

Protein Dispense Test

BIOXHIT Section 1 - Crystallization Technologies

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This report has been written by Janet Newman who supervised and designed the experiments with the help of Patrick Celie, David Egan and Anastassis Perrakis from the NKI.

Data that was obtained after the workshop was added to this report by Patrick Celie.

Protein Dispense Test

BIOXHIT Section 1 - Crystallization Technologies

Introduction

A simultaneous test of five nano-dispensing robots was run in conjunction with the High Throughput Macromolecular Crystallization Workshop held at the NKI in Amsterdam on the 14-16th December, 2005. The machines in the test were the Oryx (Douglas Instruments) the Mosquito (TTP), the Screenmaker 96+8 (Innovadyne), the Microsys (Genomic Solutions) and the 961 (Genomic Solutions). The only obvious absences from the line-up were the Phoenix (Art Robbins), and the Hydra + 1 (Matrix).

The tests were performed by the vendors on their respective machines, except for the test run on the Mosquito, which was performed by Janet Newman. The Mosquito is the NKI's in-house nano-dispensing machine, and has been in use in a general user lab for at least two years. No vendor was available to run the test on the Mosquito.

NOTE: The experiments with the Mosquito were repeated at a later stage by Joby Jenkins and Sarah Payne from TTP Labtech with the assistance of Patrick Celie from the NKI. Also Patrick Shaw Stewart (Douglas instruments) repeated the experiments after the workshop. The (improved) data from these experiments has been incorporated at a later stage and is presented in 'Updated Dispense Tests' on page 12 and 13 of this report.

The Test

Each vendor was asked to produce four 96 well plates, two were protein dispense tests, and two were crystallant dispense tests. All of the test reagents contained fluorescein, and the plates (Black Greiner 96 well Microlon plates) were analyzed using the standard Fluorescein protocol on a Fluostar Optima plate reader (BMG Labtechnologies).

The protein dispensing section of the test compared the ability of the machines to reproducibly dispense small droplets, using two protein standard solutions. Each protein was dispensed into 24x20nl, 50nl, 100nl and 200nl drops in a black 96 microwell plate. The protein solutions were concocted to mimic a standard protein ("Easy protein" – 25mg/ml lysozyme, 10mg/ml BSA, 0.5mg/ml fluorescein, 20mM tris pH 8.0) or a difficult protein ("Sticky protein" – 25mg/ml lysozyme, 10mg/ml BSA, 50% glycerol, 1% Tween 80, 0.5mg/ml fluorescein, 20mM tris pH 8.0)

