

Validation of the Ames MPF Penta 2 Assay with a Selection of Genotoxic and Non-Genotoxic Chemicals

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Abstract

The Ames MPF Penta 2 assay is a liquid, miniaturized alternative to the Ames test in Petri dishes. The assay comprises OECD Guideline 471-compliant strains (namely, the *S. typhimurium* TA98, TA100, TA1535 and TA1537 and the *E. coli* WP2 uvrA[pKM101]) and it offers several advantages with respect to the Ames test in Petri dishes, such as less hands-on time and potential automatization. Importantly, there is a significant reduction of needed test item and consumables, including the liver S9 fraction (in line with the 3R principles). We are currently validating this assay with a broad subset of the genotoxic and non-genotoxic chemicals recommended by Kirkland and co-authors in 2016. Here, we present the mutagenic response to a panel of test items, comparing the results and sensitivity with the Ames test results published by the National Toxicological Program. Moreover, we present and discuss scientific and technical issues that were identified and assessed during the course of this validation, including (but not limited to) intra- and inter-lab data reproducibility, identification of toxic compounds and treatment with (semi-)volatile compounds.

Advantages of the Ames MPF

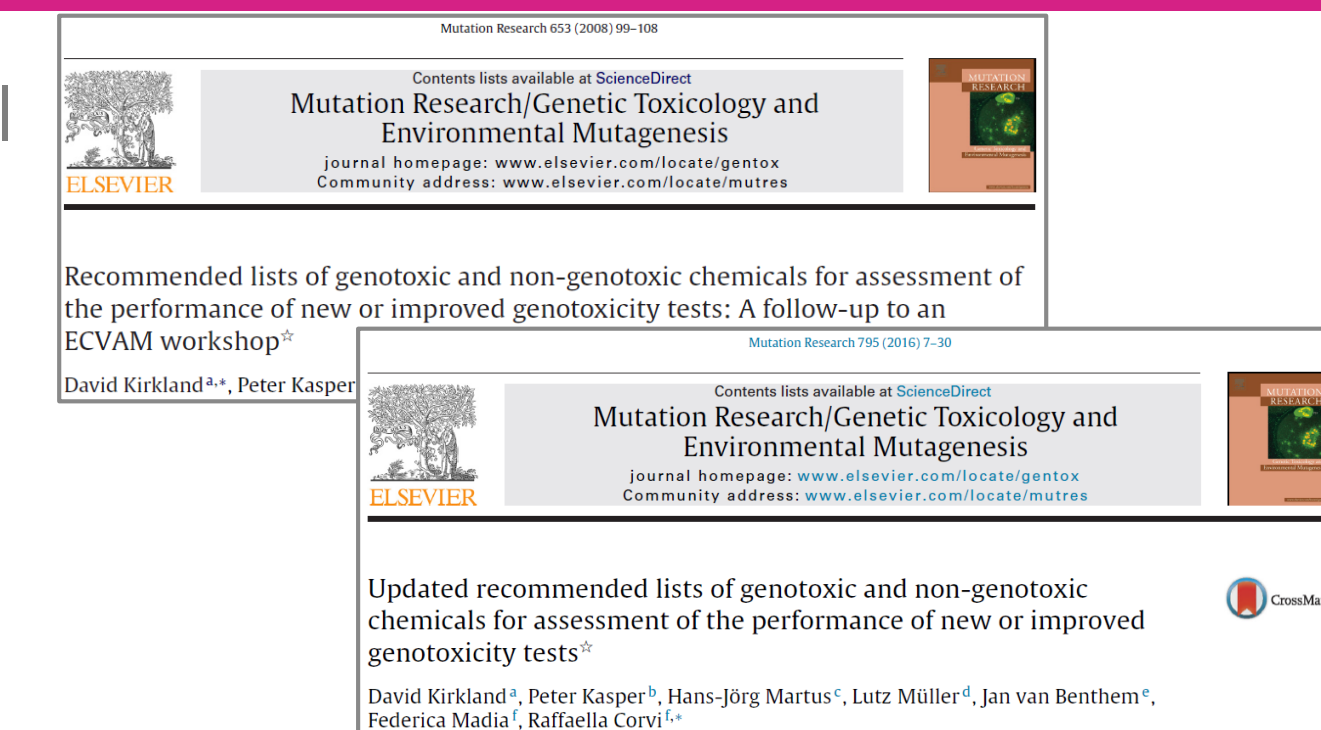
The Ames MPF assay has several advantages over the Ames test in Petri dishes, including

- 4-fold reduction of the test item needed
- 13-fold reduction of the liver S9 homogenate (3Rs)
- reduction of the hands-on time (30 minutes instead of 5 hours c.ca)
- automatable
- higher sensitivity with weak positive/equivocal compounds^[1]
- reduction of the impact on the environment

Selection of the compounds

We selected the compounds to use for our internal validation based on the lists recommended by Kirkland and co-workers.^[2,3]

Our results were compared to data of the Ames test in Petri dishes available in the literature.^[4-6]

