-Factor PA
8	4-30-20	Clorox	Conduct 8-Factor Staph scoping studies when lab reopens
9	4-21-20	EWG	Present testing plan proposal to Steering Committee

Meeting Summary:

8-Factor Staph Scoping Update

- Presentation of *Clorox S. aureus OECD QCT Screening Update* (attached)
- Clorox conducted a screening study to scope out concentrations/contact times for *S.a.* 8-factor study
    - Screening study test conditions: 3 and 5min, 800, 1000, and 1500ppm QAC
    - Concentrate, 3 test days, 10 µL inoculum, TSB with OECD soil (TSB/OECD), SB with 5% (v/v) FBS (SB/FBS)
    - Conclusions:
        - Demonstrated complete kill at 3 min for SB/FBS at 1000 ppm QAC and at 5mins at 500, 800, and 1000ppm.
        - Reasonable spread between 800 and 1000 ppm quat, and between SB/FBS and TSB/OECD at 3 mins.
        - Discontinued evaluation of 1500 ppm QAC due to lack of difference between 1000 ppm and 1500 ppm QAC.
- S. Tomasino felt that the selection of 800 ppm QAC provides a baseline LR for the current method (TSB/OECD). TSB/OECD and SB/FBS were selected because those treatments showed the largest difference in LR in the *P.a.* 8-factor study. Selection of 800

ppm QAC allows the measurement of differences between *S.a.* treatments; no more screening data are necessary.

- J. Hilgren felt that the data show effects similar to those seen with *P.a.* J. Hilgren is not aware that the UDM has been conducted to determine the equivalency between the treatments; suggests running the UDM with various QAC concentrations to determine the minimum effective concentration (MEC). Concerned about the scenario if UDM fails and OECD passes.
- S. Tomasino suggested that the EWG pursue this initiative. The Steering Committee previously agreed upon a path forward which did not include additional UDM testing. Regarding the MEC concept, generation of *S.a.* UDM data should not hold up the generation of the OECD *S.a.* data; it was agreed upon that the OECD testing would proceed.
- J. Hilgren felt that the objective of current work was to look at equivalency. There was a need to ensure there was not a change for *S.a.* as a result of the changes made for *P.a.* (e.g., unintended consequences).
- B. King indicated that Clorox's lab is closed for 2 weeks; requests availability of another lab to run the UDM. Noted that there is variability in the data for TSB/OECD.
- L. Hellickson indicated that Ecolab can perform some range finding on UDM with *S.a.*
- M. Brutofsky felt that 800 ppm QAC is the MEC for the UDM for *S.a.*
- S. Tomasino asked how many combinations of UDM testing need to be performed before agreeing on a final protocol.
- J. Hilgren felt that the EWG needs to discuss this as a group, including if there is a need to compare UDM using TSB/OECD and SB/FBS.
- M. Brutofsky confirmed that the UDM data previously generated by Ecolab for *P.a.* used cultures grown in SB and the inoculum had 5% FBS incorporated. Running the UDM using the 8-factor study parameters will be too cumbersome.
- T. Rizzardi suggested generating a kill curve with the quat for *S.a.* Clorox is willing to generate 800 ppm QAC data under the protocol before the UDM is conducted.
- S. Tomasino felt that there are enough data to select a treatment to show differences between factors; introducing the MEC complicates things. What happens if the data do not agree, do we offer a new set of factors for *S.a.*? The EWG came to EPA with a list of potential modifications and as a result, growth medium, soil load, and inoculation volume were selected. It was agreed that these limited modifications would be researched so as not to completely change the testing platform. If the 800 ppm QAC is evaluated with the UDM using SB/FBS and it does not pass, what would be the next step?
- J. Hilgren indicated that there is an assumption that there is a difference in outcome between the current OECD and the UDM. He has not seen any data to indicate that there is an equivalency problem for *S.a.* and QACs, however, he suspects that there may be.
- B. King indicted that it is unreasonable to expect *S.a.* and *P.a.* to show the same results.
- J. Hilgren expected that the MEC is different for *S.a.* and *P.a.* but felt that testing should be done to assure we have made the right changes for all chemicals.
- S. Tomasino indicated that the screening performed by Clorox is extremely important. He is not in favor of running all 8-factors. S. Tomasino and the Work Group support dropping the 20µL inoculum treatment moving forward.

- J. Hilgren indicated that he is suggesting that a lab run the UDM in advance of the *S.a.* 8-factor study.
- S. Tomasino wanted to verify the method with the sample quat for both organisms. Testing other active ingredients was discussed but not in conjunction with the UDM. Suggested having other approved laboratories verify the selected modifications, evaluate the variability, and assess the performance standards. *S.a.* is important but ancillary; it was not the top priority. Requested that the EWG make a recommendation and prepare a timeline for selection of QAC concentration for *S.a.* screen.
- S. Tomasino would like the other cleared laboratories to begin testing the modifications for *P.a.* The primary emphasis is on a change to the growth medium and EPA is generating data around that recommendation. The next few weeks will be used to conduct the data analysis and generate some *S.a.* data. Do any labs have any *S.a.* data? Labs should not feel pressured to continue screening *S.a.* until there is a comfort level with the UDM.
- B. King felt that based on S. Tomasino's proposal, *S.a.* testing does not need to slow down, however, J. Hilgren indicated that this is yet to be determined.

8-Factor Pseudomonas Update – BEAD

- S. Tomasino indicated that EPA has all the data from the *P.a.* 8-factor study. EPA will QC the data and generate summary spreadsheets for both labs. Summary must be cleared by EPA management. Plan to have data available to share with the workgroup in the next 2-4 weeks.
- J. Hilgren requested both the data summary and the raw data. Once it is complete, S. Tomasino will send the data to Pat for distribution to the workgroup.
- The workgroup agreed to suspend testing of the 20 µL inoculum volume. EPA, Clorox, Ecolab, and Lonza agreed with this decision; no laboratory disagreed.

Proficiency Testing Status Update

- Readiness: there are enough labs lined up to conduct additional *P.a.* data collection.
- K. Smith indicated that RB would conduct their second assay next week. Lab feels confident with their first test and discussed data with EPA last week.
- Other labs actively testing?
  - S. Tomasino recommended evaluating the QAC to get some baseline data.
  - ALG: evaluating the quat this week. ALG saw good preliminary data on *S.a.* using the current method (TSB/OECD) against 500 ppm NaOCl, in line with expected results.
  - Stepan: planning to test 150 ppm NaOCl and 2000 ppm quat against *P.a.*
  - Microchem: additional *P.a.* work this weekend.

Follow up discussions from 2/26 meeting

- Kill curve
  - J. Hilgren is glad that other labs are working on QAC for proficiency testing and would like to know what an acceptable range would be for PT.
  - S. Tomasino indicated that parameters for PT with the QAC are still being assessed.