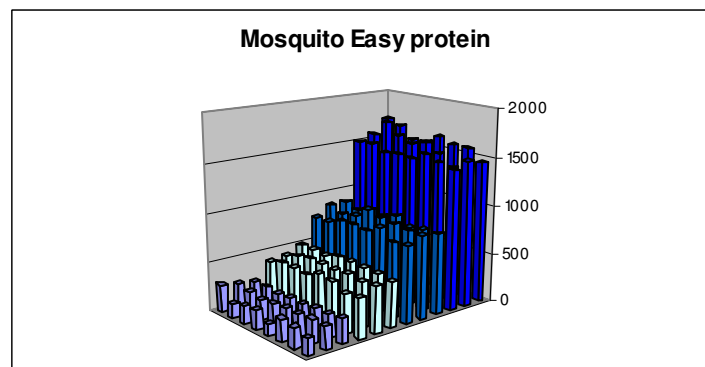
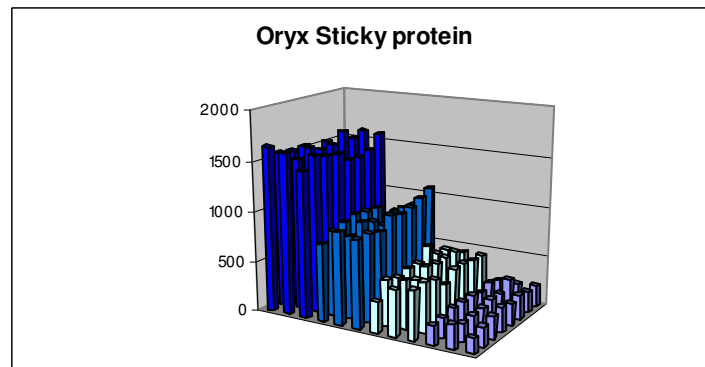
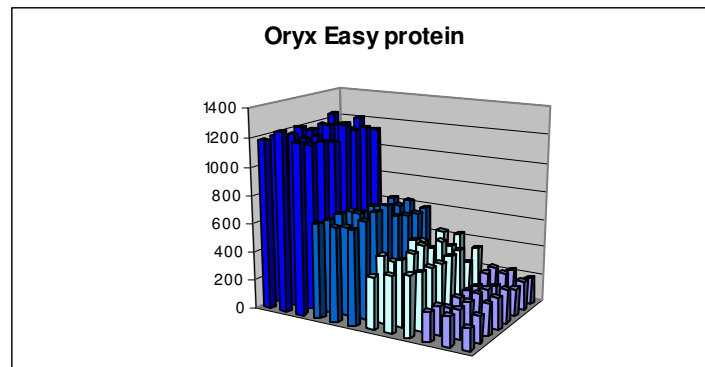
The crystallant test was designed to see how well the crystallant component of a drop would be dispensed and consisted of dispensing 100nl drops from each well of two 96 well 'stock' blocks into black 96 microwell plates. Each column of the stock block contained aliquots of the same solution. Thus each stock block contained 12 unique solutions, each which would be dispensed eight times by each robot. One of the stock blocks (PEG block) contained 20-45% PEG 10K, and 20-45% PEG 3K (each solution also contained 0.5mg/ml fluorescein, 20mM tris pH 8.0). The other block

(Isopropanol-MPD block) contained 0-50% isopropanol, and 20-70% MPD (each solution also contained 0.5mg/ml fluorescein, 20mM tris pH 8.0).

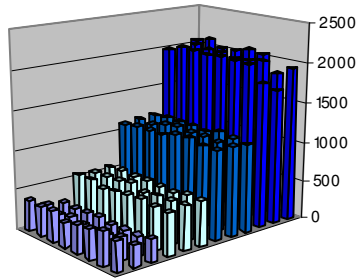
All the plates were sealed with an adhesive film after the nano-dispensing, and were later filled with 100µl 20mM tris pH 8.0 using a Robbins Hydra 96 well transfer robot. These filled plates were resealed, then wrapped in foil and mixed gently overnight. Finally, the plates were vortexed briefly (to ensure complete dissolution of any dried fluorescing reagent) and spun down before being analyzed.

Data – Protein

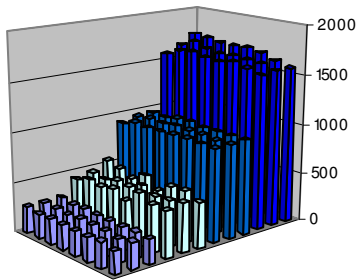
The following are charts of the raw data for each of the five machines and the two protein samples – notice that the Oryx test put down the drops 200nl → 20nl, and that the 961 put down each set of 24 drops of a given volume in 2 rows, rather than the 3 columns used by the other machines.



