

Method Comparison of Antibody Titre Sensitivity using RCPAQAP Datasets

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Introduction

The Antibody Titre EQA offered by the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) surveys laboratory technical procedures on Antibody titres using various methods. We reviewed reports from the last 5 years to ascertain any differences in sensitivity between various methods employed by laboratories.

Method

Anti-D Titre results submitted for 20 samples with target medians ranging from 1:16 to 1:256 from 2018 to 2022 were analysed. The frequency of results were divided into different method groups; manual tube and both Gel and Glass based card technologies (CAT). Survey targets were determined by consensus medians. Target medians for each method category were subsequently tabulated and compared to determine any potential sensitivity differences. A Chi square test was performed on the cumulative frequencies to ascertain the significance of the method differences.

Results

Across 20 surveys, the median of tube methods were found to have equal, or in 8 instances lower dilutions than CAT methods (Table 1). Method frequency comparisons show increased adoption of CAT methods, while usage of tube decreased in the 5 year span (Figure 1).

Individual results were further categorised by degree of difference from the target median. A bias is seen, where the majority of results that are lower than target medians were predominately using tube techniques (Figure 2).

Chi square analysis shows a statistical significance between the distribution frequencies of the tube and CAT methods ($p < 0.05$, $df = 14$), however analysis of tube differences at higher dilution targets showed no statistical significance ($p > 0.05$, $df = 5$). A control chi-square analysis between the gel and glass bead CAT methods showed no statistically significant difference between the frequencies ($p > 0.05$, $df = 5$).

