Performance and reliability testing of a new capillary based non-contact liquid handling system

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Introduction

Direct transfer of compounds from a storage plate into an assay plate can bring desirable quality improvements to screening and can increase throughput. Pre-dilution with aqueous buffers of DMSO compound solutions sometimes results in precipitation of samples which for highly active compounds may lead to false positive results if tips are not washed sufficiently. Pre-dilution is also more time consuming.

The Boehringer-Ingelheim HTS Group in Biberach Germany is looking for a reliable solution for a direct compound transfer. As a consequence of this need, an evaluation of the performance and reliability of a new capillary based non-contact liquid handling system. The CyBi®-HummingWell (Fig.1) was carried out in 2008. The CyBi®-HummingWell transfers nanolitre volumes using an array of precisely sized glass capillaries. The predefined volume to be transferred is drawn into the capillaries automatically on liquid contact and is dispensed into the source plate by compressed air pressure. The evaluation was performed in two steps. First the precision and accuracy of the CyBi®-HummingWell was tested using different cassettes (transfer volume 25 nl, 50 nl, 100 nl, 250 nl). Second, to assess stability of the device under HTS conditions, the reliability of the liquid handler was tested with 300 plates over three days under different conditions.

Figure 1: CyBi®-HummingWell device with plate stacker and wash station

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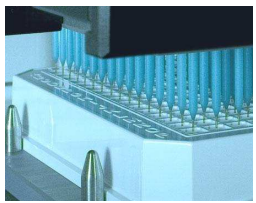
Material:

- Compound Storage Plate (Greiner 384 Small Volume PP Microplate , #784201)
- Assay Plate (Greiner 384 PS Transparent, # 781101)
- OrangeG Dye, Fluka, #75380
- Dimethylsulfoxide 99.5% GC (DMSO), Sigma, #D5879-1GA
- PBS Buffer
- Multidrop Combi Dispenser, Thermo
- Flexdrop IV, Perkin Elmer
- Centrifuge, Kendro
- Safire II Reader, Tecan
- Tecan TRAC HTS System (Figure 3)
- CyBi®-HummingWell with 2 plate stackers
- 384 Well Capillary cassettes (25nl, 50nl, 100nl, 250nl)

Figure 2: CyBi®-HummingWell capillary cassette in a 384 well plate



Precision and accuracy test:

The testing of the precision and accuracy of the CyBi®-HummingWell was performed with 4 different capillary cassettes (25nl, 50nl, 100nl, 250nl) and with 2 different source volumes, 1µl and 2µl, in the compound plate.

The testing was done according to the following procedure:

- Filling of compound plates with 1 µL / 2 µL Orange G / DMSO solution, (Flexdrop PE)
- Visual control and centrifugation
- Aspirate (25nl, 50nl, 100nl, 250nl) Orange G / DMSO out of the compound plate (HummingWell) (automatic on immersion of capillaries)
- Dispense into the dry Assay Plate (HummingWell) (by air pressure pulse)
- Dilution of transferred Orange G / DMSO with 50µl PBS Buffer (Multidrop Combi)
- Centrifugation (1000rpm)
- Signal Readout with Safire II (Absorbance 485nm)

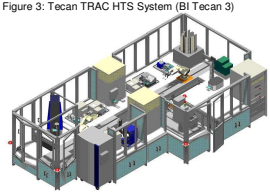
Table 1: Precision an accuracy data for the 25nl, 50nl, 100nl and 250nl cassette

Cassette	50nl			25nl								
Source Vol.	2µl			1µl			2µl			1µl		
Plate	1	2	3	1	2	3	1	2	3	1	2	3
MW [OD]	0.79	0.80	0.78	0.78	0.78	0.77	0.52	0.49	0.48	0.48	0.47	0.47
Volume [nl]							24.8	23.9	23.6	23.9	24	24
STD	0.04	0.04	0.04	0.04	0.04	0.06	0.03	0.03	0.03	0.03	0.03	0.03
CV	4.92	5.44	5.35	5.46	4.90	7.39	6.51	5.86	5.86	6.49	6.15	6.07
Min [OD]	0.70	0.67	0.69	0.66	0.63	0.56	0.41	0.4	0.38	0.39	0.36	0.36
Max [OD]	0.97	1.03	0.99	1.02	0.92	1.11	0.58	0.54	0.54	0.6	0.53	0.55