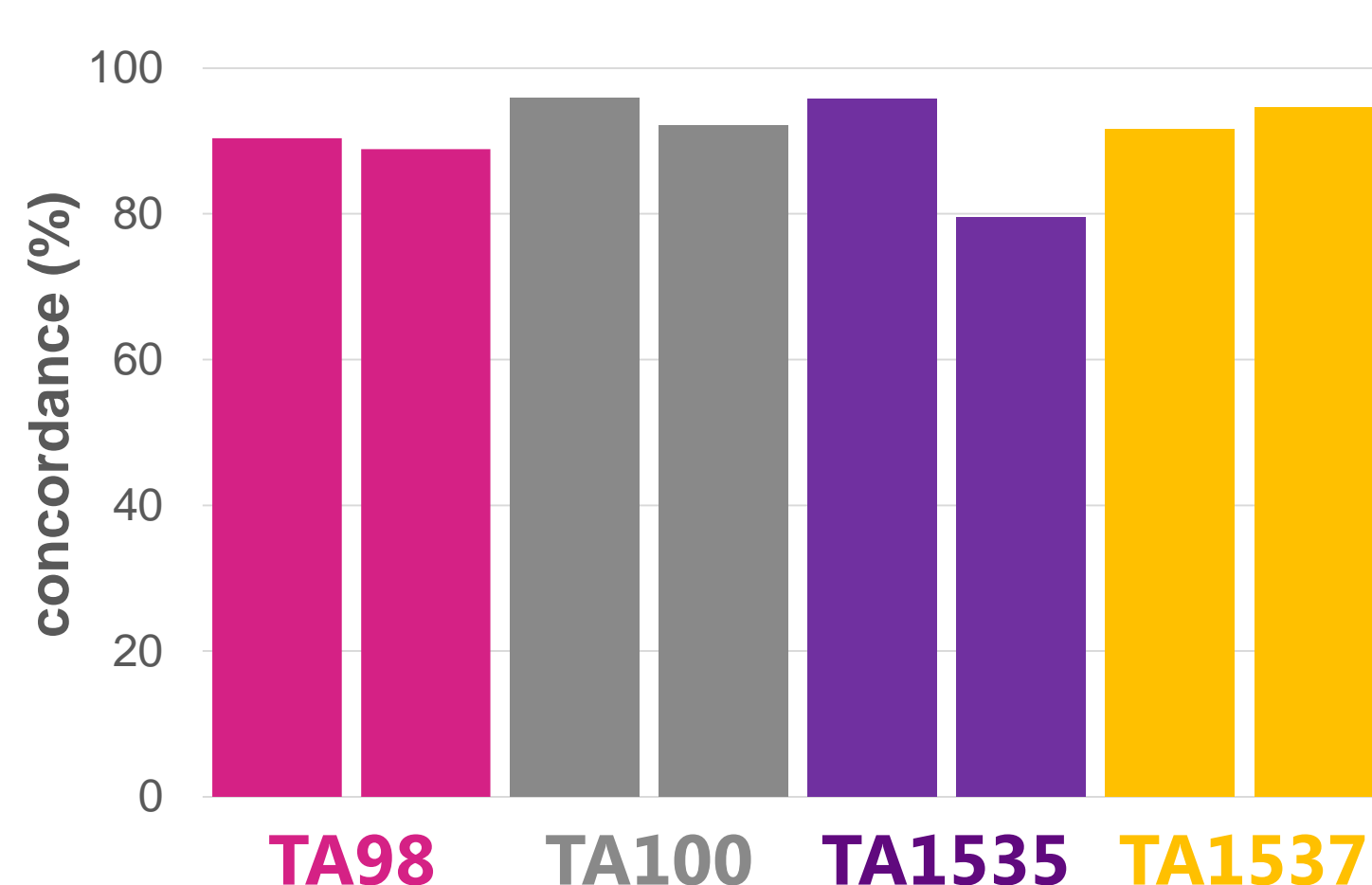


Concordance, Sensitivity, Specificity

Four *Salmonella* strains were considered in the absence and in the presence of S9 (left and right column, respectively):

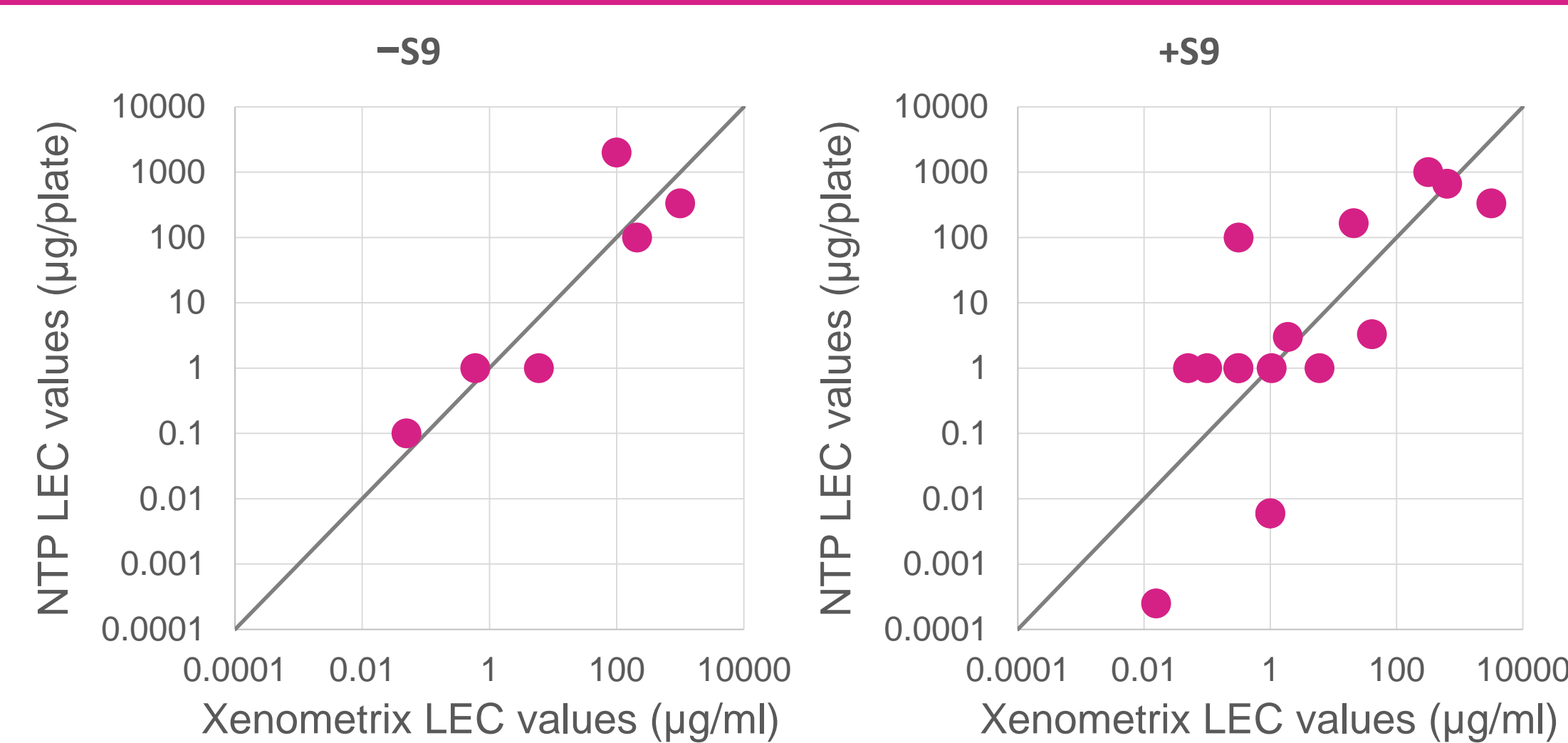
- TA98 (52–54 substances)
- TA100 (49–51 substances)
- TA1535 (47–44 substances)
- TA1537 (36–37 substances)

Overall, the calculated sensitivity (TP) and specificity (TN) were 92.0% and 82.9%, respectively. 57.