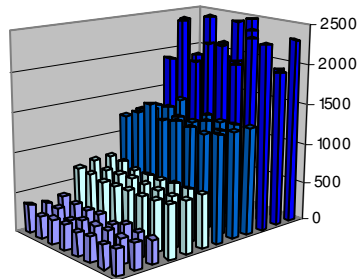
Mosquito Sticky protein



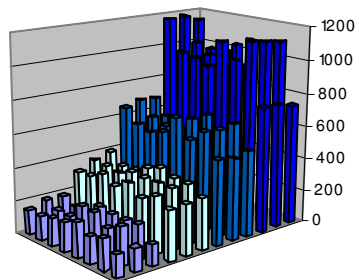
Screenmaker 96+8 Easy protein



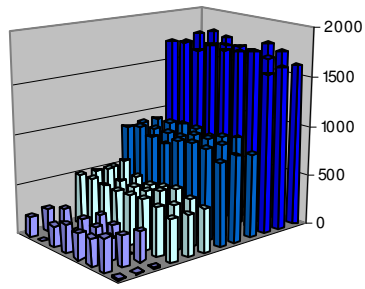
Screenmaker 96+8 Sticky protein



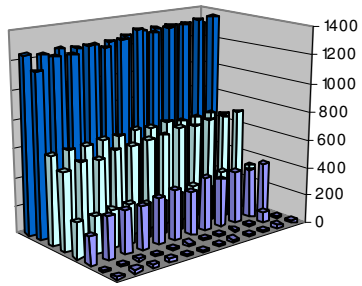
Microsys Easy protein



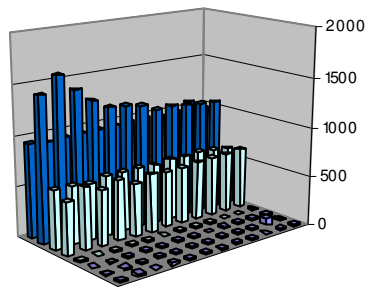
Microsys Sticky protein



961 Easy protein



961 Sticky protein



The Results - Protein

Two values can be extracted from the protein dispense tests, a precision value for each volume/protein combination, and an accuracy value. The precision is given by the coefficient of variation (CV), which is the (standard deviation)/(average) for each set of measurements. The accuracy of each machine is calculated by comparing the average each set of 24 measurements with a standard curve of that protein.

CV(%)	Oryx		Mosquito		96+8		Microsys		961	
	Easy	Sticky	Easy	Sticky	Easy	Sticky	Easy	Sticky	Easy	Sticky
20nl	12.7	13.7	15.7	22.8	3.7	8.0	18.7	76.4	65.3	65.6
50nl	13.3	11.4	8.1	3.2	7.9	4.6	8.5	8.4	16.5	13.5
100nl	6.5	8.1	5.1	2.0	1.5	4.5	9.5	4.0	5.9	4.6
200nl	2.9	4.5	5.4	4.5	3.0	10.1	12.5	3.3	3.8	17.1

Volume (nl)	Oryx		Mosquito		96+8		Microsys		961	
	Easy	Sticky	Easy	Sticky	Easy	Sticky	Easy	Sticky	Easy	Sticky
20nl	24	22	26	30	30	32	21	17	2	2
50nl	49	48	57	58	57	70	42	52	36	2
100nl	77	94	101	121	116	141	72	96	79	63
200nl	144	164	187	217	201	234	121	185	156	119

0-50% 51-80% 81-120% 120-150% 151%+

Table 1. Summary of the protein dispense test for five nano-dispensing robots.

The upper table shows the CV for each robot/volume/protein combination. CVs greater than 10% are shown in a red font.