THE CLOROX COMPANY

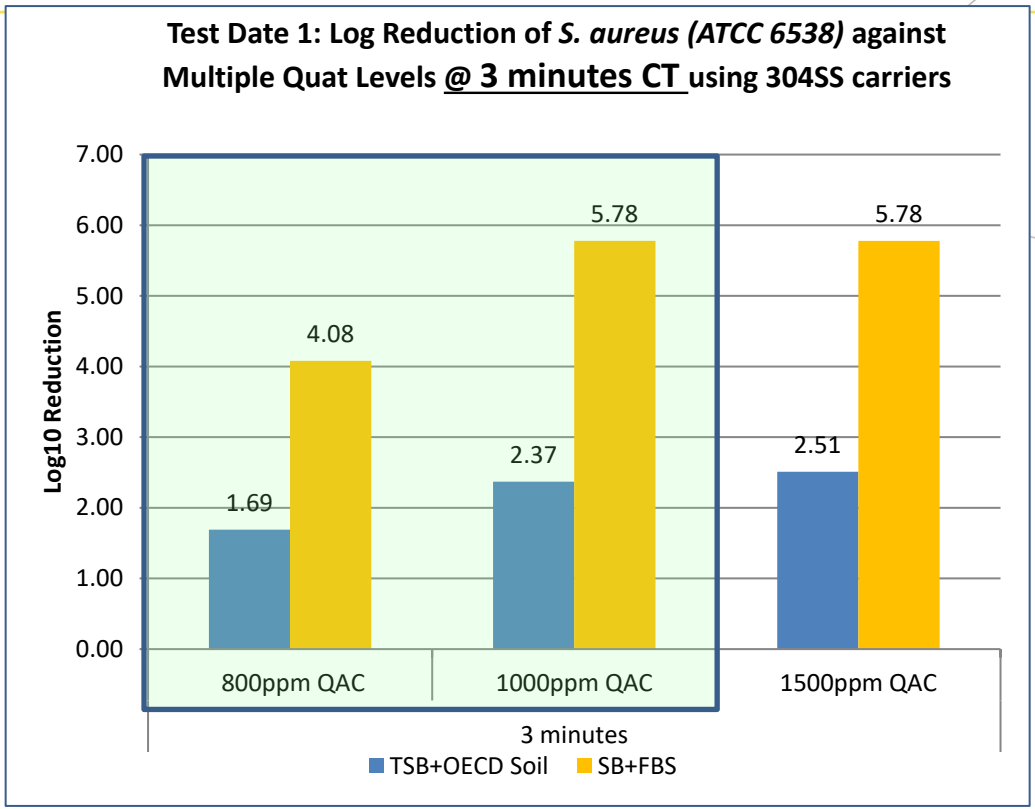
S. aureus QCT Range Finding Screening Update

Bill K and Mrudula S

EWG Bi-Weekly-OECD Technical Planning Meeting/ 03-09-20

Test Parameters

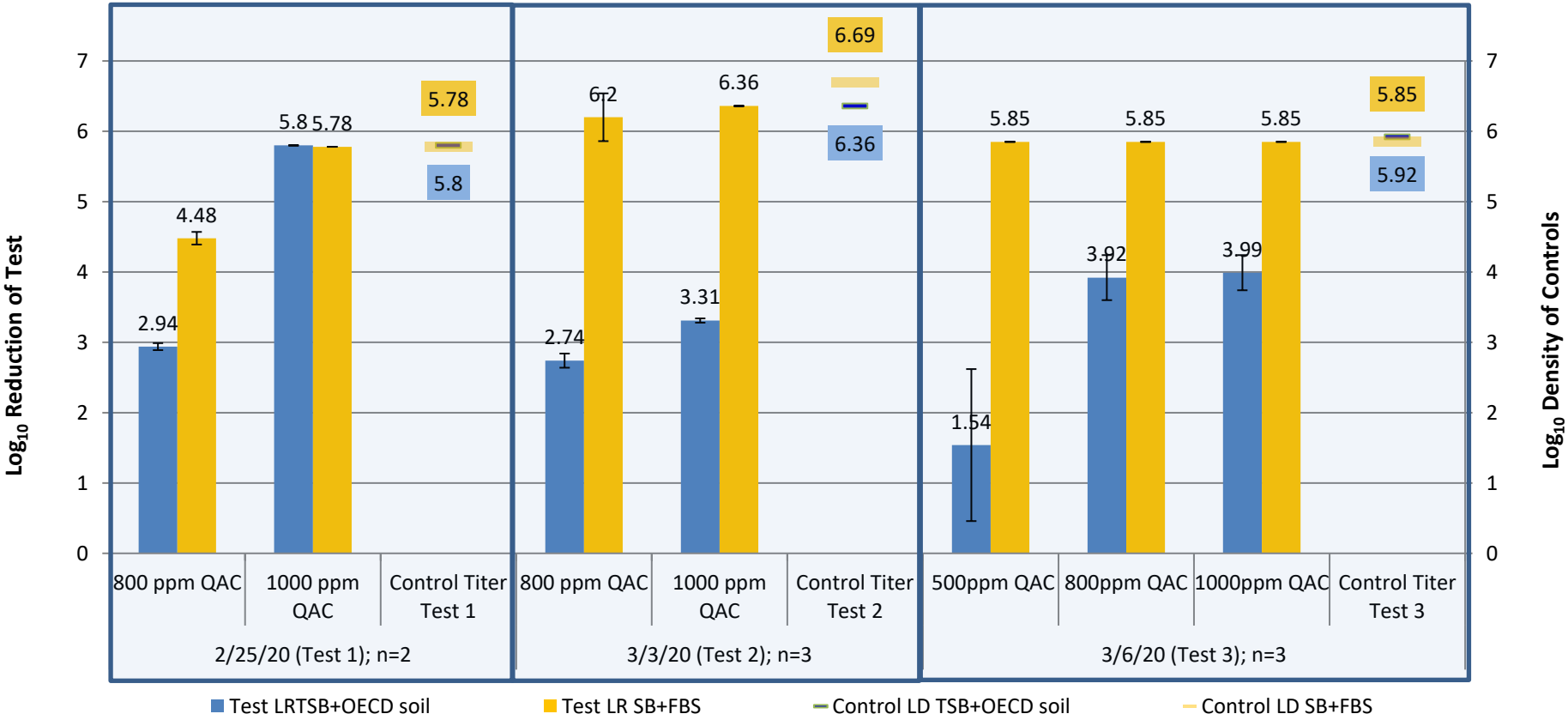
Test Parameters	
Test Organism	24+ 2hr *S. aureus* in TSB and SB
Test Quat Solutions	Test 1: 800ppm, 1000ppm and 1500ppm (prepared in 366ppm OECD HW) Test 2:800ppm and 1000ppm (prepared in ~384 ppm OECD HW) Test 3: 500ppm, 800ppm and 1000ppm (prepared in ~384 ppm OECD HW)
Contact times	Test 1: 3 minutes (see graph on right) and 5 mins (see graph on Slide 4) Tests 2 & 3: 5 mins (see graph on Slide 4)



Summary of Results - Test Dates 1, 2 & 3 (5 minutes contact time):