Limit of Detection

The lowest effective concentrations for the positive test items indicate that the LoD values of the Ames MPF assay are comparable to those of the NTP's. We are not scaling the values (the comparison would look better for the Ames MPF assay).



Discordant Results

Three types of discordant results were observed during the course of the validation:

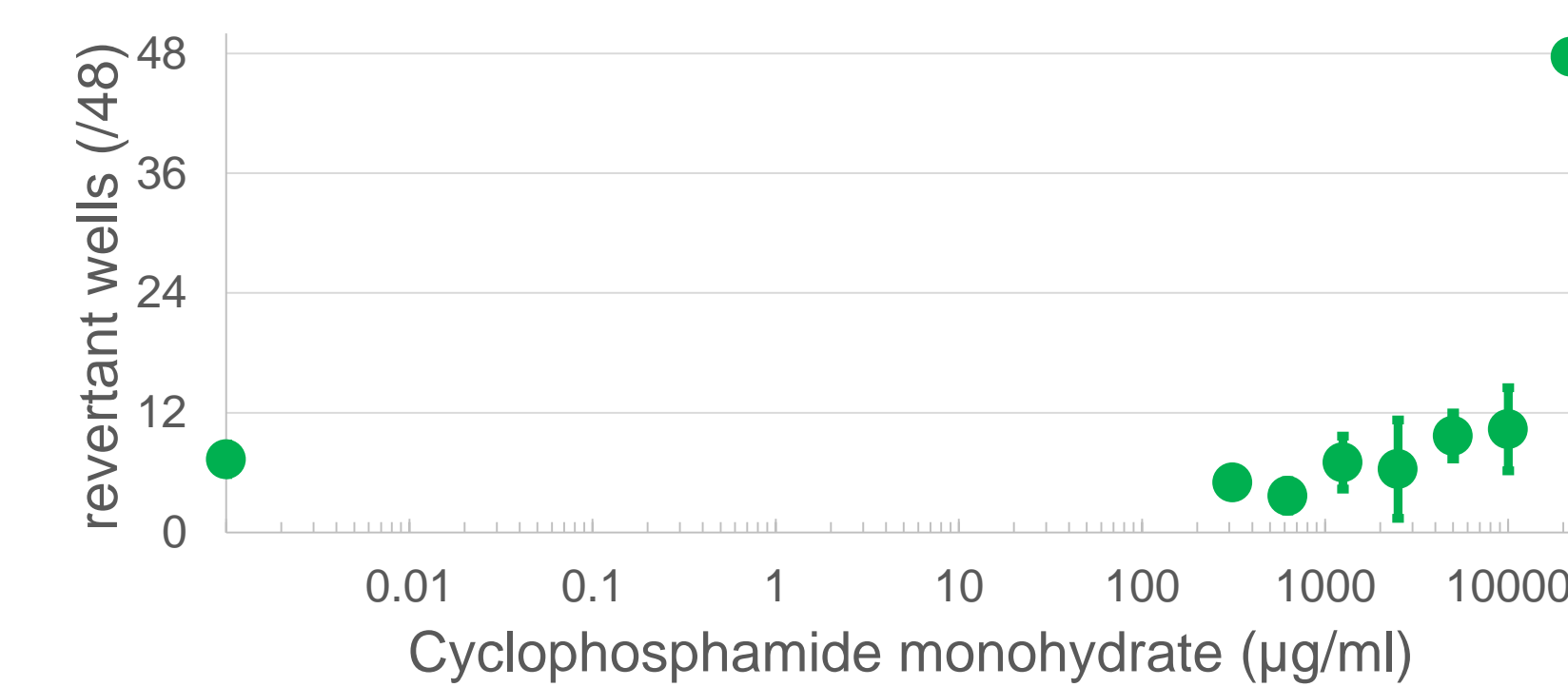
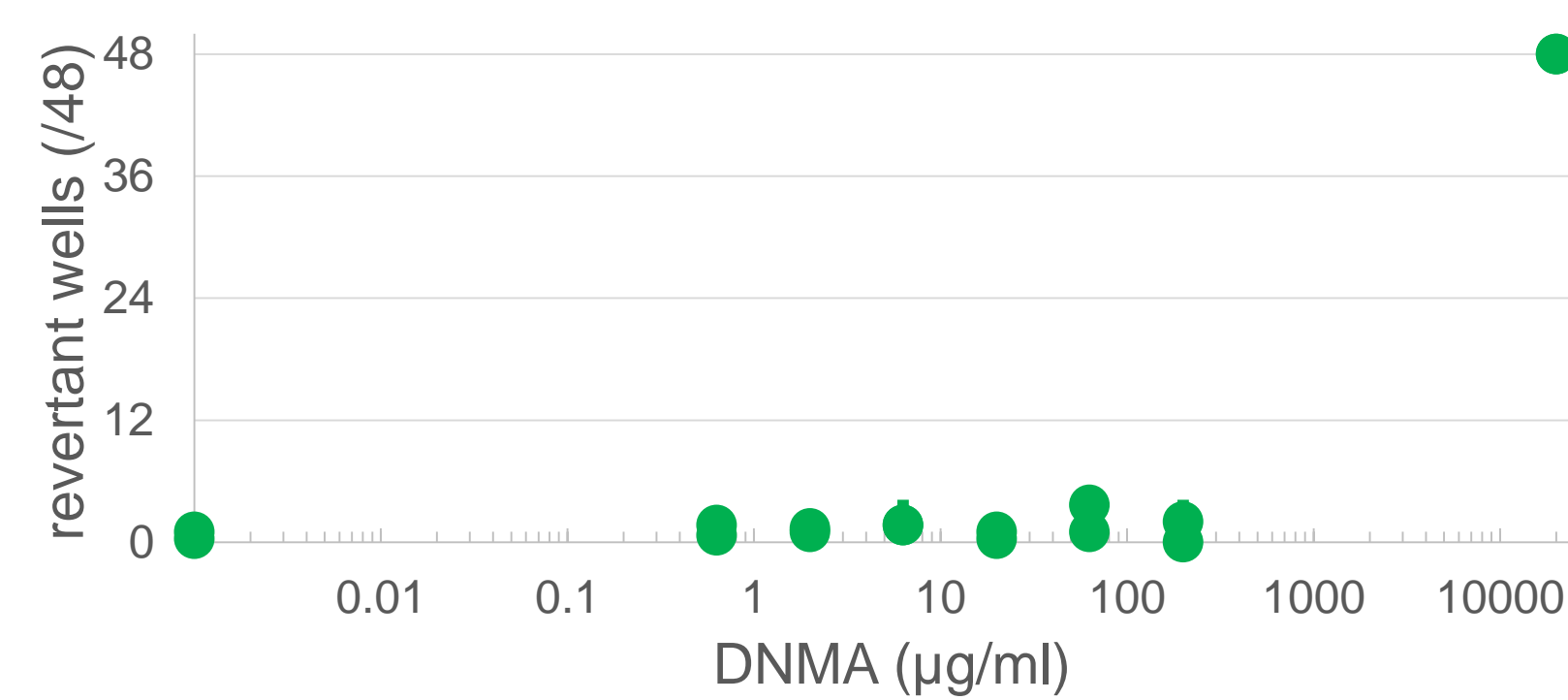
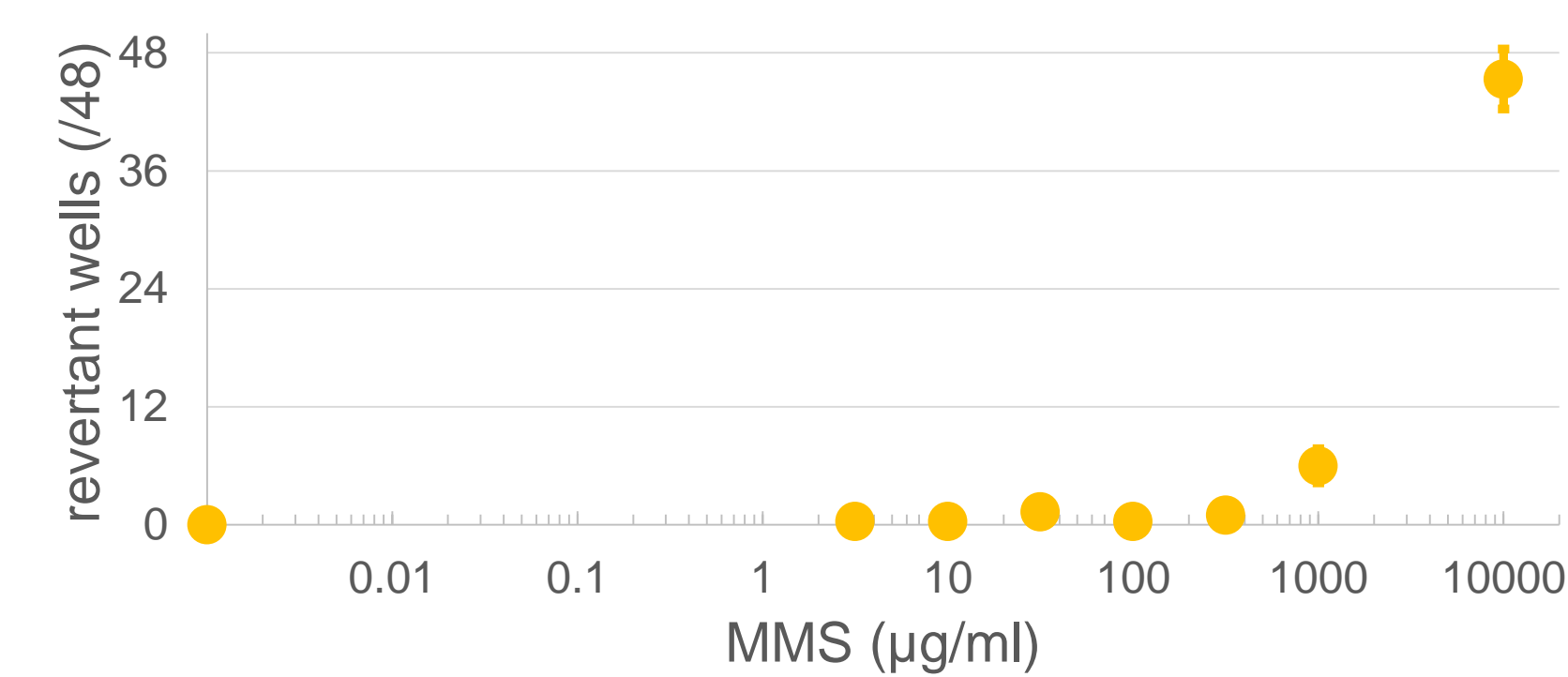
different concentrations