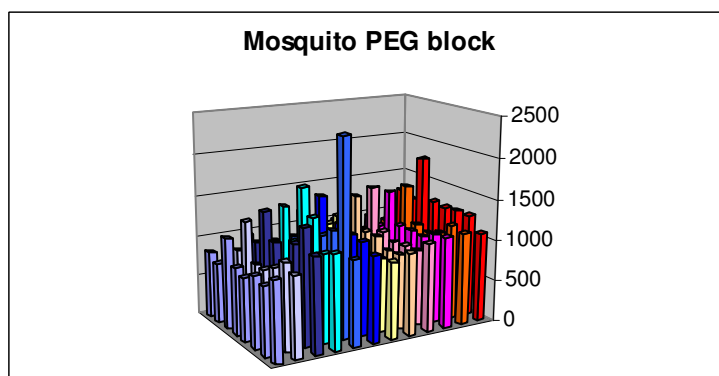
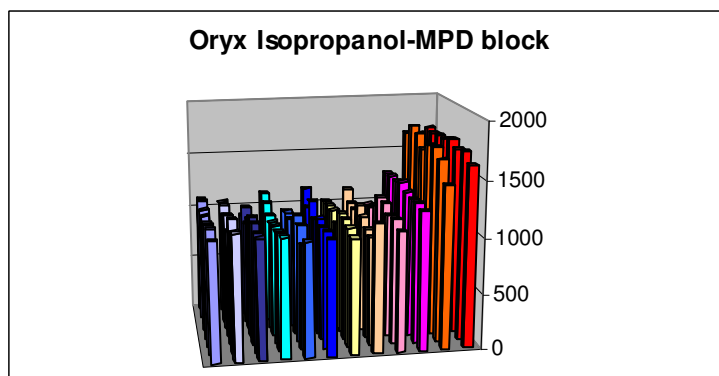
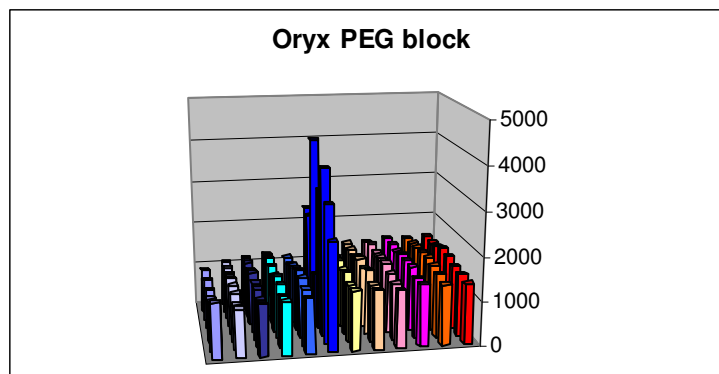
The lower table shows the volume (from the average fluorescence of each robot/volume/protein combination) calculated from a standard curve of the respective protein. The volumes that are within 20% of the expected result are coloured green, the volumes that are within 50% are coloured orange, and the volumes that deviate by more than 50% from the expected value are coloured red, as shown in the key.

Discussion – Protein

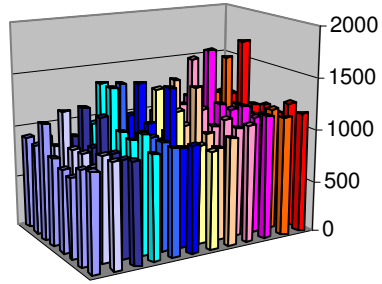
The results from the protein test showed that most of the machines did much better than expected at the lower volume ranges. The CVs for the Screenmaker 96+8 Easy protein were particularly impressive. It was also notable that most of the machines did not have any real problems with the “sticky protein”. Malcolm Collins, who ran the tests on the Genomic Solutions machines, stated before the tests that the 961 machine had not been tuned for low volumes, and he doubted that the machine would put down 20nl or 50nl drops. He was half-right – the 961 did not succeed in putting down the smallest drops with either sample, and could put down 50nl drops only with the Easy protein sample. The accuracy values are at best a guide, as the tests were run with sub-optimal quantities of fluorescein – in hindsight, the stocks should have had 10 fold more fluorescein than they did (5mg/ml rather than 0.5mg/ml). The standard curve was certainly not linear at the concentration range used and is at best a reality check, rather than a gold standard. However, the CVs should be unaffected, and the trends in the results are probably reliable.

Data – Crystallant

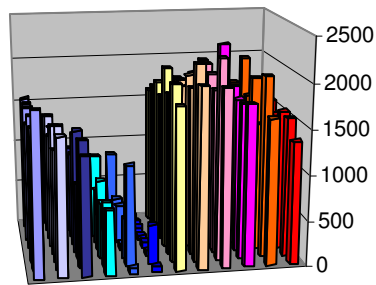
The PEG stock block contained 20%, 25%, 30%, 35%, 40% and 45% PEG 10K in columns 1-6, and 20%, 25%, 30%, 35%, 40% and 45% PEG 3K in columns 7-12. The Isopropanol-MPD block had water in column 1, then 10%, 20%, 30% 40% and 50% isopropanol in columns 2-6. Columns 7-12 had 20%, 30%, 40%, 50% 60% and 70% MPD respectively. Each of the 8 rows of a given column were pipetted into the block from the same bulk solution, and should be identical. Some of the machines could not aspirate directly from the deepwell blocks, and in this case an intermediate shallow 96 stock plate was made by 1 to 1 transfer from the deepwell block. The Microsys dispensing system did not partake in this part of the test, and it is probably fair to guess that it would not have performed well on the more viscous (30% or more PEG 10K) as its dispensing technology is similar to the Screenmaker 96+8. The different angle views of the plotted data were chosen to try to maximize the information that can be gleaned from looking at the graphs.



