OECD Quantitative Method for Evaluating Mycobactericidal Activity of Microbicides

Review of the 2013 Collaborative Study

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Background

- In 2013, 6 labs completed a method performance evaluation of the OECD method using *Mycobacterium terrae*
- Labs followed EPA SOP-MB-25-01, based on the OECD test guidelines dated 08/05/11
- A log reduction (LR) of 4 has been proposed as the quantitative performance standard for tuberculocides
  - Target range for the untreated control counts: 4.5-5.5 logs

Terminology

- Control LD: $\log_{10}$ of the total CFUs on a control carrier
- *TestLD*: mean of the 4 Control LDs enumerated per test day
- LR: difference between the *TestLD* and the mean of the $\log_{10}$ CFUs for the 3 treated carriers enumerated on the same test day
- S_r: repeatability standard deviation
- S_R: reproducibility standard deviation

Test Conditions and Treatments

- Test microbe: *Mycobacterium terrae*
  - ATCC # 15755, strain W-45
- Standard test parameters
  - 5 minute contact time
  - 375 ppm hard water as the diluent
  - 3-part soil load added to the inoculum
- Treatments
  - Sodium hypochlorite (reference standard)
  - Quaternary ammonium compounds
  - Phenol
  - Citric acid
  - Hydrogen peroxide/peroxyacetic acid

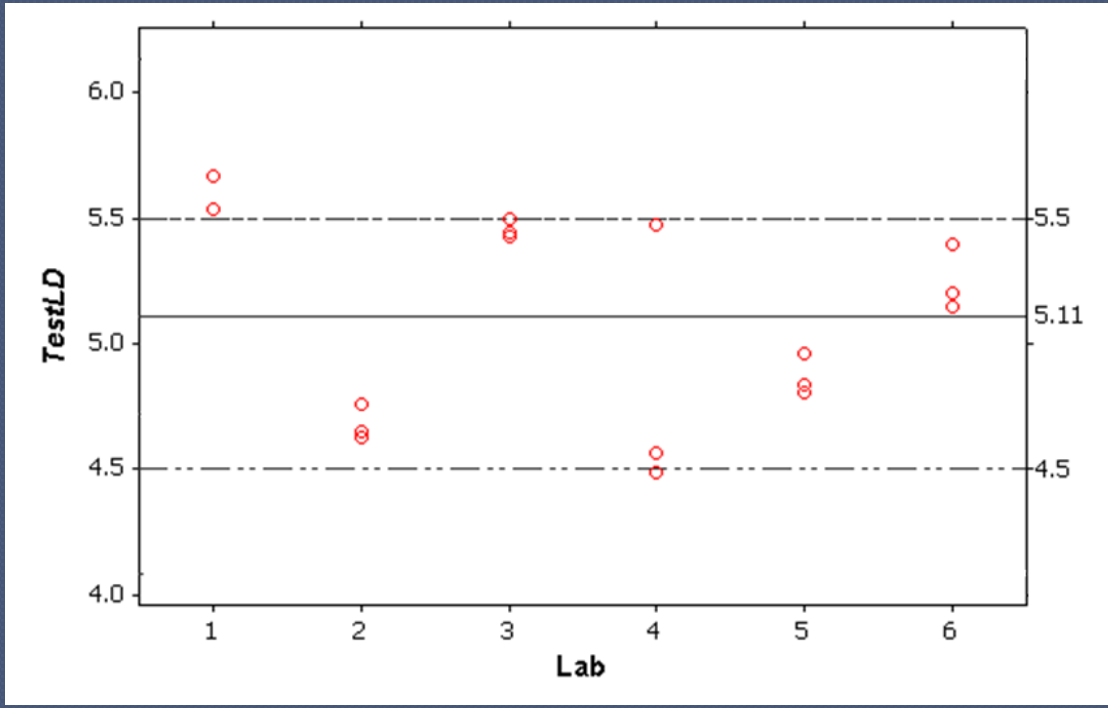
Collaborative Study Plan

- Step 1: Conducting the OECD method using one reference standard at two levels of presumed efficacy
  - 500 ppm and 2,000 ppm NaOCl
- Step 2: Conducting the neutralization confirmation assay
  - Each lab evaluated one test chemical
- Step 3: Conducting the main OECD method performance evaluation
  - 1 concentrated product, 3 ready to use products