different results within the literature

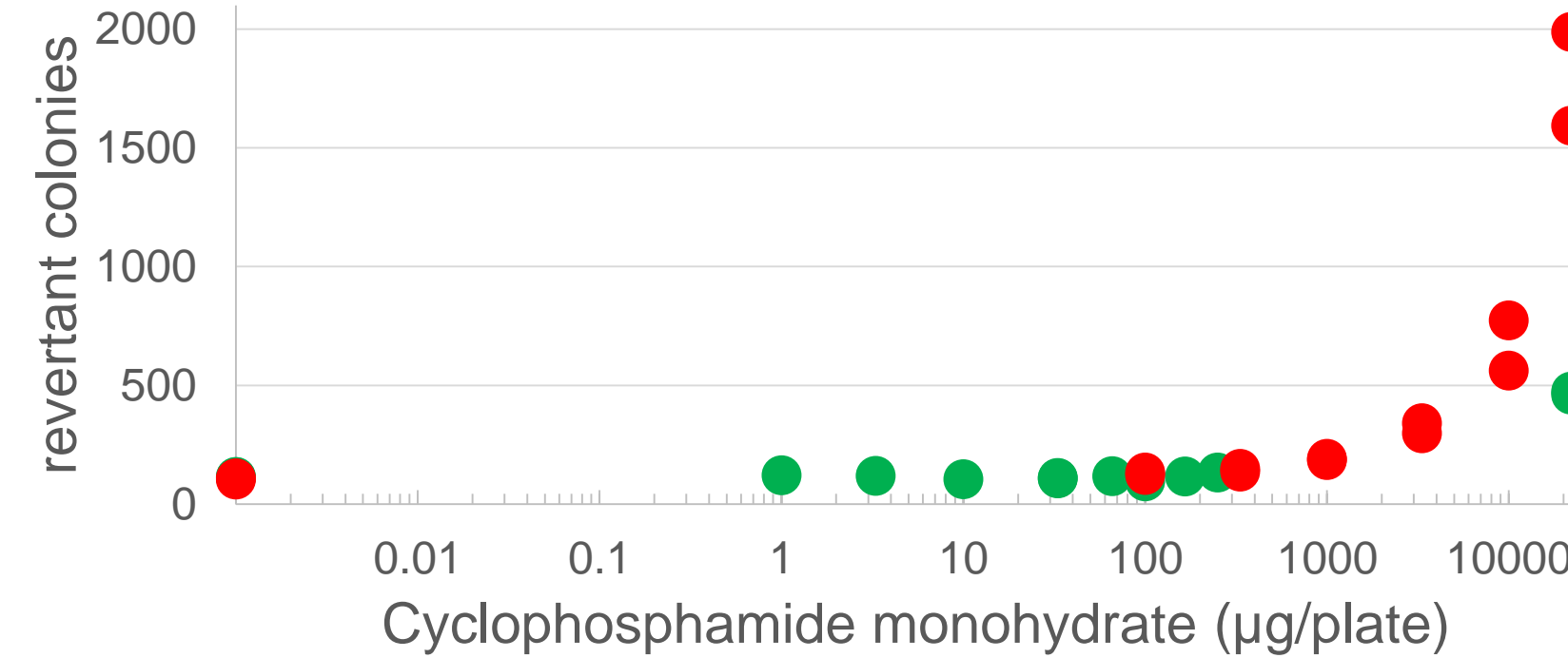
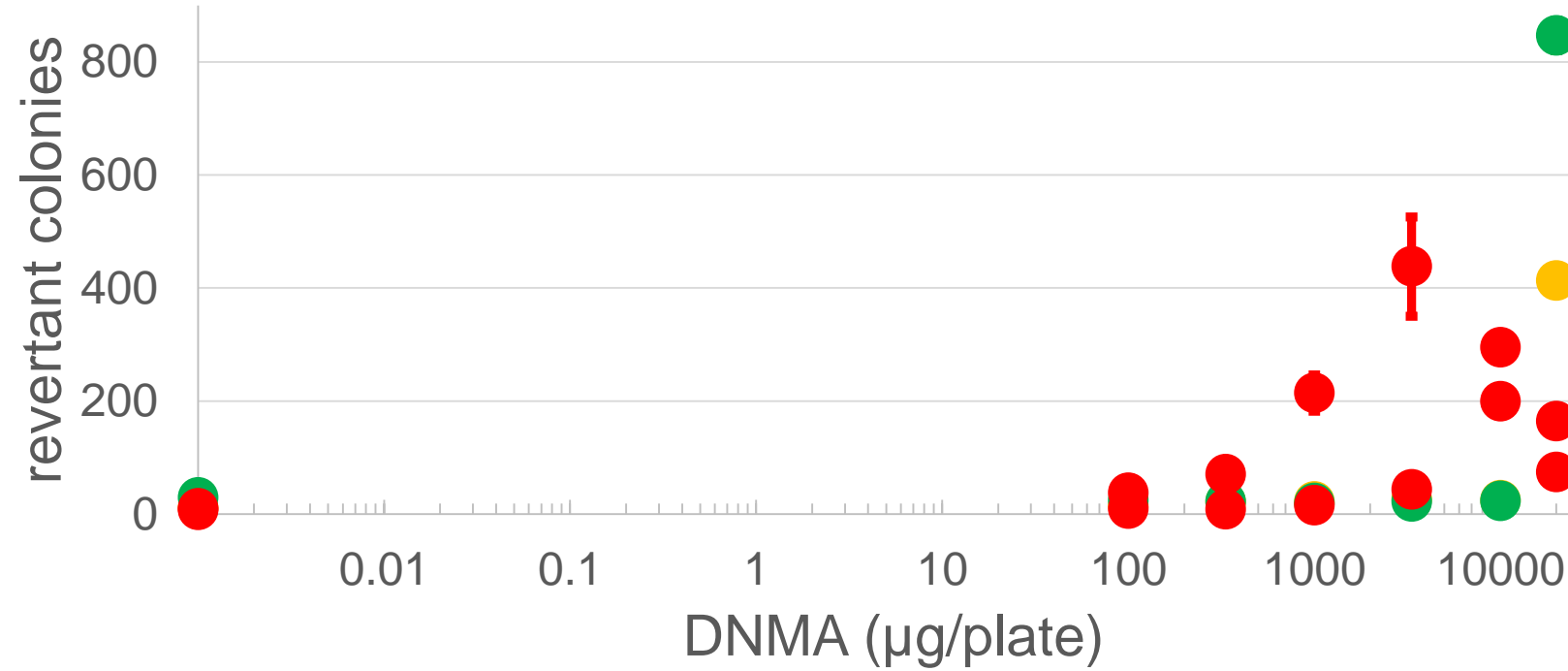
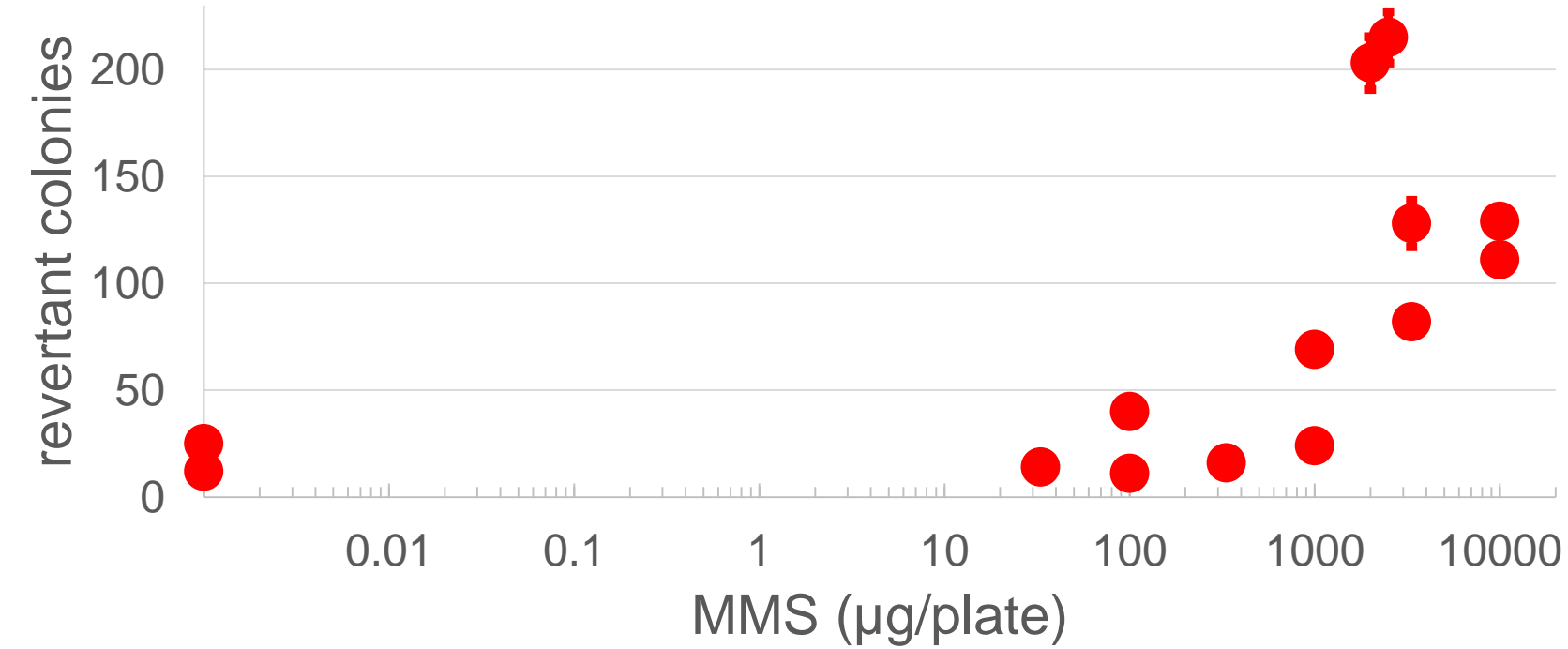
different results compared to the literature

