## Citric acid stability and microorganism viability in solutions for use in cough testing

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### Microorganism counts in citric acid solutions reduce across a week of storage time

#### Introduction

Citric acid cough testing is used to examine airway sensitivity and requires inhalation of a nebulised solution. However, the stability of these solutions over time and the need for aseptic preparation methods is unclear, with inconsistency in available guidance<sup>1-4</sup>. Solution stability is necessary to ensure consistency in the strength of solution that is inhaled. Avoiding inhalation of harmful microorganisms is also important to ensure the respiratory safety of individuals being tested. Pilot data suggests that preparation methods influence microorganism viability and that solutions may become more acidic with storage over time<sup>5</sup>. The current study refined pilot methods to explore the impact of concentration and preparation methods on the stability and microorganism viability of

#### **Methods**

Twenty-seven 0.6 mol/L, three 2 mol/L and three 0.1 mol/L citric acid solutions were prepared and stored under different conditions. All solutions were diluted with sterile physiologic saline. Conditions varied by preparation method (aseptic or benchtop compounded), storage container and storage temperature. The aseptically prepared solutions were sourced from a national pharmaceutical company and were manufactured in advance of testing. The benchtop compounded solutions were prepared by a licensed compounding pharmacy on the same day as testing occurred. The solutions were contained in sterile syringes or sterile<sup>6</sup> borosilicate glass bottles. After baseline testing the solutions were then stored at room temperature or in a fridge at 4°C. Flow cytometry was used to analyse microorganism viability. A pH meter was used to determine the stability of the solutions. Testing occurred at two timepoints, initially after prepared solutions were decanted into containers (baseline) and after seven days (day 7).

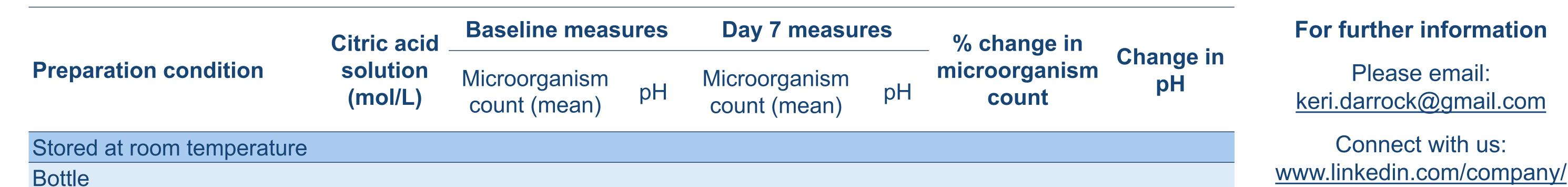
#### Results

Lower microorganism cell counts were found in the aseptically prepared samples compared to the benchtop compounded samples at baseline (see Table 1). Microorganism counts at day 7 reduced by a minimum of 29% across all samples, with counts in benchtop samples at day 7 closer to those found in aseptic samples. A greater reduction in microorganism counts was seen in solutions stored in bottles compared to syringes. The pH of solutions reduced by a maximum of 0.12 across all samples. Storage temperature did not differentially affect microorganism count or pH.

#### Conclusions

Decreased microorganism counts across all samples after seven days suggests that storage of solutions for a week before testing may be responsible for low cell counts at day 7. Given the aseptically prepared solutions were prepared in advance of the benchtop prepared solutions, it is likely that the lower microorganism counts reflect a reduction in cells as seen in the solutions prepared on the same day as baseline testing. The stability of citric acid solutions remains unclear, as the finding that solutions reduced by the same pH likely indicates measurement error. A pH meter's sensitivity can be reduced at high acidity levels (low pH)<sup>7</sup> so further investigation is warranted with use of a more sensitive method of measuring pH. Comparison of aseptically prepared and benchtop compounded solutions when prepared on the same day is needed to determine the benefit of either method. Extending the period for testing to 14 days would also reveal if microorganism cell counts continue to decrease and if cell counts across methods converge. If so, the complexity and expense of aseptic preparation may not be required if an optimum storage period is confirmed.

# **Table 1***Microorganism total cell counts and solution pH across samples*



Aseptically prepared	0.6	486	1.56	247	1.44	49%	-0.12
Benchtop compounded	0.6	2750	1.54	680	1.44	75%	-0.10
	0.1	2461	2.00	575	1.88	77%	-0.12
	2.0	2016	1.00	718	0.88	64%	-0.12
Syringe							
Aseptically prepared	0.6	608	1.56	430	1.44	29%	-0.12
Benchtop compounded	0.6	3147	1.56	2194	1.44	30%	-0.12
Stored in fridge							
Bottle							
Benchtop compounded	0.6	2764	1.56	751	1.44	73%	-0.12
Syringe							
Benchtop compounded	0.6	3498	1.55	1289	1.43	63%	-0.12

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<sup>1</sup>Falconer & Steadman, 2017; <sup>2</sup>Falconer et al., 2014; <sup>3</sup>Morice et al., 2007; <sup>4</sup>World Health Organisation, 2014; <sup>5</sup>Darrock et al., unpublished; <sup>6</sup>Swenson et al., 2018; <sup>7</sup>Cheg & Zhu, 2005