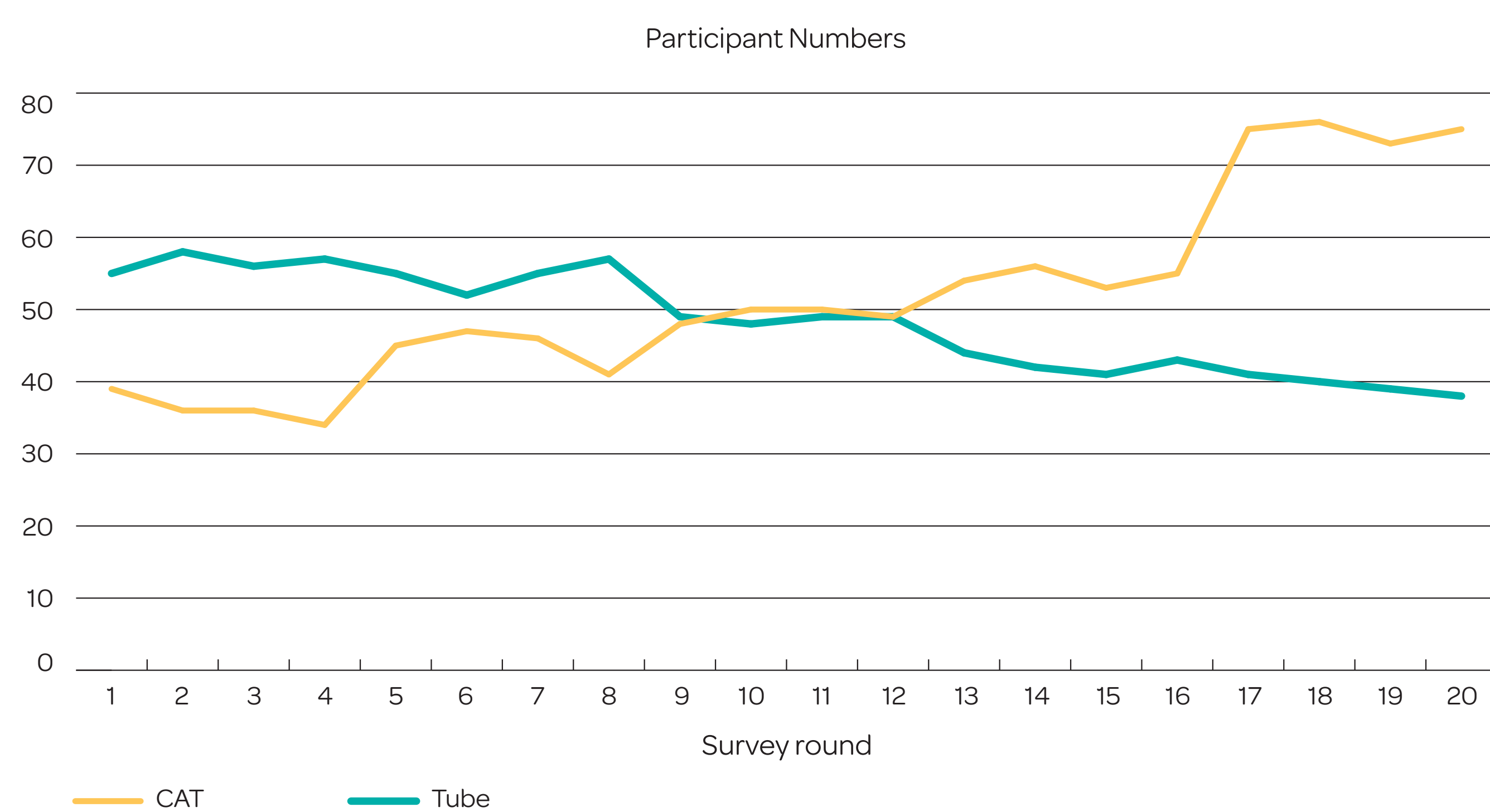


Figure 1. Number of participants for each method over the survey rounds, starting from 2018 to 2022.