Days 1,2 & 3 Test Data	Concentration (ppm)	Test LR $_{TSB+OECD\ soil}$	Test LR $_{SB+FBS}$	*Sd Test* $_{TSB+OECD\ soil}$	*Sd Test* $_{SB+FBS}$	Control LD $_{TSB+OECD\ soil}$	Control LD $_{SB+FBS}$	*Sd Control* $_{TSB+OECD\ soil}$	*Sd Control* $_{SB+FBS}$
2/25/20 (Test 1); n=2	800 ppm QAC	2.94	4.48	*0.05*	*0.09*				
	1000 ppm QAC	5.8	5.78	*0*	*0*				
	Control Titer Test 1					**5.8**	**5.78**	*0.05*	*0.04*
3/3/20 (Test 2); n=3	800 ppm QAC	2.74	6.2	*0.1*	*0.34*				
	1000 ppm QAC	3.31	6.36	*0.03*	*0*				
	Control Titer Test 2					**6.36**	**6.69**	*0.06*	*0.03*
3/6/20 (Test 3); n=3	500ppm QAC	1.54	5.85	*1.08*	*0*				
	800ppm QAC	3.92	5.85	*0.32*	*0*				
	1000ppm QAC	3.99	5.85	*0.25*	*0*				
	Control Titer Test 3					**5.92**	**5.85**	*0.02*	*0.03*

Log Reduction of *S. aureus* against 800 and 1000ppm Quat (Day 1 & 2) and 500, 800 and 1000ppm (Day 3) @ 5 minutes CT



Equivalence Work Group Teleconference Final Minutes

April 8, 2020; 11:00-12:00pm

Attending:

Steve Tomasino, EPA BEAD	Tony Rizzardi, Ecolab	Olivia Arends, Stepan
Kimberly Nesci, EPA BEAD	John Hilgren, Ecolab	Bao Thach, Stepan
Marc Carpenter, EPA BEAD	Lisa Hellickson, Ecolab	Karen Ramm, Microchem
Rebecca Pines, EPA BEAD	Dave Jones, Lonza	Tim Curtis, Mason/Pilot
Pat Quinn, EWG	Milady Brutofsky, Lonza	Denise Burnside, SRC
Rhonda Jones, EWG/SRC	Sharon Hayden	Bill King, Clorox
Rick Shimshock, ALG	Diane Falbo, SCJ	David Kang, MBT
Matt Sathe, ALG		

Action Items (Due Date reflects actions completed as of 4/8/20):

No.	Due Date	Person	Description
1	Ongoing	Labs	Notify BEAD of any supply/equipment issues or back orders and any issues/cull rate when ordering new carriers
3	Ongoing	Labs	Perform Proficiency Test (PT) using two NaOCl concentrations on 3 test dates. Send raw data worksheets to BEAD after test date 1.
4	DONE	Ecolab	Replicate prior MEC UDM work on Quat/Hypochlorite Concentrate with *Staph*.
5	DONE	BEAD	Seek EPA management approval to release raw data/summary of 8-Factor PA
6	DONE	Clorox/Ecolab	Provide study outlines for upcoming testing
7	4-10-20	S. Tomasino	Distribute BEAD outline for upcoming testing/timeline
8	4-10-20	P. Quinn	Schedule work group call to prepare for Steering Committee Meeting
9	4-21-20	EWG	Present testing plan proposal to Steering Committee
10	4-22-20	Ecolab	Conduct additional Quat "failing conc." testing on OECD method
11	4-22-20	Clorox	Conduct 8-Factor Staph scoping studies
12	4-22-20	Work Group	Final Selection of 4-Factor Staph testing concentrations
13	5-1-20	BEAD	Replicate Ecolab MEC UDM work on Quat Concentrate with Pseudomonas

Meeting Summary:

8-Factor – *Pseudomonas* data presentation (https://www.regulations.gov/document?D=EPA-HQ-OPP-2018-0850-0016)

- S. Tomasino presented the 8-Factor *Pseudomonas* data generated by Lonza and BEAD to evaluate the impact of changing growth media, soil type, and inoculum volume on 2000 and 3000ppm Quat Concentrate (See Table 1 & 2). Dr. Al Parker, MSU, is currently analyzing the data. The following preliminary findings were discussed:
  - The testing demonstrated adequate control carrier counts in both labs. Some typical variability was experienced day-to-day with the bacterial counts.
  - The combinations were sensitive to active levels with 3000ppm having better performance than 2000ppm.
  - The increase in inoculum volume demonstrated little change in the reduction values though it was noted that the 20uL inoculum trials contained twice as much soil load as the 10uL which may have caused more stringency confounding those results. The OECD soil test was more impacted by this than the FBS.

- The largest log reduction change occurred with the Synthetic Broth (SB) and 5% FBS combination.
- High variability was seen between labs for TSB and FBS (Rows 3 and 4 in Table 2) testing. If these conditions are kept in the method, the work group recommended additional investigation to mitigate these reproducibility issues. The SB testing had almost no variation between labs, so the work group was excited that this may translate into a significant reduction in reproducibility of the method overall. It was felt this could be due to the firm, more easily harvested pellicle formed in SB.
- S. Tomasino agreed that the change to SB and FBS appear to move both concentrations into a passing range. He further noted that an improvement in the reproducibility noted with this combination could lead to a reduction in the performance criteria.
- The work group agreed the data trend supports adoption of SB and FBS in replace of the current method's media and soil type.

Ecolab presentation of *Staph* UDM results (*attached*) – Lisa Hellickson

- L. Hellickson, Ecolab, presented the study design (Slide 2) and results for their evaluation of 5 concentrations of the Quat Concentrate against *S. aureus* in the AOAC Use-Dilution method. The testing was conducted on one test date to aid in the selection of testing concentrations for the upcoming 4 Factor *Staph* testing and to provide the information to assess equivalency. Of the concentrations tested, the minimum effective concentration appears to be 1000ppm. It was noted that there was a very minimal difference in number of positive carriers from 500-1000ppm so Clorox plans to evaluate additional concentrations in their scoping work for the 4 Factor Staph study.
- The work group requested volunteers to repeat this testing as well as the original *Pseudomonas* testing to increase the confidence in this work and the equivalency assessments.

Follow up discussions on 4-Factor – *Staph* Testing

- B. King shared they hope to get back into the lab next week following COVID-19 quarantine to initiate the scoping testing.
  - The work group has agreed based on the Pseudomonas testing to drop the 20uL inoculum testing so the design drops from an "8 Factor" to a "4 Factor" study.
  - A study outline will be provided with the minutes for review.
  - 250, 500, 1000ppm Quat Concentrate will be tested to evaluate passing, marginal, and failing UDM performance levels.
  - One technician will conduct testing on one test date. Each treatment will have 3 carriers. Due to social distancing limitation of staff, production two crops of inoculum during the test date will be needed. The results will be evaluated for impact.
  - This scoping will be used to inform a formal protocol for the 4-Factor Staph testing as performed on multiple dates in at least 2 labs like the *Pseudomonas* testing.
- Ecolab shared their plan to conduct additional *Pseudomonas* work in the OECD method on a failing Quat Concentrate to assure correlation of failing outcomes between methods and with the new changes. They will provide a study outline to distribute with the minutes. They plan to initiate testing on April 10th.
- The next call agenda will include time for presentation of the Clorox and Ecolab data.