Ames MPF

Color code:
Negative
Equivocal
Positive



NTP Ames

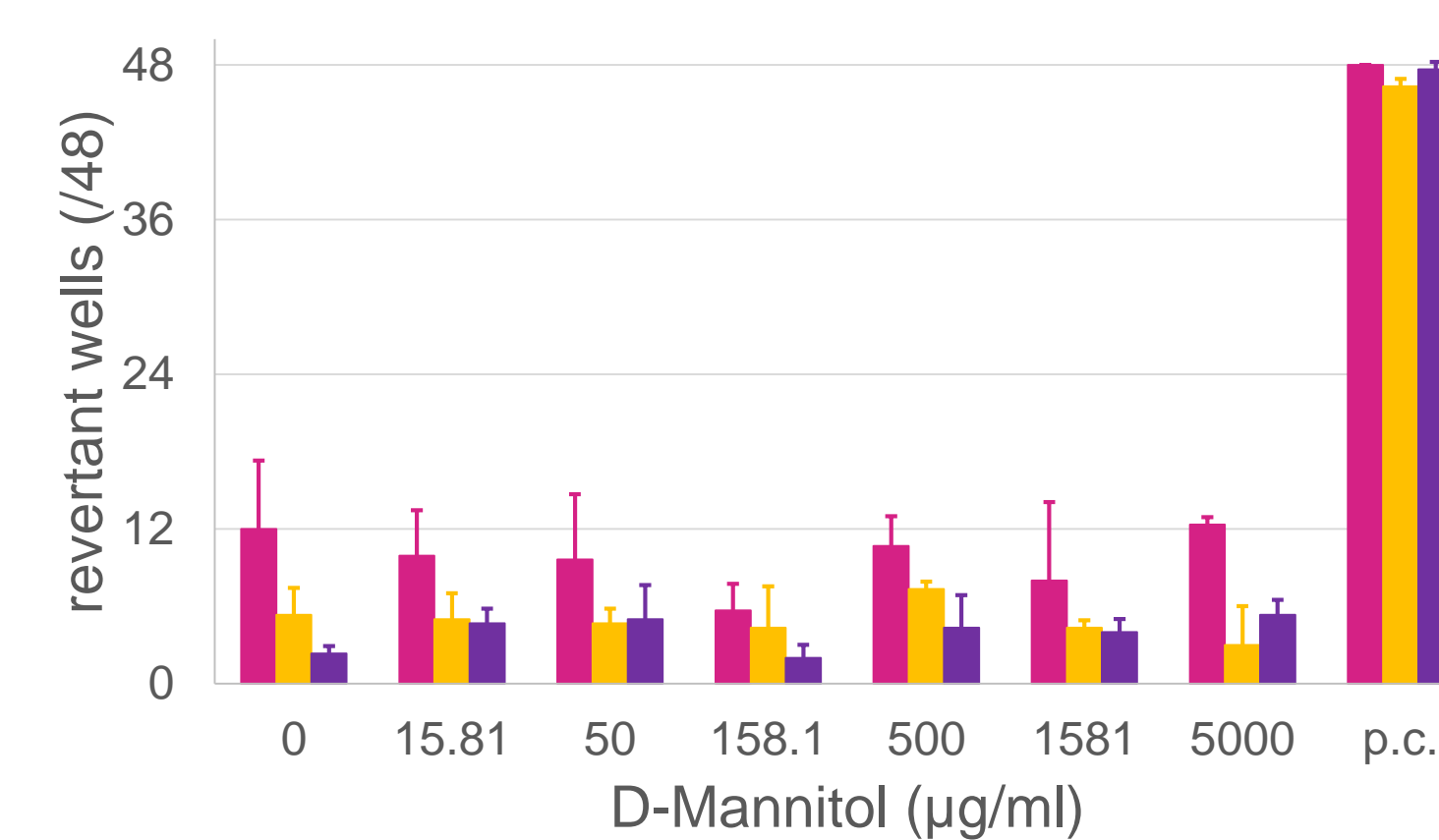
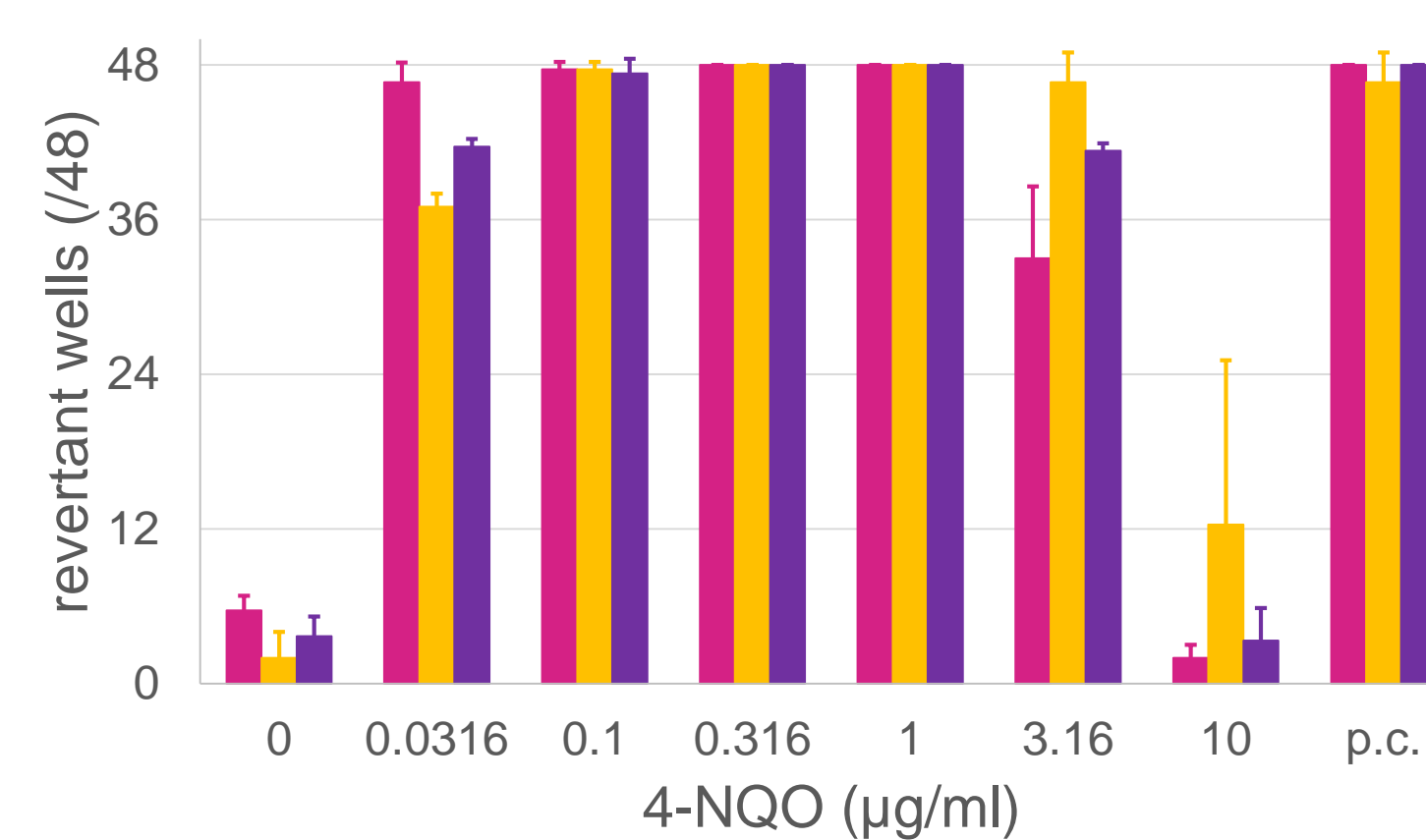