Cassette	250nl			100nl								
Source Vol.	2µl			1µl			2µl			1µl		
Plate	1	2	3	1	2	3	1	2	3	1	2	3
MW [OD]	3.27	3.05	3.42	3.33	3.28	3.15	1.57	1.56	1.53	1.54	1.47	1.49
Volume [nl]	230	240	219	240	230	233	85.1	87.9	84.7	92.8	88.5	85.5
STD	0.10	0.09	0.15	0.15	0.14	0.10	0.04	0.05	0.05	0.09	0.08	0.07
CV [%]	3.08	3.05	4.25	4.65	4.26	3.04	2.82	3.13	3.3	5.56	5.09	4.37
Min [OD]	3.01	2.48	3.11	2.95	2.94	2.68	1.36	1.35	1.35	1.34	1.21	1.31
Max [OD]	3.60	3.38	3.86	3.80	3.70	3.42	1.68	1.65	1.65	1.79	1.76	1.73

Table 1 gives an overview of the precision and accuracy of the HummingWell device with different capillary cassettes and with 2 different compound source volumes. No outliers were observed. This shows that direct compound transfer can be performed with the HummingWell from a very small source volume of 1µl.

Figure 3: Tecan TRAC HTS System (BI Tecan 3)



2. Reliability of the HummingWell:

The following expanded testing routine was performed under HTS conditions, to assess the reliability of this liquid handler. The testing was done according to the following procedure:

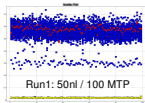
- Compound plates (CP) filled with 2 µL Orange G / DMSO
- Aspirate Orange G / DMSO from CP (HummingWell) (automatic on immersion of capillaries)
- Dispense into the dry Assay Plate (AP) (HummingWell) (by air pressure pulse)
- Capillary Wash with 50% DMSO/Water
- Dispense 50µl Dilution buffer into AP (*Multidrop)
- Centrifugation (*VSPIN)
- Signal Readout Absorbance 492nm (*EnVision)

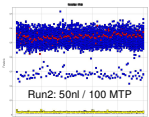
(* steps performed overnight with Tecan TRAC System)

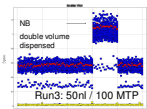
Table 2: HTS Runs performed with HummingWell

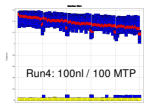
Run No.	Transfer Volume	Number of MTP	Transferred Compounds
1	50nl	100	36 800
2	50nl	100	36 800
3	50nl	100	36 800
4	100nl	100	36 800
5	100nl	100	36 800
6	100nl	100	36 800

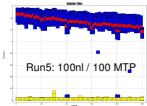
Graph 1-6: The measured signal (OD492nm) for each well is shown as a blue spot in the y-axis against the MTP number in the x-axis. Red spots are high controls, yellow spots are low controls. Pipetting error can be easily seen as blue spots below the main body of data.











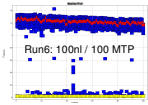


Table 3: Overview of pipetting errors (outliers)

Run No.	Transfer Volume	Outliers
1	50nl	0
2	50nl	1
3	50nl	1
4	100nl	31 (26 x Capillary M14)
5	100nl	8 (4 x Capillary M14)
6	100nl	59 (57 x Capillary M14)

Conclusion

The precision of the HummingWell is much better then the specification of 10%. The accuracy data from the 100nl cassette were higher then 10%, but still in an acceptable range. The accuracy from the 25nl and 250nl cassette are below 10%. The robust transfer of compounds from very low source volumes (1µl) is possible with this device.

The results from the 50nl reliability test demonstrate the robust transfer of about 110.000 wells with only 2 outliers. A single capillary which often gave a lower pipetting volume was observed, but this could have been fixed easily by changing this capillary.

The data from the 100nl cassette show a different situation. One capillary (M14) did not work reproducibly. Some other capillaries failed randomly. The reasons for these outliers are not fully clear. In routine operation the M14 capillary would have been changed in this case.

Finally, we believe that good quality control of the cassettes, validated cleaning and storage procedures from the vendor and intermediate function tests by the user, will all help to support the successful use of this technology in drug discovery.