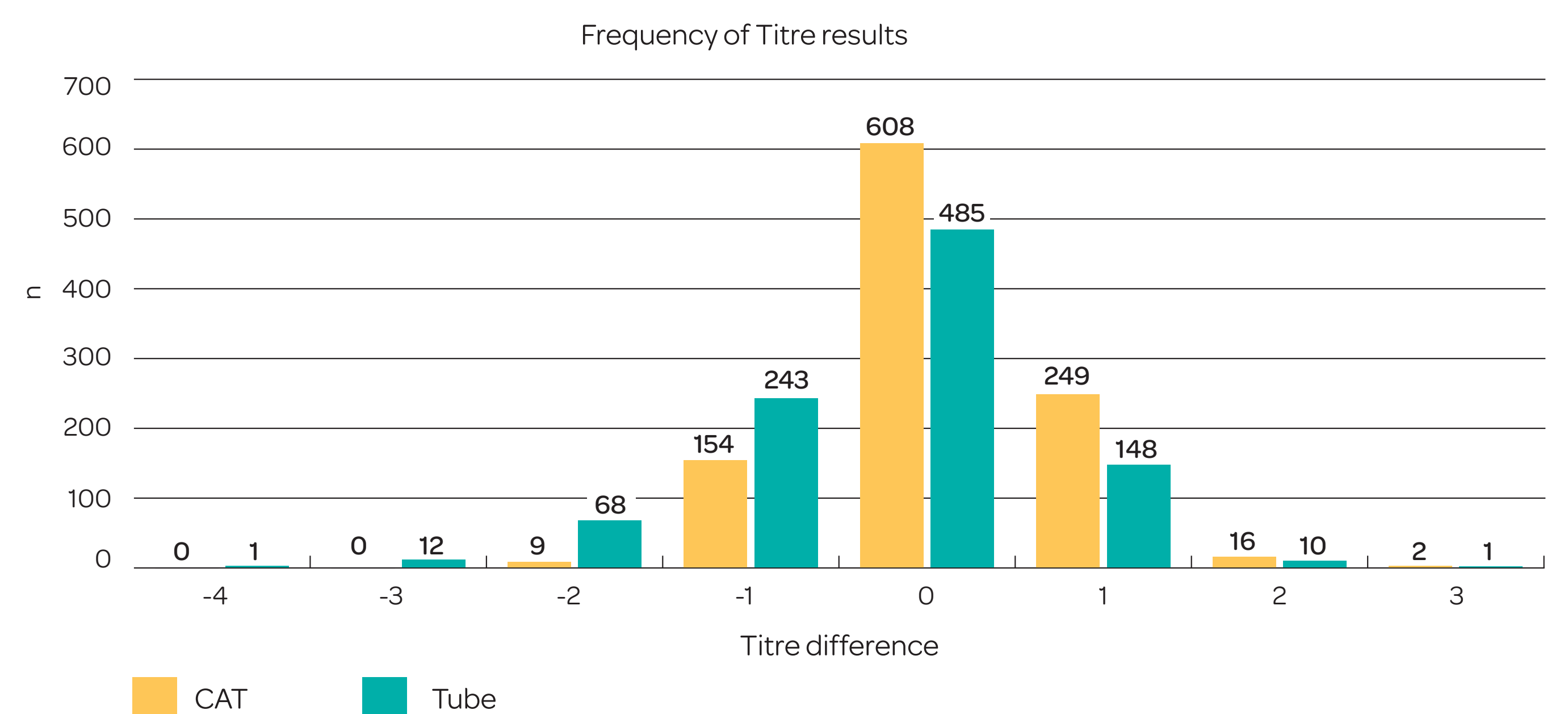


Figure 2. Frequency of results of both CAT and Tube methods compared to target dilutions. 159 of results using tube methods were higher than the target median, compared to 324 that were below.

Discussion

Titration of antibodies detected in maternal serum is critical for the timing of clinical management and intervention in the context of Haemolytic Disease of the Foetus and Newborn (HDFN). Furthermore, differences in sensitivity of titre methods must be considered to define the critical values set by the testing laboratory in the context of patient management¹.

The analysis of the RCPAQAP datasets is in line with published literature indicating CAT methodology as a more sensitive platform compared to conventional tube^{1,2,3}. While the degree of difference in sensitivity can vary up to eightfold in some studies², our data showed mainly a one to twofold dilution difference between the methods, and a single instance of a four-fold difference to the target median (figure 2). Despite demonstrating a significant increasing sensitivity difference at dilutions in the 1:32 to 1:128 range, the correlation was not significant at higher dilution targets (>1:128) based on this dataset.

We acknowledge some of the limitations with this survey data. Information about reagent diluent and the indicator cell zygosity used by participants were not captured before 2022, which may have contributed to the final titre values^{4,5}. Variation in operator experience and laboratory procedures may also be a contributing factor. As reaction strengths also weaken over time, this can have implications depending on logistical and operational factors.

It is also of note that target medians across all surveys are of relatively high titre dilutions, with only one survey target of 1:16. Further surveys with lower titre targets to obtain a more holistic comparison between the methods are under consideration.

Conclusion

Based on returned data, there was a significant difference observed between manual tube and CAT methods in obtaining antibody titre dilutions and comparatively no significant sensitivity difference between the CAT gel and CAT glass methods. This should be considered when observing trends and setting critical titre values in patient management and monitoring for HDFN.

Table 1. Titre dilution medians of each method category. The number of results for each method are displayed. Tube medians that are lower than CAT are in bold.

Survey Round	2022				2021				2020				2019				2018			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
CAT	256 (n=75)	128 (n=73)	64 (n=76)	128 (n=75)	64 (n=55)	128 (n=53)	64 (n=56)	16 (n=54)	64 (n=49)	128 (n=50)	256 (n=50)	128 (n=48)	128 (n=41)	64 (n=46)	256 (n=47)	64 (n=45)	128 (n=34)	64 (n=36)	128 (n=36)	512 (n=39)
Tube	128 (n=38)	128 (n=39)	64 (n=40)	64 (n=41)	64 (n=43)	64 (n=41)	32 (n=42)	16 (n=44)	64 (n=49)	64 (n=49)	128 (n=48)	128 (n=49)	128 (n=57)	64 (n=55)	256 (n=52)	64 (n=55)	128 (n=57)	32 (n=56)	128 (n=58)	256 (n=55)

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