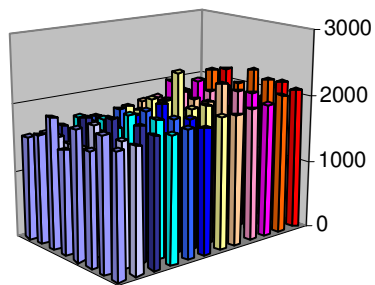
Mosquito Isopropanol-MPD block

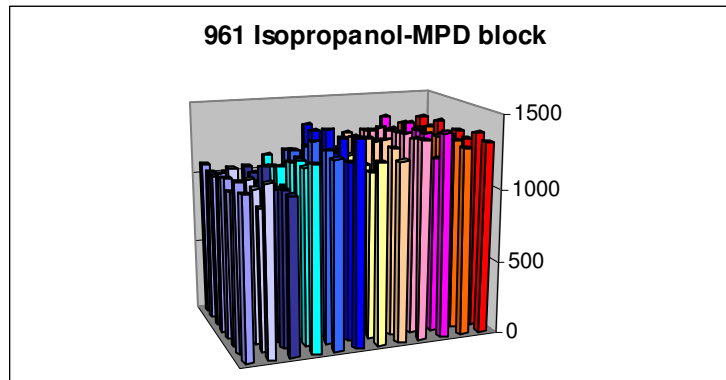
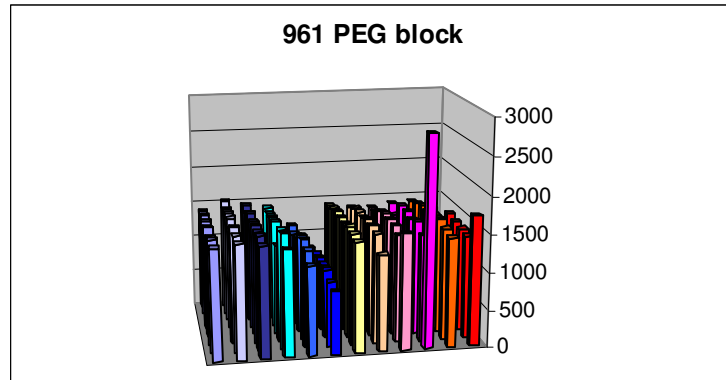


Screenmaker 96+8 PEG block



Screenmaker 96+8 Isopropanol-MPD block





The Results – Crystallant

This test had no standard curves associated with it, as 24 different standard curves (one for each of the different stocks) would have been required. Instead the volume calculation is a simple comparison of the average for each stock/machine combination with the average over all machines for that stock. The precision value in each case is calculated over the 8 points in each stock/machine combination. Only four of the nano-dispensing robots took part in this test – the Oryx, the Mosquito, the Screenmaker 96+8, and the 961.

CV (%)	PEG 10K						PEG 3K					
	20%	25%	30%	35%	40%	45%	20%	25%	30%	35%	40%	45%
Oryx	15.6	3.6	3.3	4.1	9.3	34.4	3.2	4.2	4.8	3.1	3.5	4.7
Mosquito	14.0	18.0	17.9	22.6	40.4	16.1	12.4	15.6	19.4	14.1	18.5	15.4
96+8	8.6	9.2	21.2	39.7	89.5	118.9	8.4	8.4	9.1	12.5	13.2	6.8
961	3.0	1.9	1.9	6.9	26.8	7.0	1.8	5.2	4.4	29.9	2.8	11.0