Update on PT/Readiness Status

- BEAD provided the table below to summarize the status of the 9 volunteer labs to complete testing. Five labs are now complete. RB provided an update via email that they had finished one test date successfully and will continue to complete the work as soon as local conditions allowed.
- S. Tomasino encouraged completed labs to run additional testing now on the quat to replicate the recent data from the 8-Factor study to keep skills current and add to the data set.

Lab	Readiness
1	In-progress
2	Completed
3	Completed
4	Completed
5	No data
6	Complete
7	Completed
8	In-progress
9	In-progress

Preparation for April 21st Steering Committee Meeting

- R. Jones shared the agenda was discussed by a smaller group including A. Lowit, K. Nesci, R. Jones, P. Quinn, and J. Hilgren which included goals, objectives, path to completion of the project, and need for a proposal/timeline of the remaining tasks.
- S. Tomasino requested a subgroup of the Committee get together to prepare work group recommendations to outline the test modifications that should be down selected, additional testing needed, timing of final SOP revision, and sequence to prepare for the final collaborative.
- R. Jones recommends a larger evaluation of quat formulations, other actives, and other product forms before industry can confirm these are the only changes needed to achieve equivalence.
- S. Tomasino also recommends a multi lab data collection to verify the SB and FBS data and measure reproducibility using 2000, 3000 and a level that fails UDM. He proposes a 3-4 lab collection.
- R. Jones stated we need to determine the testing list, optimum order and items can be done concurrently. S. Tomasino would like to forecast the resource allocation over the next few quarters for this effort.
- S. Tomasino will coordinate a review and distribute the revised, draft Agenda for the 4/21 SC meeting, as well as advising where BEAD is able to participate.
- P. Quinn will schedule the meeting prior to April 21st Steering Committee and distribute to the work group.
- S. Tomasino will work with Al Parker at Montana State to assist Al with the presentation on preliminary findings during the Steering Committee meeting.

Next Meeting: April 22, 2020 at 11amEST

Method Modification Screening Study

Preliminary Data Summary – Do Not Cite or Quote

(03/20/2020)

Purpose:

In order to address technical concerns expressed by industry regarding the Quantitative Method, the Equivalency Workgroup and Steering Committee recommended the evaluation of specific method modifications to improve alignment with existing methods. A study was conducted to screen the proposed modifications to determine their impact on log reduction (LR) of *Pseudomonas aeruginosa* when tested against a quaternary ammonium compound (QAC). This document provides a basic summary of the findings. Additional analysis of the data will be required to determine statistical significance of the modifications on LR. It is anticipated that these data will be used to support additional verification testing.

Study Attributes:

- The study was conducted in a standardized fashion by two laboratories (EPA - Lab 1 and Lonza - Lab 2) per a study protocol accepted by the Equivalency Workgroup.
- EPA's Draft Standard Operating Procedure (SOP) MB-25-05; OECD Quantitative Method for Evaluating Bactericidal and Mycobactericidal Activity of Microbicides Used on Hard, Non-porous Surfaces (version date 08/06/19) was followed for data collection.
- *P. aeruginosa* was the test microbe.
- Two concentrations (2000 ppm and 3000 ppm) of a QAC sample supplied by industry were evaluated as the antimicrobial treatments.
    - The diluent for the QAC treatments was OECD 375 ppm hard water
- The contact time was 5 minutes.
- Physically screened and cleaned 304 stainless steel carriers were used per the revised carrier quality specifications provided by the OECD Carrier Quality Workgroup. The number of carriers used per treatment was adjusted for the purpose of the study.
- Control carrier counts and LR were the main test variables.
- A mean control carrier count level of 5.0-6.0 logs Colony Forming Units (CFU)/carrier for each of the eight treatments was required for a valid data set.
- See Table 1 for experimental aspects evaluated under this study; modified aspects of the current method are identified.

Table 1. Experimental Aspects

Aspect	Current Method	Proposed Modification
Growth medium	Tryptic Soy Broth (TSB)	Synthetic Broth (SB)
Soil type	Three-part OECD Soil	5% Fetal Bovine Serum (FBS)
Carrier inoculation volume	10 µL	20 µL

- See Table 2 for a list of eight method treatments and the number of carriers tested.
    - A randomized/factorial study design was employed.
    - Each of the eight method treatments were tested on the same day.
    - Three replications were conducted by each lab.

Table 2. Treatments for the Method Modification Screening Study			
Method Treatment (growth medium/soil type/inoculation volume)	**Antimicrobial Treatment** (number of carriers)		**Control Carriers** (number of carriers)
	QAC (2000 ppm)	QAC (3000 ppm)	
1. TSB and OECD soil and 10 µL*	2	2	2
2. TSB and OECD soil and 20 µL	2	2	2
3. TSB and FBS and 10 µL	2	2	2
4. TSB and FBS and 20 µL	2	2	2
5. SB and OECD soil and 10 µL	2	2	2
6. SB and FBS soil and 10 µL	2	2	2
7. SB and OECD soil and 20 µL	2	2	2
8. SB and FBS and 20 µL	2	2	2
Total carriers:	16	16	16

*current method in SOP MB-25-05

General Observations:

- Control Carrier Counts – both labs achieved adequate control carrier counts (5.0-6.0 logs CFU/carrier) across the eight treatments.
- Mean LR values with standard deviation error bars are provided in Figure 1 (Lab 1) and Figure 2 (Lab 2).
- Overall, the 3000 ppm treatment exhibited slightly higher LR values compared to the 2000 ppm treatment.
- Across labs and QAC treatments, the mean LR values for the *current method* (TSB/OECD soil/10 µL) were less than 2; the change to 20 µL did not increase the LR values.
- Across labs and QAC treatments, a consistent change (increase) in LR values was shown for the **synthetic broth (SB)** and **fetal bovine serum (FBS)** combination with LR values of approximately 5.0.
- Greatest variability in results between labs was observed for the TSB and FBS combination.

Next Steps:

- The data have been forwarded to Montana State University for statistical analysis (see Appendix 1 – 2020 Method Modification Screening Study Data for *P. aeruginosa*).
- The data and the analysis will be provided to the Equivalency Workgroup and Steering Committee.
- It is anticipated that the Equivalency Workgroup will provide recommendations on next steps (e.g., down-select modifications for collaborative testing) to the Steering Committee.