Step 1: Reference Standard

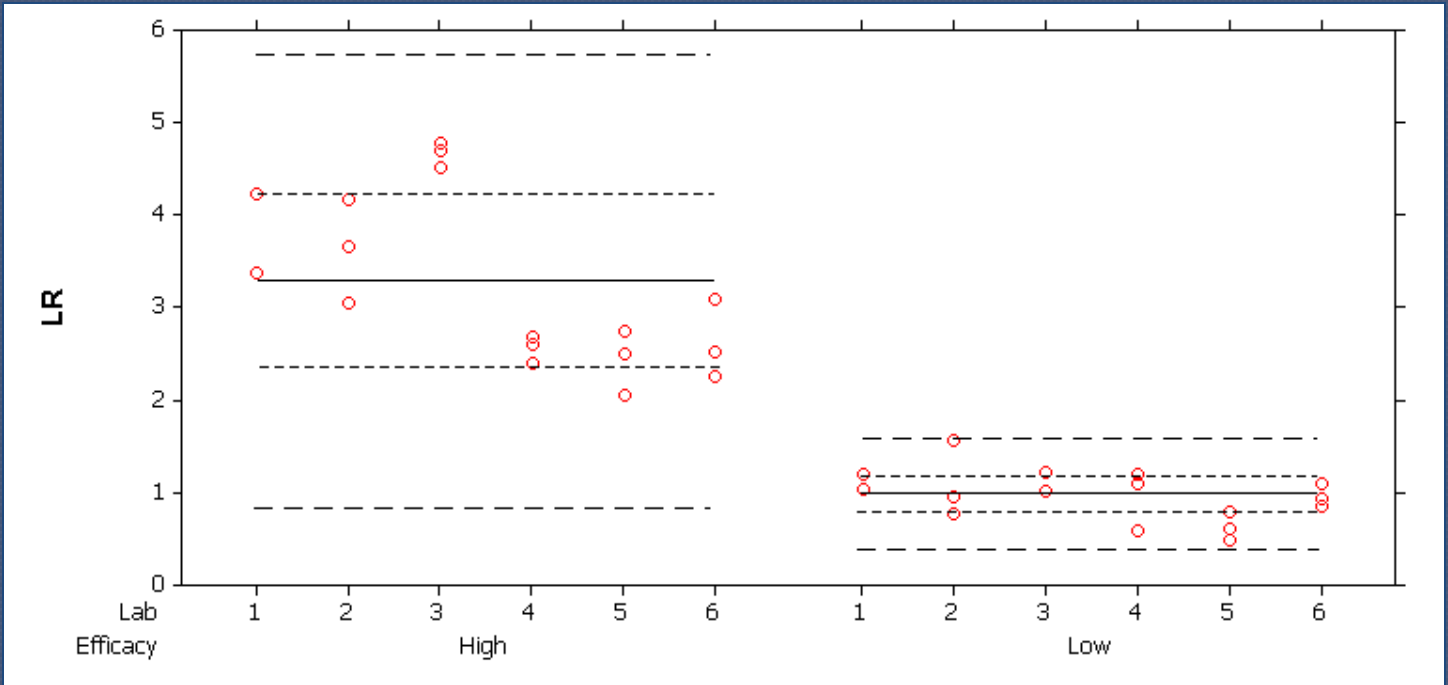
- 2 efficacy levels for sodium hypochlorite (at presumed high and low efficacy levels)
  - High efficacy level = 2,000 ppm
  - Low efficacy level = 500 ppm

Reference Standard – TestLDs



- Each point is the *TestLD* = mean of the 4 Control LDs from a single test.
- Solid horizontal line indicates the mean *TestLD* of 5.11.
- Dashed lines at 4.5 and 5.5, the limits of validity for *TestLD* specified in the protocol.