CV (%)	Isopropanol						MPD					
	0%	10%	20%	30%	40%	50%	20%	30%	40%	50%	60%	70%
Oryx	2.0	4.9	6.7	4.3	6.1	4.9	4.6	6.0	7.4	1.6	5.4	1.8
Mosquito	11.9	13.1	14.9	16.7	15.8	20.1	16.7	18.1	16.3	16.1	13.5	17.0
96+8	9.7	9.9	8.9	7.3	8.3	8.7	11.4	9.7	7.5	7.0	9.5	7.1
961	3.2	8.1	3.7	8.3	6.0	5.9	3.2	2.6	3.1	4.9	1.4	2.3

Volume (nl)	PEG 10K						PEG 3K					
	20%	25%	30%	35%	40%	45%	20%	25%	30%	35%	40%	45%
Oryx	79	88	95	108	123	229	99	97	96	99	103	105
Mosquito	73	76	91	108	122	92	67	71	70	72	73	92
96+8	129	112	93	58	42	9	129	132	133	118	120	104
961	119	123	121	125	113	70	105	100	100	110	103	99

Volume (nl)	Isopropanol						MPD					
	0%	10%	20%	30%	40%	50%	20%	30%	40%	50%	60%	70%
Oryx	91	91	86	82	80	82	81	80	80	89	107	109
Mosquito	74	81	82	89	82	84	77	81	83	83	76	77
96+8	145	140	145	140	141	135	152	144	141	137	134	130
961	90	88	87	89	97	99	90	95	96	91	84	84

0-50% 51-80% 81-120% 120-150% 151%+

Table 2. Summary of the crystallant dispense test for four robots

The upper two tables show the CVs for the 96 different robot/stock combinations. Each robot/stock combination was dispensed eight times. The CVs that are above 10% are printed in a red font.

The lower two tables show the volumes for the 96 different robot/stock combinations. Each volume should be 100nl, and the values shown are calculated by dividing the average fluorescence for each robot/stock combination (eight measurements) by the average fluorescence for each stock using all four machines (32 measurements).

These values are a rough guide at best. The calculated volumes that fall between 80-120nl are coloured green, those that fall between 50-80 or 120-150nl are coloured orange, and those either less than 50nl or more than 150nl are coloured red, as shown in the key.

Discussion – Crystallant

There are quite a few high CVs in this test – some of these are expected, for example the combination of PEG 10K/Screenmaker 96+8. Some were not expected – there is no real reason why the Oryx robot should have a high CV (15.6%) for 20% PEG 10K, when it has low CVs (3.3-9.3%) for 25-40% PEG 10K. There is one value in the set of measurements in the Oryx/20% PEG 10K combination that, if excluded from the

CV calculation, drops the CV to 8% (values are 887,802,986,888,847,91,1019,1276). This value was recorded in well H1, which means that it was likely to have been the 8th drop of the crystallant dispensed – and there is no ready explanation for why this value is unexpectedly large.

The CVs for the Mosquito are likewise disappointingly high across the whole spectrum of 24 crystallant solutions. Looking at the data, it seems that the values recorded in row C was consistently high – so there may have been a problem with that particular tip position. Excluding that row from the CV calculation gives CV values of 8.9%, 11.5%, 17.1%, 24.0%, 43.9%, 12.1%, 6.6%, 4.6%, 6.5%, 6.9%, 12.3%, 5.1% for the 20-45% PEG 10K, 20-45% PEG 3K respectively and of 6.7%, 7.5%, 12.9%, 14.4%, 7.3%, 18.7%, 11.7%, 15.8%, 4.6%, 5.9%, 4.0%, 4.2% for the 0-50% isopropanol and 20-70% MPD respectively.

However, as has been pointed out, none of the vendors really had the opportunity to tweak their machines to get the best data they could for the test, so it is unfair to massage the Mosquito data and not any of the other data.

An instance where some improvement would occur with some tweaking is the Screenmaker 96+8 and the Isopropanol-MPD block. Here the CV data suggest that the machine deals with the solutions easily, but the accuracy data suggests that the machine needs to be calibrated to deliver a lower volume on each dispense.

Conclusion

It seems that the machines in general deal well with protein solutions, and have more of a challenge coping with the range of viscosities and surface tensions found in the average set of crystallization solutions. The machines all did surprisingly well with the smaller drops, with some notable examples performing very credibly even at 20nl dispense volumes.