Figure 1. Lab 1 Data: Log reduction (LR) of *Pseudomonas aeruginosa* when Tested against 2000 ppm and 3000 ppm QAC

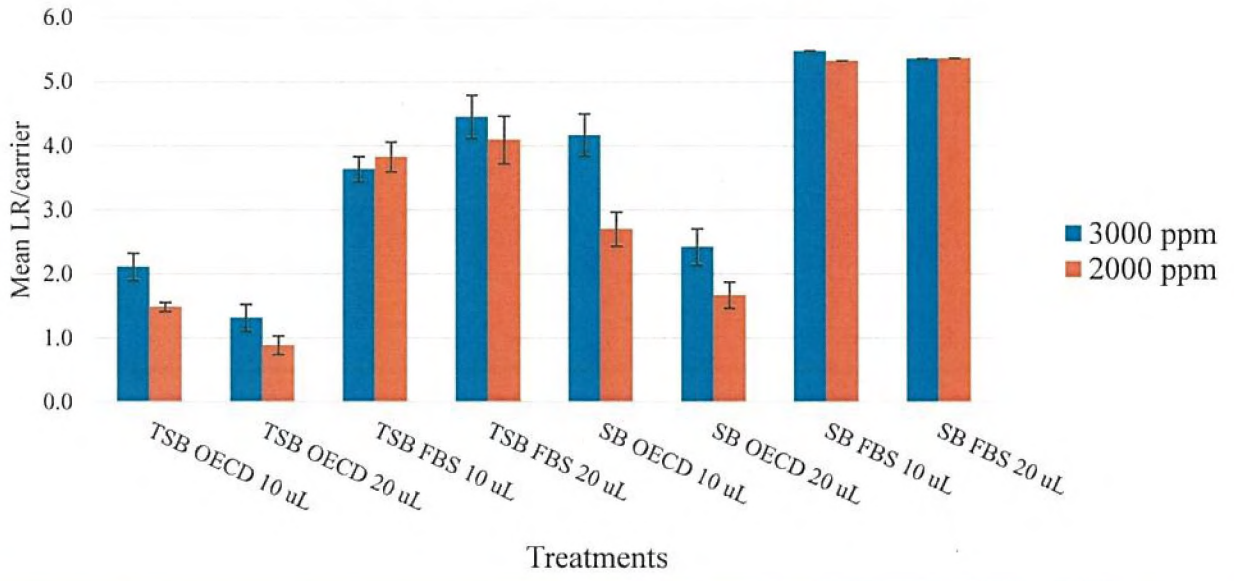
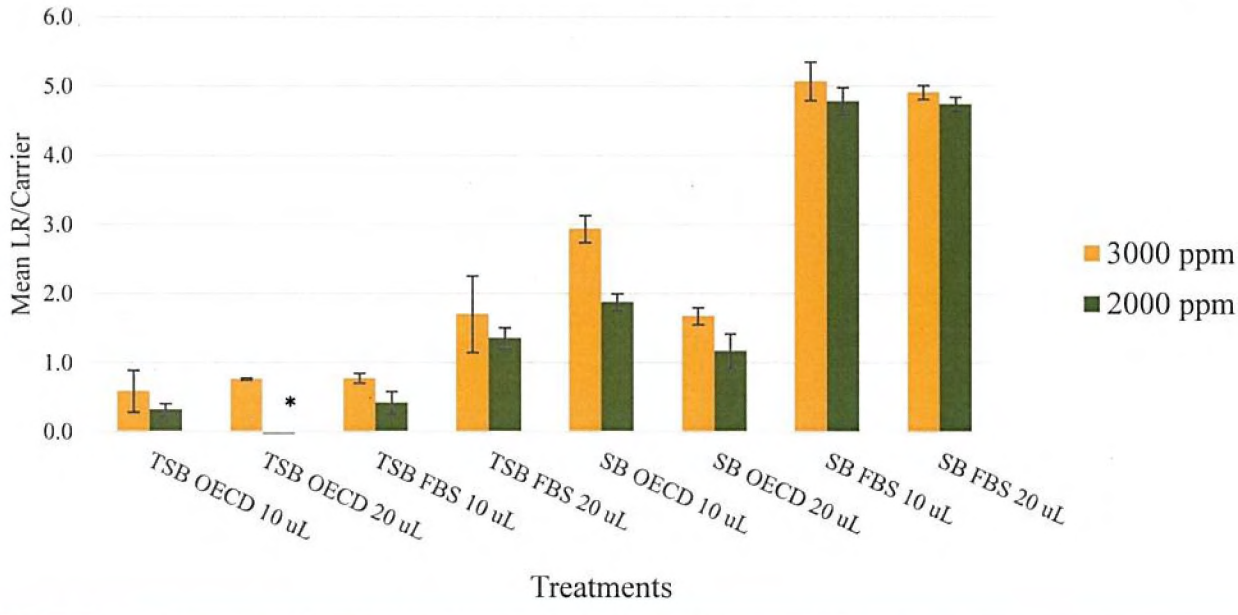


Figure 2. Lab 2 Data: Log reduction (LR) of *Pseudomonas aeruginosa* when Tested against 2000 and 3000 ppm QAC



*No log reduction observed for the TSB/OECD/20 µL treatment at 2000 ppm QAC.

QUAT UDM RESULTS

Josh Luedtke & Lisa Hellickson

TEST CONDITIONS

- Goal: Determine the Minimum Efficacy Concentration (MEC) of Quat against *Staphylococcus aureus* using the OECD Bactericidal Test.
- Test: Use Dilution Method
- Test Organism: *Staphylococcus aureus* ATCC 6538
- Product Diluent: 375 ppm AOAC Hard Water
- Concentrations Tested: 100 ppm, 250 ppm, 500 ppm, 750 ppm, 1000 ppm quat
- Soil Load: 5% Fetal Bovine Serum
- Exposure Temperature: 20 ± 1°C
- Exposure Time: 5 minutes
- All concentrations tested in one test date

UDM RESULTS

Concentration	Test Organism	Number of positive tubes/Number of Carriers	Pass/Fail
100 ppm quat	*Staphylococcus aureus* ATCC 6538	38/60	Fail
250 ppm quat		26/60	Fail
500 ppm quat		6/60	Fail
750 ppm quat		5/60	Fail
Suggested MEC → 1000 ppm quat		3/60	Pass

Note: Carrier Enumeration Control Mean Log Density = 6.34 log10 CFU/carrier

Step 1: Use UDM to scope Quat Concentrations (Ecolab):

- **Identify the minimum effective concentration of QAC against S. aureus (ATCC 6538) using the UDM**
  - 5 mins, 5% FBS, 375 ppm AOAC hard water
  - Do this by testing multiple concentrations using 10-tube tests
  - Not sure what concentrations to start with
    - S. aureus should be easier to kill than Pseudo, so maybe try:
      - 400, 600, 800, 1000, 1200 ppm?
  - In the end, you want a table that looks like this (see next slide) – with

Example step 1 results

QAC concentration	UDM Result # Neg Tubes	OECD Result Log Reduction
400 ppm	3/10 (fail)	
600 ppm	5/60 (fail)	
800 ppm	9/10 (pass)	
1000 ppm	10/10 (pass)	
1200 ppm	10/10 (pass)	

MEC = 800 ppm

Step 1 is finding range of concentrations that span from strong fail to strong pass that can be used in Step 2 OECD testing

Step 2: OECD testing of quat concentrations using same times & concentrations and book end combos of soil and broth (Clorox)

- Run OECD using same or overlapping range of concentrations as used in the Step 1 UDM scoping.
  - 5 mins, 3-part OECD soil, 375 ppm OECD hard water
  - Select best and worst case combinations of medium & soil

Example step 2 results

QAC concentration	UDM Result # Neg Tubes	OECD Result Log Reduction
400 ppm	39/60 (fail)	<1.55 (fail)
600 ppm	50/60 (fail)	1.71 (fail)
800 ppm	58/60 (pass)	1.80 (fail)
1000 ppm	59/60 (pass)	2.59 (fail)
1200 ppm	60/60 (pass)	4.5 (pass)