Reference Standard – TestLRs



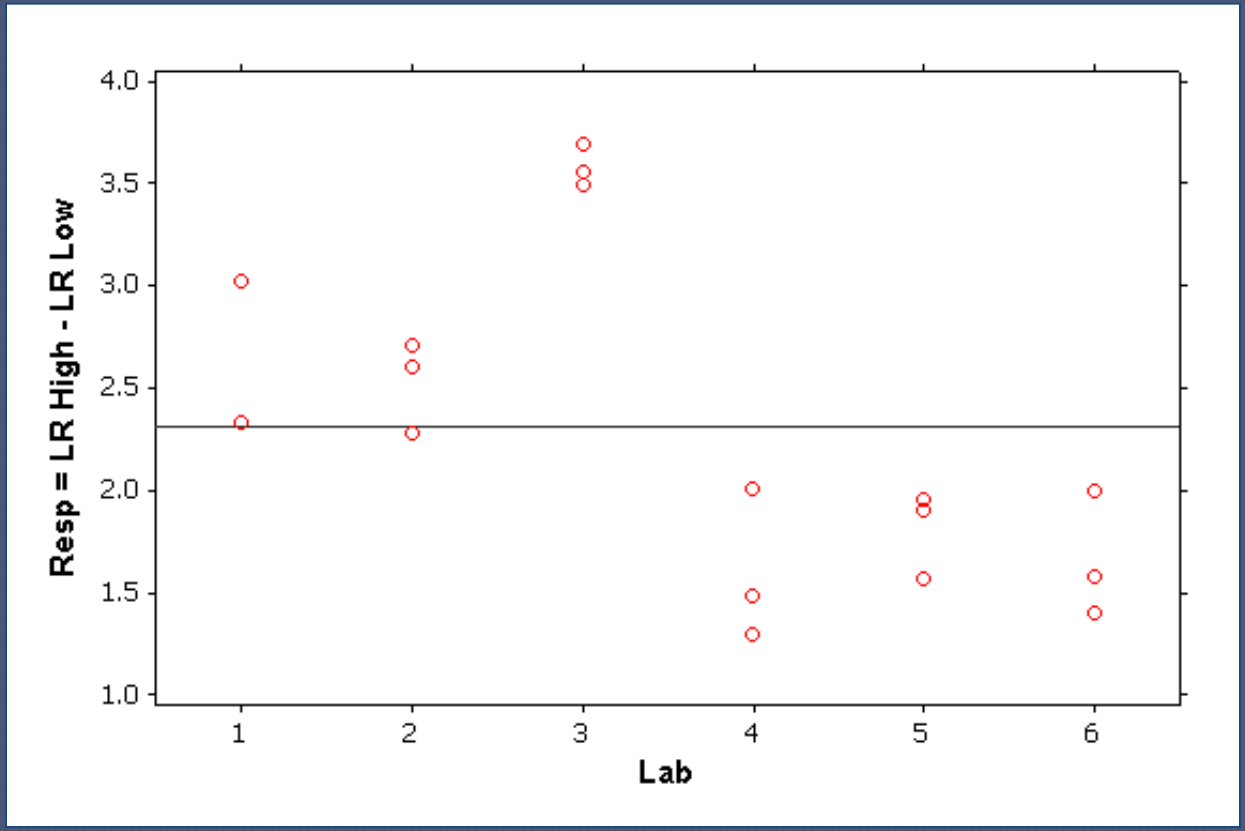
- Each point is a log reduction for a single test.
- Solid horizontal lines indicate the mean LR for each treatment.

Reference Standard – Summary by Lab

	TestLD		LR: Low Efficacy		LR: High Efficacy		Responsiveness	
Lab	**Mean**	**S_r**	**Mean**	**S_r**	**Mean**	**S_r**	**Mean**	**S_r**
1	5.602	0.092	1.115	0.119	3.796	0.607	2.681	0.488
2	4.677	0.069	1.098	0.418	3.628	0.565	2.530	0.226
3	5.454	0.055	1.083	0.120	4.667	0.138	3.584	0.101
4	4.844	0.548	0.964	0.326	2.562	0.141	1.598	0.368
5	4.869	0.089	0.628	0.149	2.435	0.347	1.807	0.211
6	5.251	0.130	0.968	0.120	2.626	0.426	1.658	0.306

Responsiveness for each lab is:
(mean LR for all high efficacy tests – mean LR for all low efficacy tests)