Updated DispenseTests

Dispense tests with the Mosquito were repeated by TTP Labtech-representatives at a later stage after the workshop. Also some experiments with the Oryx were repeated after the workshop. The (improved) data has been incorporated into the tables below.

Protein dispense test, including adjusted data for the Mosquito (easy and Sticky) and Oryx (Sticky protein)

CV(%)	Oryx		Mosquito		96+8		Microsys		961	
	Easy	Sticky	Easy	Sticky	Easy	Sticky	Easy	Sticky	Easy	Sticky
20nl	12,7	16,8	17,9 (9,5)*	22,6 (8,6)*	3,7	8,0	18,7	76,4	65,3	65,6
50nl	13,3	8,2	7,6	4,8	7,9	4,6	8,5	8,4	16,5	13,5
100nl	6,5	8,6	6,8	2,2	1,5	4,5	9,5	4,0	5,9	4,6
200nl	2,9	6,9	3,1	2,6	3,0	10,1	12,5	3,3	3,8	17,1

Volume (nl)	Oryx		Mosquito		96+8		Microsys		961	
	Easy	Sticky	Easy	Sticky	Easy	Sticky	Easy	Sticky	Easy	Sticky
20nl	24	25	24	24	30	32	21	17	2	2
50nl	49	59	64	55	57	70	42	52	36	2
100nl	77	112	120	119	116	141	72	96	79	63
200nl	144	217	220	240	201	234	121	185	156	119

0-50% 51-80% 81-120% 120-150% 151%+

* when the first column of the 20 nl drops is not taken into account, the CV falls within the 10% range. There are 3 columns (24 drops) prepared with each volume. Apparently a multidispense (one aspiration and 3 dispenses) at 20nl volume causes an inaccuracy at the first dispense.

Protein dispense test, including adjusted data for the Mosquito (All) and Oryx (PEG 10K)

CV(%)	PEG 10K						PEG 3K					
	20%	25%	30%	35%	40%	45%	20%	25%	30%	35%	40%	45%
Oryx	7,3	6,2	7,7	5,3	17,4	7,2	3,2	4,2	4,8	3,1	3,5	4,7
Mosquito	6,4	8,1	8,1	10,3	3,5	6,8	9,4	5,1	8,2	3,2	5,5	3,6
96+8	8,6	9,2	21,2	39,7	89,5	118,9	8,4	8,4	9,1	12,5	13,2	6,8
961	3,0	1,9	1,9	6,9	26,8	7,0	1,8	5,2	4,4	29,9	2,8	11,0

CV(%)	Isopropanol						MPD					
	0%	10%	20%	30%	40%	50%	20%	30%	40%	50%	60%	70%
Oryx	2,0	4,9	6,7	4,3	6,1	4,9	4,6	6,0	7,4	1,6	5,4	1,8
Mosquito	13,9	8,4	4,3	5,0	5,1	6,6	2,9	1,7	3,0	5,4	4,6	4,4
96+8	9,7	9,9	8,9	7,3	8,3	8,7	11,4	9,7	7,5	7,0	9,5	7,1
961	3,2	8,1	3,7	8,3	6,0	5,9	3,2	2,6	3,1	4,9	1,4	2,3

Volume*	PEG 10K						PEG 3K					
	20%	25%	30%	35%	40%	45%	20%	25%	30%	35%	40%	45%
Oryx	92	88	88	84	86	66	99	97	96	99	103	105
Mosquito	95	94	89	96	84	91	91	83	78	74	81	81
96+8	129	112	93	58	42	9	129	132	133	118	120	104
961	119	123	121	125	113	70	105	100	100	110	103	99

Volume*	Isopropanol						MPD					
	0%	10%	20%	30%	40%	50%	20%	30%	40%	50%	60%	70%
Oryx	91	91	86	82	80	82	81	80	80	89	107	109
Mosquito	81	91	94	110	108	98	81	99	114	113	110	125
96+8	145	140	145	140	141	135	152	144	141	137	134	130
961	90	88	87	89	97	99	90	95	96	91	84	84

0-50% 51-80% 81-120% 120-150% 151%+