It will be interesting to see if the OECD method predicts the same MEC, or if it is drastically different (like we observed with Pseudomonas)

Step 3: Run 4*3 or 4*2 Factor OECD with Staph aureus (Clorox)

QAC concentration	Ecolab UDM Result # Neg Tubes	Clorox OECD Results Log Reduction			
		TSB/FBS	SYN/FBS	TSB/3PS	SYN/3PS
100 ppm	22/60 (fail)	NA			
250 ppm	34/60 (fail)				
500 ppm	54/60 (fail)				
750 ppm	55/60 (fail)	NA			
1000 ppm	57/60 (Pass)				

Clorox OECD/SA-v1.0:

- 3 Quat concentrations: 250, 500, & 1000 ppm
- 4 Factors per day
- 1 quat conc per day
- 3 reps per data point
- Potential completion: ~April 20, 2020

UDM: Staph & Quat (5 minutes, 5% FBS, 375 ppm AOAC hard water)

Benefits of this strategy

- Ensures

Equivalence Work Group Teleconference Final Minutes

April 22, 2020; 11:00-12:00pm

Attending:

Steve Tomasino, EPA BEAD	John Hilgren, Ecolab	Olivia Arends, Stepan
Kimberly Nesci, EPA BEAD	Lisa Hellickson, Ecolab	Karen Ramm, Microchem
Marc Carpenter, EPA BEAD	Dave Jones, Lonza	Tim Curtis, Mason/Pilot
Rebecca Pines, EPA BEAD	Milady Brutofsky, Lonza	Denise Burnside, SRC
Pat Quinn, EWG	Diane Falbo, SCJ	Bill King, Clorox
Rhonda Jones, EWG/SRC	Hunter Brigman, Microchem	Mrudula Srikanth, Clorox
Rick Shimshock, ALG	Becky Lien, SRC	Kyle Smith, RB
Matt Sathe, ALG		

Action Items (Due Date reflects actions completed as of 4/22/20):

No.	Due Date	Person	Description
1	Ongoing	Labs	Notify BEAD of any supply/equipment issues or back orders and any issues/cull rate when ordering new carriers
3	Ongoing	Labs	Perform Proficiency Test (PT) using two NaOCl concentrations on 3 test dates. Send raw data worksheets to BEAD after test date 1.
4	4-21-20	Done	Present testing plan proposal to Steering Committee
5	4-22-20	Done	Conduct additional Quat "failing conc." testing on OECD method
6	4-22-20	Done	Conduct 4-Factor Staph scoping studies
7	5-20-20	B.King/S. Tomasino	Draft a study design for Staph testing on UDM and modified OECD method to generate additional data between labs and with other AI
8	5-20-20	M. Brutofsky	Check for historical UDM data on Staph
9	When approved	BEAD	Replicate Ecolab MEC UDM work on Quat Concentrate with Pseudomonas (completed; aligns with Ecolab; await mgmt. approval to share)

Meeting Summary:

Pseudomonas: **OECD & UDM Consolidated Equivalency Data** (Presentation attached)

- L. Hellickson (Ecolab) presented new OECD and UDM test results (one day) for several lower Quat Concentrate levels. The failing UDM performance of these levels was mirrored in the modified OECD test method (Synthetic Broth and FBS). A composite table of all data generated to date on *Pseudomonas* was presented showing pass/fail equivalence between the UDM method and the modified OECD method.
- In Slide 2, the error was "0" so no error bars appear on the graph. S. Tomasino noted this is predictable and it is good to see the modified OECD method is sensitive to changes in concentration. He further shared BEAD had replicated the UDM testing of the 1500ppm – 3500ppm Quat Concentrate and the data aligned with the Ecolab data. This data and other small studies with *Pseudomonas* will be shared once approved for release.
- S. Tomasino expressed an idea that pellicle and its management may be a contributing factor to method variability, and perhaps it's more important to the TSB cultures versus SB-based cultures. He asked L. Hellickson for her observations regarding pellicle. L. Hellickson stated she removed the pellicle through aspiration and the Synthetic Broth (SB) pellicle is more cohesive

and easily removed as compared to the TSB pellicle, which is less tightly associated, larger, and more fragile.

***Staphylococcus*: 4-Factor – Staphylococcus Scoping/Next Steps** (Presentation attached)

- B. King (Clorox) presented their 4-Factor study on *Staphylococcus* using the Quat Concentrate alongside work by Ecolab and Lonza. Due to COVID restrictions, the study was separated across three test dates so three test cultures were used, but each value was conducted on one test date using 3 carriers. Additional replication is needed within and between labs to confirm the outcome.
- The data suggests the growth media has less impact on the reduction outcome than the soil load. The OECD soil represented a greater challenge (lower LR) than FBS. The UDM results ranged from 6 positive carriers for 500 ppm, 5 positive carriers for 750 ppm, and 3 positive carriers for 1000 ppm; however, using the modified OECD (SB + FBS and TSB+FBS) demonstrated a higher level of LR.
- Though additional test replication is needed, the group will have to determine how to address or resolve this discrepancy (i.e., has method stringency been reduced for *S. aureus*). Clorox plans additional testing as conditions allow. Lonza also plans to conduct testing.
- J. Hilgren stated the need to check the proposed changes on the required organisms and with other active ingredients (AI). R. Jones concurred.
- R. Pines questioned how this will be addressed if additional testing confirms the difference (related to apparent reduced method stringency when using SB with 5% FBS with S. aureus). R. Jones suggested reproducibility may play a role with determining equivalence in the 500-1000 ppm concentrations as we saw with the Proficiency Testing of the low chlorine level (a range of 0.5-4 log reduction was seen across the test labs). No additional suggestions were offered.
- S. Tomasino shares this may not be a method issue but an artifact trying to compare qualitative and quantitative methods. We need additional data and to take into account the variation between labs. We need to keep method modifications to a minimum – to those the group selected – and we may have to accept some differences as long as the method is adequate for protecting public health
- The work group agreed B. King will draft a study design narrowing to key concentrations for UDM and modified OECD testing. Pending approval, S. Tomasino will review, then the plan will be discussed at the next meeting so participants can go forward to generate additional data. M. Brutofsky will check to see if they have historical UDM data at these concentrations.

Steering Committee Meeting Report was postponed due to time until the May 20th meeting.

Review Draft OECD Project Plan

- J. Hilgren (Ecolab) presented a draft project plan from our current status through implementation of the modified OECD method for regulatory use. The chart is sorted in three sections to illustrate the proposed parties responsible for the lab work across the project. The next Steering Committee meeting is proposed for late June/early July.
- Additional review and discussion of the plan was not possible due to time. It was noted more discussion is needed on the PT testing and the possibility of switching to the quat Concentrate to judge proficiency and to qualify more labs to aid in the testing. The group will discuss this in

more detail on May 20th call. K. Nesci stated that BEAD and AD will need to be involved in implementation.