Repeatability

As per the literature for the miniaturized Ames IITM assay (inter-laboratory consistency: 89.5%),^[7] the Ames MPF assay is repeatable!

	-S9		+S9	
	R	#tc	R	#tc
TA98	100.0	7	77.8	9
TA100	100.0	27	95.8	24
TA1535	100.0	6	88.9	9
TA1537	100.0	11	100.0	9

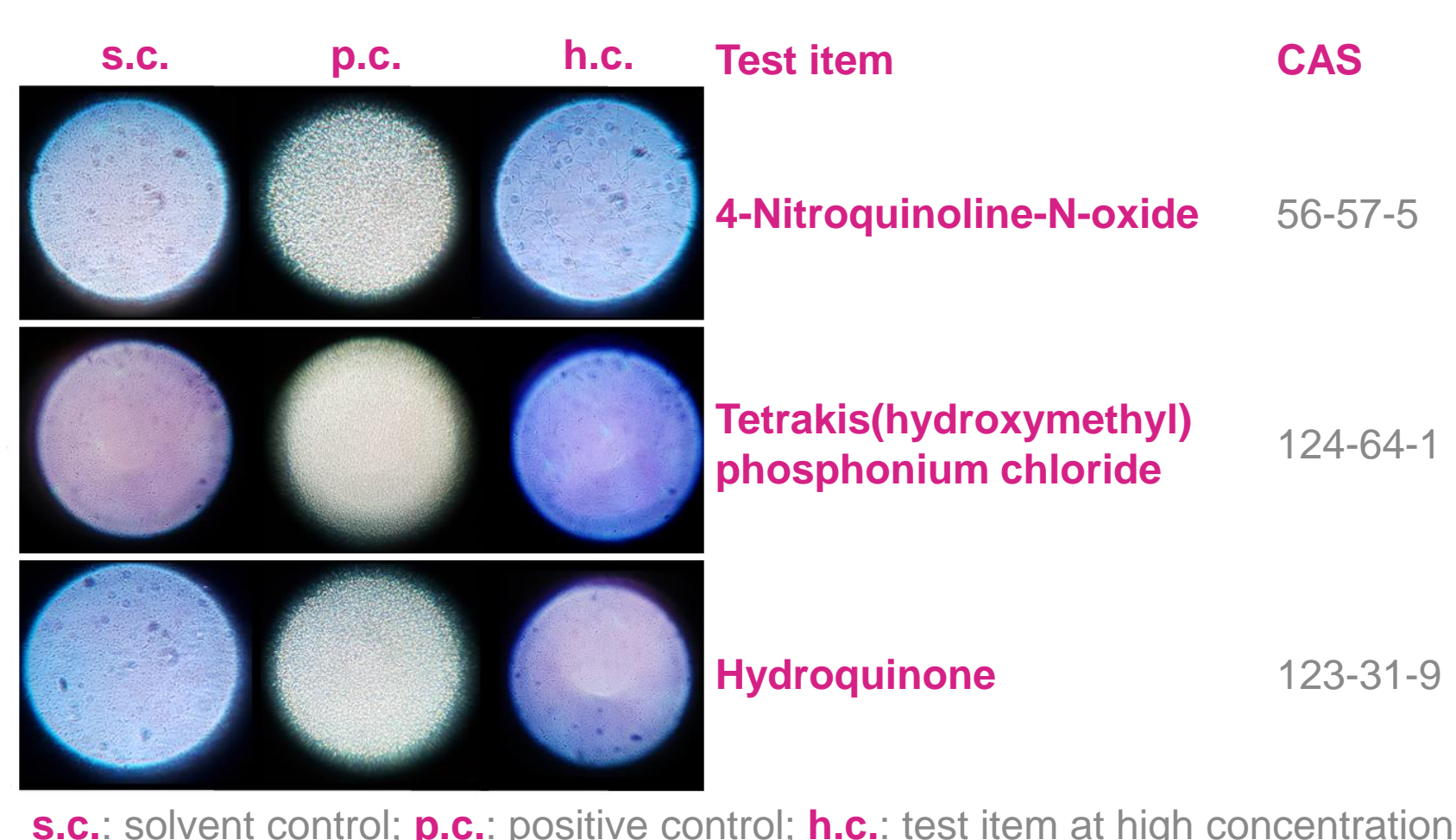
R: repeatability (%)
#tc: number of tests considered