Responsiveness

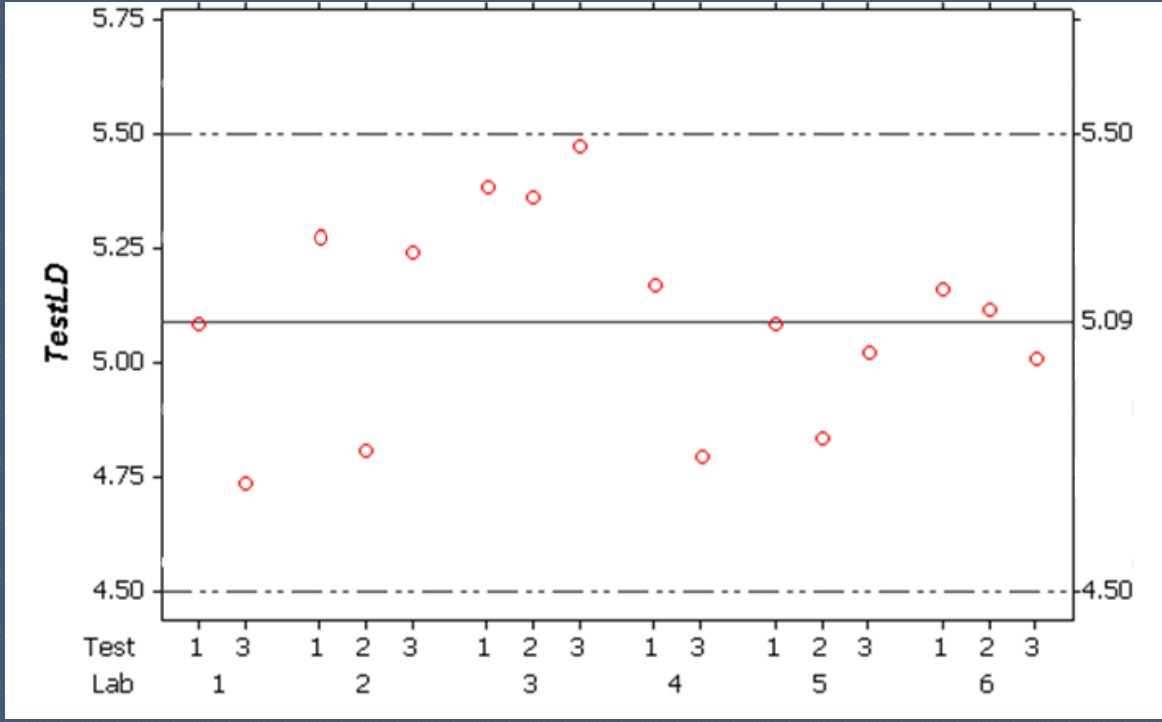


- Each point is the responsiveness for a single test (LR for high efficacy treatment – LR for low efficacy treatment).
- Solid horizontal line indicates mean responsiveness .

Reference Standard – Conclusions

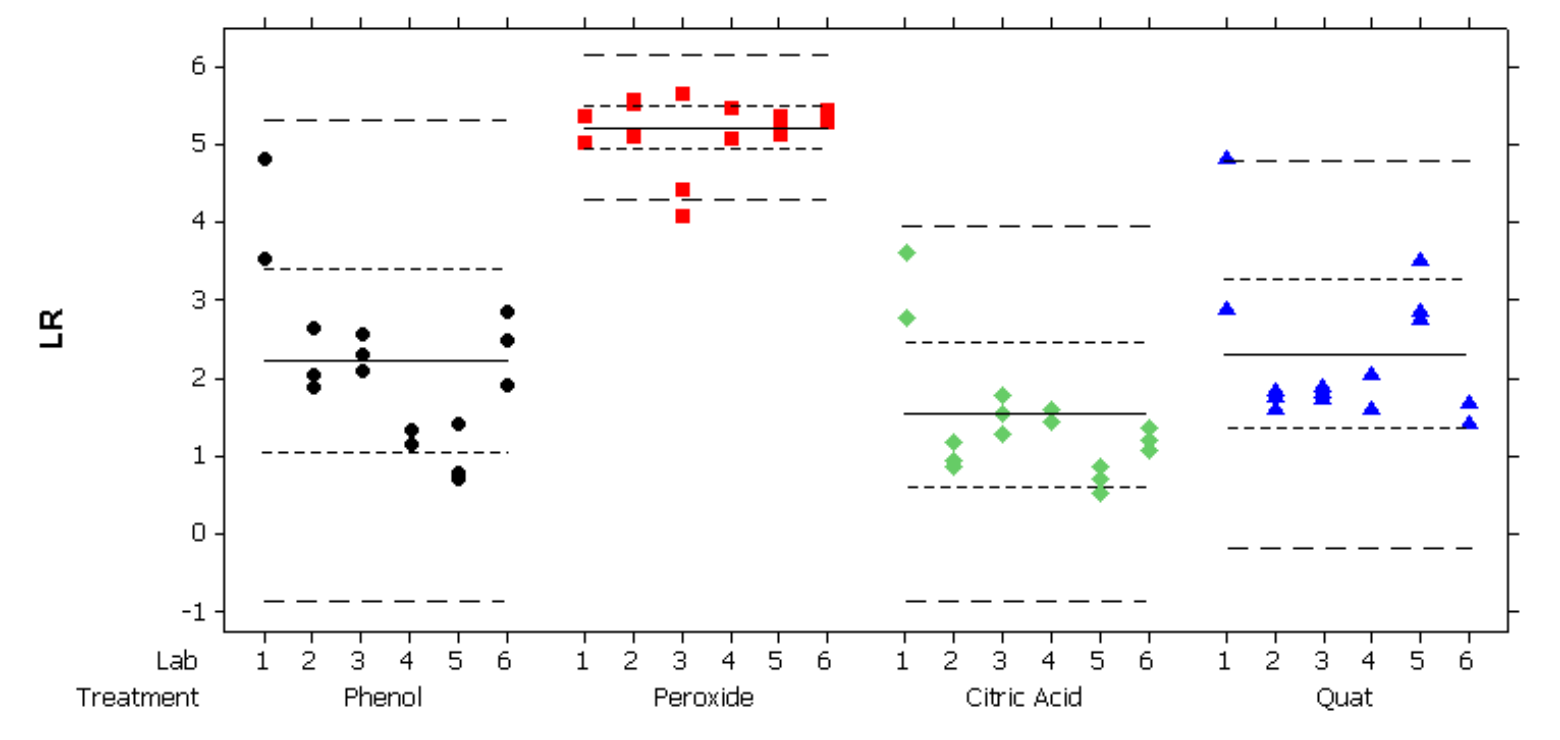
- S_r and S_R values for *TestLD* were small.
- For both efficacy levels, S_r and S_R values for LR were small.
- The mean LR at the high efficacy level was statistically significantly larger than the mean LR at the low efficacy level (p-value ≤ 0.001), confirming responsiveness.
- The greater variability associated with the high efficacy treatment may be due to the moderate mean LR (3.28) achieved.