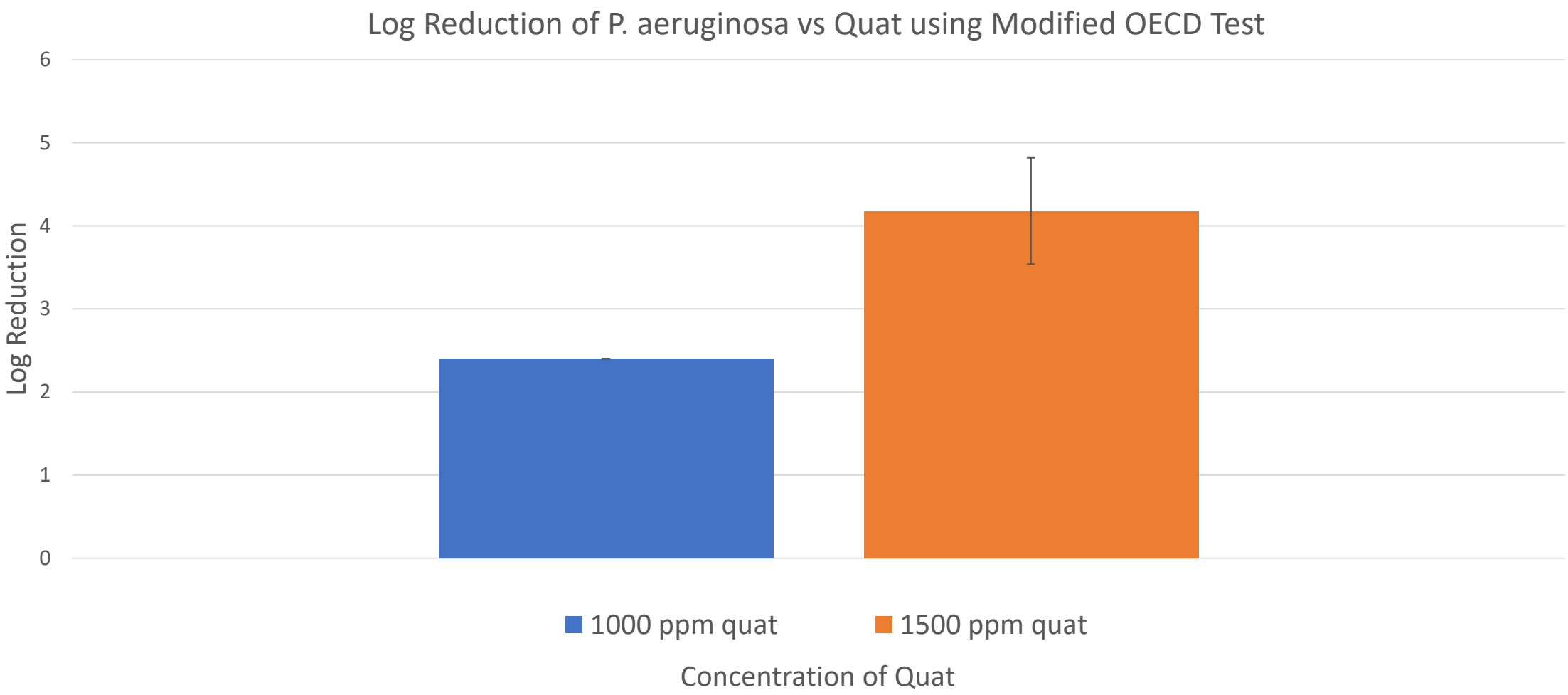
Next Meeting: May 20, 2020 at 11amEST

Test Conditions

- Determine if concentrations of quat that failed a Use-Dilution Test will also fail a revised OECD Bactericidal Test when tested with *Pseudomonas aeruginosa*.

Test Parameter	Proposed Test Condition
Test method	OECD Bactericidal Test with modifications for growth media and soil
Growth media for test culture	Synthetic Broth
Test System	Pseudomonas aeruginosa ATCC 15442
Quat Concentrate	MB-50
Concentrations of Quat	1000 ppm quat and 1500 ppm quat
Diluent	375 ppm OECD Hard Water
Soil Load	5% Fetal Bovine Serum
Exposure Time	5 minutes
Exposure Temp	Room Temperature
Carrier Material	304 Stainless Steel Carriers
Number of Carriers per Test Condition and Controls	3

Modified OECD Test Results



Pseudomonas data overview (5 min exposure, 375 ppm hard water)

All data generated by Ecolab except where noted

	SB, FBS	TSB, 3PS, 10 µL	SB, FBS, 10 µL
Quat Concentration	**UDM Result** # Neg Tubes/Total Tubes	**Current OECD Result** Log Reduction	***Modified* OECD Result** Log Reduction
800 ppm	7/10	--	--
1000 ppm	--	--	2.40 ("fail")
1200 ppm	8/10	--	--
1500 ppm	52/60 (fail)	<1.55 ("fail")	4.18 ("fail")
2000 ppm	55/60 (pass)	1.71 ("fail")	5.33 Lab 1 ("pass") 4.78 Lab 2
2500 ppm	56/60 (pass)	1.80 ("fail")	--
3000 ppm	58/60 (pass)	2.59 ("fail")	5.48 Lab 1 ("pass") 5.07 Lab 2
3500 ppm	58/60 (pass)	3.99 ("fail")	--

New Ecolab data

BEAD & Lonza data

Comparison of UDM vs OECD for Staph aureus on Quats

A summary of Ecolab, Lonza, & Clorox data

Bill King, Mrudula Srikanth, Lisa Hellickson,
John Hilgren, Milady Brutofsky

(EWG Work Group Meeting - April 22, 2020)

Initial work plan to determine Minimum effective quat concentrations for Staph aureus on UDM :

- **Identify the minimum effective concentration of QAC against S. aureus (ATCC 6538) using the UDM**
  - 5 mins, 5% FBS, 375 ppm AOAC hard water
  - Do this by testing multiple concentrations using 10-tube tests
  - Not sure what concentrations to start with
    - S. aureus should be easier to kill than Pseudo, so maybe try:
      - 400, 600, 800, 1000, 1200 ppm?
  - In the end, you want a table that looks like this (see next slide)

1. Preliminary Range Finding (Ecolab)

QAC concentration	UDM Result # Neg Tubes	OECD Result Log Reduction
400 ppm	3/10 (fail)	
600 ppm	5/60 (fail)	
800 ppm	9/10 (pass)	57/60 Staph
1000 ppm	10/10 (pass)	
1200 ppm	10/10 (pass)	

MEC = 800 ppm

Step 1 is finding range of concentrations that span from strong fail to strong pass that can be used in Step 2 OECD testing

2. Initial UDM vs. OECD comparison (Ecolab)

QAC concentration	UDM Result # Neg Tubes	OECD Result Log Reduction
400 ppm	39/60 (fail)	<1.55 (fail)
600 ppm	50/60 (fail)	1.71 (fail)
800 ppm	58/60 (pass)	1.80 (fail)
1000 ppm	59/60 (pass)	2.59 (fail)
1200 ppm	60/60 (pass)	4.5 (pass)

It will be interesting to see if the OECD method predicts the same MEC, or if it is drastically different (like we observed with Pseudomonas)