Toxicity

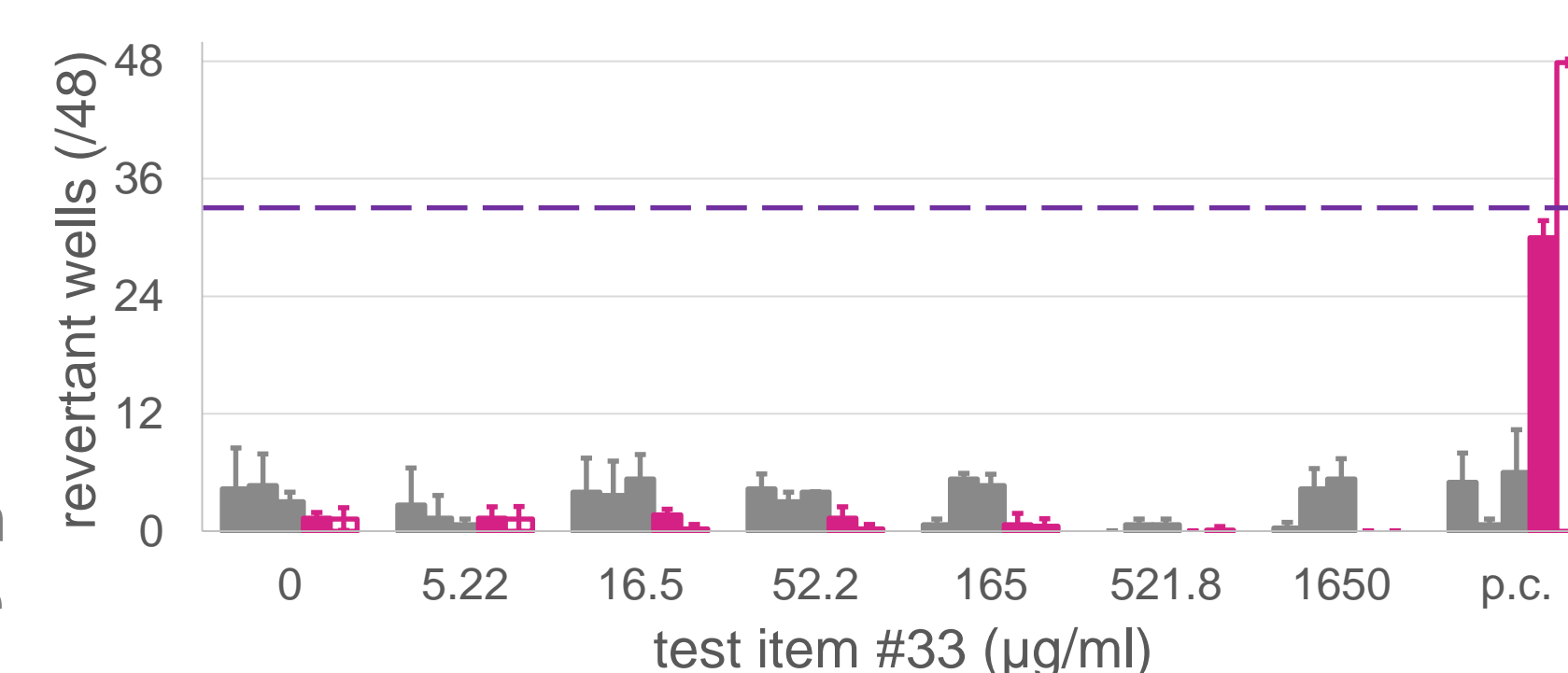
Toxicity can be assessed with several ways, including

- dose-dependent decrease in the number of positive wells
- specific purple color shade
- bubbles/droplets in the wells
- re-plating of the well content on Petri films
- inspection of the wells under the microscope.



Volatile Test Items

Volatile compounds and mixtures can be tested taking advantage of easy, fast and cost-effective adaptations to the protocol as it is described in the Xenometrix "Instructions for Use". Results obtained with these adaptations for a test item are shown in pink and white in the graph on the right.



Do you want to know more? Please contact us (ds@xenometrix.ch, nn@xenometrix.ch)!!!

^[1]: Flückiger-Isler and Kamber. 2012. *Mutat Res* 747(1):36–45. ^[2]: Kirkland et al. 2008. *Mutat Res.* 653(1–2):99–108; ^[3]: Kirkland et al. 2016. *Mutat Res.* 795(1):7–30; ^[4]: NTP Chemical Effects in Biological Systems (website); ^[5]: Dunkel et al. 1984. *Environ Mutagen.* 6(Suppl 2):1–251; ^[6]: Zeiger et al. 1988. *Environ Mutagen.* 11(Suppl 12):1–158; ^[7]: Flückiger-Isler et al., 2004. *Mutat Res.* 558(1–2):181–97.