Method Performance – Test LDs



- Each point is the *TestLD* = mean of the 4 Control LDs from a single test.
- Solid horizontal line indicates the mean *TestLD* of 5.09.
- Dashed lines at 4.5 and 5.5, the limits of validity for *TestLD* specified in the protocol.

Method Performance – LRs



- Each point is a log reduction for a single test.
- Solid horizontal lines indicate the mean LR for each treatment.

Method Performance – Summary by Lab

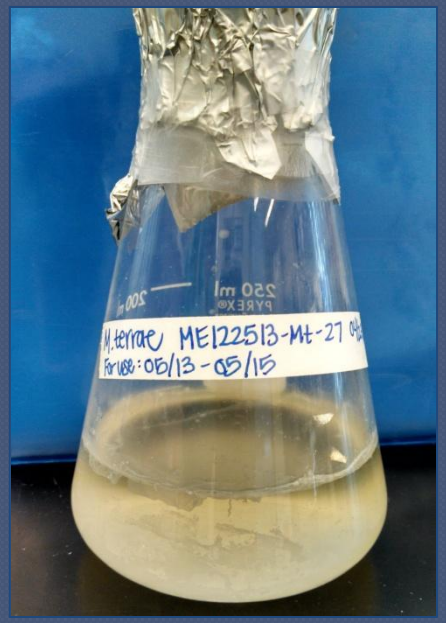
Lab	*TestLD*		LR-A		LR-B		LR-C		LR-D	
	Mean	S_r	Mean	S_r	Mean	S_r	Mean	S_r	Mean	S_r
1	4.908	0.248	4.187	0.914	5.209	0.248	3.210	0.596	3.858	1.379
2	5.108	0.262	2.194	0.398	5.409	0.262	0.997	0.168	1.734	0.114
3	5.404	0.058	2.327	0.228	4.729	0.825	1.540	0.252	1.818	0.077
4	4.981	0.266	1.248	0.134	5.282	0.266	1.533	0.116	1.834	0.311
5	4.978	0.132	0.975	0.389	5.279	0.132	0.710	0.176	3.038	0.412
6	5.092	0.079	2.421	0.477	5.393	0.079	1.225	0.147	1.598	0.153

Method Performance – Conclusions

- S_r and S_R for *TestLD* were small.
- For all treatments, S_r and S_R for LR were small.

Next Steps

- Increase the control carrier count range to 5.0-6.0 CFU/carrier.
- Determine an optical density range in which to harvest the test culture to achieve carrier counts in the appropriate range.
- Optimize the growth conditions for the *M. terrae* culture
  - Grow culture with agitation (150 rpm)
- Optimize harvesting procedure for *M. terrae*
  - Homogenize the culture prior to carrier inoculation



Acknowledgements

- Collaborating laboratories
  - EPA, Microbiology Laboratory Branch
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  - MICROBIOTEST
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  - Metrex
  - Eurofins
- Statistical Analysis
  - Al Parker, Montana State University

Questions?