3. Full UDM vs OECD 4 Factor Comparisons (Ecolab & Clorox)

QAC concentration	Ecolab UDM Result SB/FBS	Clorox OECD Results Log Reduction			
		TSB/FBS	SB/FBS	TSB/3PS	SB/3PS
100 ppm	22/60 (fail)	NA			
250 ppm	34/60 (fail)	4.82	5.99	1.23	<2.44
500 ppm	54/60 (fail)	5.62	6.08	2.31	3.47
750 ppm	55/60 (fail)	NA			
1000 ppm	57/60 (Pass)	5.59	5.87	3.71	3.51

Modified OECD Staph Results

- 3 Quat concentrations: 250, 500, & 1000 ppm
- 4 Treatments per day
- 1 quat conc per day
- 3 carriers per tmt
- April 20, 2020

UDM: Staph & Quat (5 minutes, TSB, 5% FBS, 375 ppm AOAC hard water)

3 Lab UDM vs OECD results and next steps (5 min exposure, 375 ppm hard water)

		SB, FBS	TSB, 3PS	TSB, FBS	SB, FBS	SB, 3PS
QAC concentration	**UDM** # Neg Tubes/Total Tubes	**Current OECD** Log Reduction	**Modified OECD #1** Log Reduction	**Modified OECD #2** Log Reduction	**Modified OECD #3** Log Reduction
100 ppm	22/60 (fail)	--	--	--	--
250 ppm	34/60 (fail)	1.23 (Clorox)	4.82 (Clorox)	5.99 (Clorox)	<2.44 (Clorox)
500 ppm	54/60 (fail)	2.31 (Clorox)	5.62 (Clorox)	6.08 (Clorox)	3.47 (Clorox)
750 ppm	55/60 (fail)	--	--	--	--
1000 ppm	57/60 (Pass)	3.71 (Clorox) 2.55 (Lonza)	5.59 (Clorox)	5.87 (Clorox) 5.68 (Lonza)	3.51 (Clorox)
1500 ppm	--	2.85 (Lonza)	--	5.68 (Lonza)	--
2000 ppm	--	4.42 (Lonza)	--	5.26 (Lonza)	--
2500 ppm	--	5.28 (Lonza)	6.27 (Lonza)	6.44 (Lonza)	--

Ecolab UDM data

Observations:

- Modifying OECD using FBS and/or SB increased log reductions
- Clorox data was generated on 3 carriers on 1 test date. Additional replication is needed to confirm outcome.

Minimum effective concentration for Pseudo was 2000 ppm

UDM vs modified OECD Results – Staph aureus

Initial findings with limited data (One Test Date – 3 carriers) for 4 factorial OECD vs UDM in the Pass/Fail grey zone:

1. Standard UDM (SB/FBS) vs Standard OECD (TSB/3PS) methods:
   - OECD fails Staph @ 1000ppm
   - UDM passes Staph @ 1000 ppm.

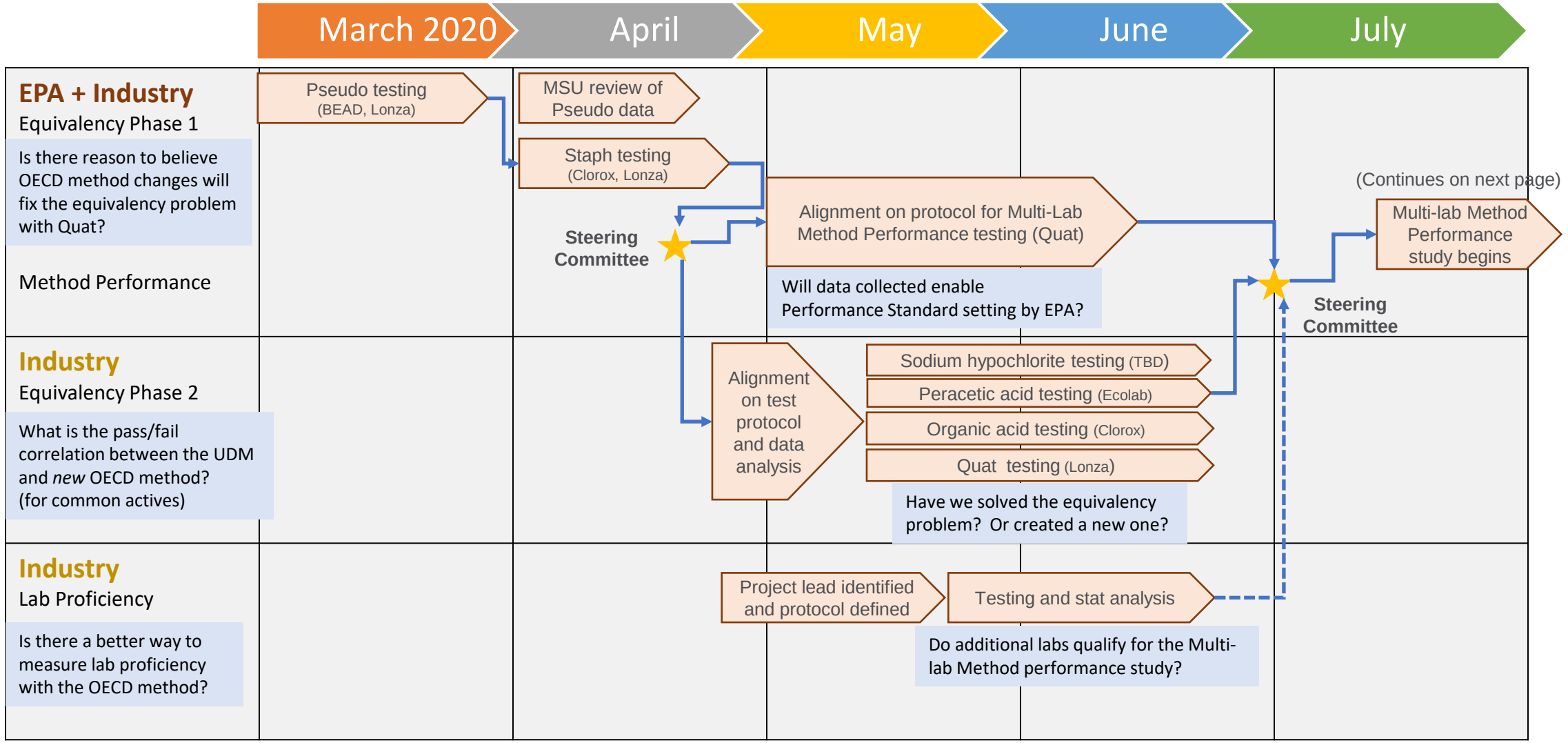
2. Responsiveness & equivalence at different quat levels:
   - Sig difference in UDM outcomes going from 250 to 500ppm
   - Insignificant UDM differences for 500, 750, & 1000ppm
   - All OECD carriers with FBS pass @ 250, 500, & 1000 ppm

3. Effects of 3PS vs FBS on OECD outcomes:
   - Does FBS lower the bar for Staph vs standard OECD with 3PS?
   - SB correlates with slightly higher log reductions than TSB – more data required

4. Discuss next steps in Staph testing???

More testing is needed to confirm these outcomes! Volunteers?

DRAFT project plan (page 1 of 2)



Draft project plan (page 2 of 2)