SHOULD I WASH OR REUSE QUADRA PIPETTE TIPS?

The answer depends on the application. If you are doing sensitive testing, such as patient HIV, where no carryover must be guaranteed, then using virgin tips is the answer. The use of virgin tips on each sample may be correct academically, but it may not be cost effective. This is particularly true in high throughput drug screening. Not only the cost of disposable tips, but also the throughput time lost in changing tips are factors to be considered. When can pipette tips be reused without jeopardizing the acceptable limits of the assay?

This is defined by the individual investigator for the protocol being used. It is a judgement call. There is no one answer. This report provides information on carryover as a result of two test protocols, both using radioactivity as the tracking method. The first method pipetted 50µL of a "hot" label that had a 85,000 counts per minute. The Quadra pipette tips were washed three (3) times (Mix Function - Aspirate / Dispense), in the Quadra wash station. 50µL of a "cold" buffer solution was pipetted and counted on a MicroBeta. The counts were less than 30cpm, which is equivalent to the background of the counter. The actual results were as follows:

For the carry over tests, a Quadra96® Model 320 was used. It also had the tip washing station that automatically changed the rinse water. A solution of 1% BSA in water was labeled with Tritium. 50µl of this solution was transferred to a test plate. The tips were then rinsed in water, using 3 aspirate and dispenses of 450µl (tip volume). At the end of the wash cycle, the Quadra has the ability to blow air through the tips to remove excess rinse water. The washed tips were then used to transfer 50µl of the unlabeled solution to a second test plate. Both sets of test plates were then counted in a MicroBeta. The results were as follows:

Plate # 1	**50µl of Label**
Mean Value:	86789.94
Standard Deviation:	1813.99
C_V %:	2.09%

Plate # 1A **Pipetting cold buffer after three wash cycles for the tips**

10.0	7.8	14.4	10.0	13.2	18.7	12.1	23.0	15.0	25.0	19.0	6.0
8.9	13.3	12.2	48.9	12.1	21.9	13.2	17.6	15.0	17.0	13.0	16.0
8.9	17.7	20.0	15.5	20.8	12.1	11.0	16.5	15.0	15.0	20.0	10.0
13.3	11.1	13.3	15.5	17.6	20.8	14.3	8.8	16.0	11.0	12.0	17.0
7.2	17.5	14.4	18.5	12.4	22.7	17.5	15.4	17.3	15.2	19.3	21.3
8.2	10.3	16.4	16.4	10.3	9.3	13.4	15.4	15.2	11.2	16.3	18.3
10.3	18.5	18.5	16.4	21.6	13.4	19.6	22.7	22.3	17.3	17.3	15.2
14.4	8.2	8.2	12.3	14.4	18.5	17.5	11.3	13.2	18.3	16.3	8.1

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Plate # 2 50µl of Label

Mean Value:	83565.25
Standard Deviation:	2859.0672
C_V %:	3.42%

Plate # 2A Pipetting cold buffer after three wash cycles for the tips

14.4	17.7	14.4	13.3	27.4	24.1	15.4	8.8	23.0	14.0	13.0	12.0
23.3	18.9	17.7	27.7	11.0	15.4	13.2	17.6	14.0	17.0	11.0	11.0
21.1	17.7	17.7	18.9	20.8	14.3	17.6	16.5	13.0	13.0	20.0	15.0
14.4	18.9	12.2	15.5	13.2	23.0	17.6	8.8	13.0	14.0	15.0	10.0
11.3	18.5	16.4	12.3	16.5	12.4	17.5	10.23	16.3	11.2	17.3	14.2
11.3	15.4	17.5	15.4	18.5	18.5	27.8	18.5	12.2	15.2	15.2	8.1
18.5	14.4	16.4	16.4	11.3	13.4	12.4	21.6	7.1	19.3	12.2	14.2
12.3	14.4	18.5	8.2	10.3	15.4	9.3	12.4	21.3	15.2	11.2	14.2

Plate # 3 50µl of Label

Mean Value:	84740.563
Standard Deviation:	2440.1746
C_V %:	2.88%

Plate # 3A Pipetting cold buffer after three wash cycles for the tips

25.5	28.8	25.5	26.6	36.2	26.3	25.2	15.4	22.0	16.0	25.0	19.0
33.3	28.8	17.7	22.2	23.0	27.4	23.0	19.7	28.0	15.0	32.0	25.0
31.3	21.1	37.7	22.2	25.2	25.2	24.1	11.0	21.0	18.0	25.0	16.0
27.7	26.6	24.4	29.9	37.3	31.8	28.5	27.4	17.0	27.0	20.0	17.0
23.6	21.6	23.6	22.6	25.7	25.7	14.4	31.9	22.3	32.5	26.4	15.2
24.7	21.6	16.4	12.3	12.4	11.3	21.6	21.6	23.4	15.2	25.4	20.3
19.5	27.7	28.8	17.5	15.4	24.7	25.7	13.4	17.3	13.2	26.4	15.2
11.3	20.5	23.6	29.8	24.7	19.6	10.3	22.7	18.3	16.3	16.3	8.1

This test demonstrates the effectiveness of tip washing on this type of application. While no carry over cannot be guaranteed, it is cost effective to evaluate washing the tips on specific protocols. Choosing different solvents for tip washing offers another option.

A second protocol was used to look at tip washing in another manner. A similarly labeled solution was transferred, using the Quadra Model 320. This time, the portion of the pipette tips handling the label were cut off and placed in scintillation vials, cocktail was added, and they were counted in a Wallac LSC.

The first set of 8 tips were cut off and counted after pipetting, but prior to any washing. The second set of 8 tips were washed with three aspirate and dispenses at 450µl, then cut off. The third set of 8 tips were washed a total of six aspirates / dispenses.

Transfer 50 µl Label (Tritium) - Count Tips in LSC with and without wash

	Plate # 1	Plate # 2	Plate # 3
"Hot" Plate CPM / well	84746	87040	84728
Tips only No Wash	10839 (12.7%)	5496 (6.3%)	6704 (7.9%)
Tips after 3 Wash / Aspirates	152 (0.18%)	158 (0.10%)	457 (0.54%)
Tips after 6 Wash / Aspirates	110 (0.13%)	90 (0.10%)	198 (0.23%)

This test implies that some of the counts were retained in the polypropylene, since the first test indicated they were not carried over to the next plate after washing.

Prior to running carry over tests, it was deemed necessary to establish a base line of what pipetting accuracy could be expected at various volumes. To measure the accuracy of each tip, a dye solution of methyl orange was pipetted into a 96-well plate, using the Quadra 96 well pipettor. An SLT Rainbow reader was used to read the optical density of each well. Each plate was read three times, and averaged to minimize the effect of reader accuracy. Optimum reader accuracy is obtained at 200µl. Pipetting was accomplished by first aspirating the buffer volume (200µl minus the Test Volume), an air gap, and then the test volume of dye solution 10µl, 25µl, 50µl and 100µl. The results were as follows:

TABULATION OF RESULTS

	10µl		25µl		50µl		100µl	
Plate #	C_v	OD	C_v	OD	C_v	OD	C_v	OD
6006	1.87	1.438	1.25	0.538	1.18	1.002	1.02	1.900
5158	2.25	1.504	1.28	0.543	0.97	0.981	1.24	1.826
5192	2.27	1.369	1.50	0.528	0.99	0.961	1.24	1.803
5162	2.41	1.487	1.26	0.516	1.19	0.976	1.30	1.816
5228	2.51	1.430	1.78	0.532	1.79	0.987	1.43	1.785
Average								
C_v	**2.26**		**1.41**		**1.20**		**1.24**	

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