Performance and reliability testing of a new capillary based non-contact liquid handling system under real HTS conditions

Martin Röhl, Frank H. Büttner, Ralf Heilker, Hans-Dieter Schubert, Martin J. Valler, Robert Ries
/ Boehringer Ingelheim Pharma GmbH & Co. KG, 88397 Biberach Germany/ High Throughput Screening, Department of Lead Discovery

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. The Boehringer-Ingelheim HTS Group in Biberach Germany is looking for a reliable solution for 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®-Well vario Capillary Head (Fig.1), was carried out in 2009. The CyBi®-Well vario Capillary Head 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 destination plate by compressed air pressure. The evaluation was performed in two steps. First the precision and accuracy of the CyBi®-Well vario Capillary Head 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 1200 plates.

Material

- Compound Storage Plate (Greiner 384 Small Volume PP Microplate, #784201)
- Assay Plate (Greiner 384 PS clear, # 781101)
- OrangeG Dye, Fluka, #75380
- Dimethylsulfoxide 99.5% GC (DMSO), Sigma, #D5879-1GA
- De-ionized water
- Flexdrop® IV, Perkin Elmer
- Centrifuge, Thermo
- Safire II Reader, Tecan
- EnVision® Reader, Perkin Elmer
- CyBi®-Well vario Pipettor with 2 plate stackers
- CyBi®-Drop 3D Dispenser
- Capillary Head for CyBi®-Well vario
- Capillary Cassettes (25 nl, 50 nl, 100 nl, 250 nl)

Figure 1:
CyBi®-Well vario pipettor workstation with exchangeable Capillary Head and two plate stacker

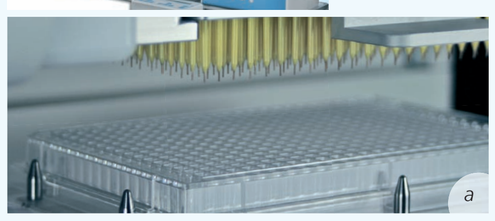


Figure 2 (a/b):
The Capillary Cassette is an array with 384 precisely sized glass capillaries with a predefined transfer volume. The cassette can be easily exchanged by the user.

Methods

Precision and accuracy test:

The testing of the precision and accuracy of the CyBi®-Well vario Capillary Head was performed with 4 different capillary cassettes (25 nl, 50 nl, 100 nl, 250 nl). The testing was done according to the following procedure:

- Aspirate Orange G/ DMSO out of a reagent trough (automatic on immersion of capillaries)
- Dispense into the dry assay plate (by air pressure pulse)
- Dilution of transferred Orange G/ DMSO with 50 µl de-ionized water (Multidrop Combi)
- Centrifugation (1000 rpm) and signal readout with Safire II (Absorbance 485 nm)

Reliability of the Capillary Head

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 storage plates (CP) filled with 2 µL Orange G / DMSO (Flexdrop)
- Aspirate Orange G/ DMSO from CP (Capillary Head/ automatic on immersion of capillaries)
- Dispense into the dry assay plate (AP) (Capillary Head/ by air pressure pulse)
- Capillary wash with DMSO/ Water (1:1)
- Dispense 50 µl Dilution buffer into AP (CyBi®-Drop 3D)
- Centrifugation and Signal Readout (Absorbance 492 nm/ EnVision Reader)

	25nl					50nl					100nl					250nl			
plate	1	2	3	4		1	2	3	4		1	2	3	4		1	2	3	4
average (OD)	0,63	0,64	0,63	0,62		0,99	0,99	0,99	0,99		0,59	0,59	0,59	0,59		1,44	1,44	1,43	1,43
sd	0,03	0,04	0,04	0,03		0,04	0,04	0,04	0,04		0,01	0,01	0,01	0,01		0,02	0,02	0,02	0,02
cv %	5,38	5,92	5,67	5,60		4,20	4,04	4,19	3,99		1,96	1,94	1,97	2,00		1,25	1,26	1,28	1,29
max	0,70	0,72	0,70	0,69		1,11	1,09	1,16	1,10		0,62	0,62	0,62	0,62		1,49	1,48	1,48	1,48
min	0,50	0,51	0,51	0,50		0,88	0,87	0,88	0,88		0,56	0,56	0,55	0,54		1,37	1,38	1,37	1,38
Volume (nl)	28	29	28	28		45	45	45	45		94	94	94	94		228	228	228	228

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

Results

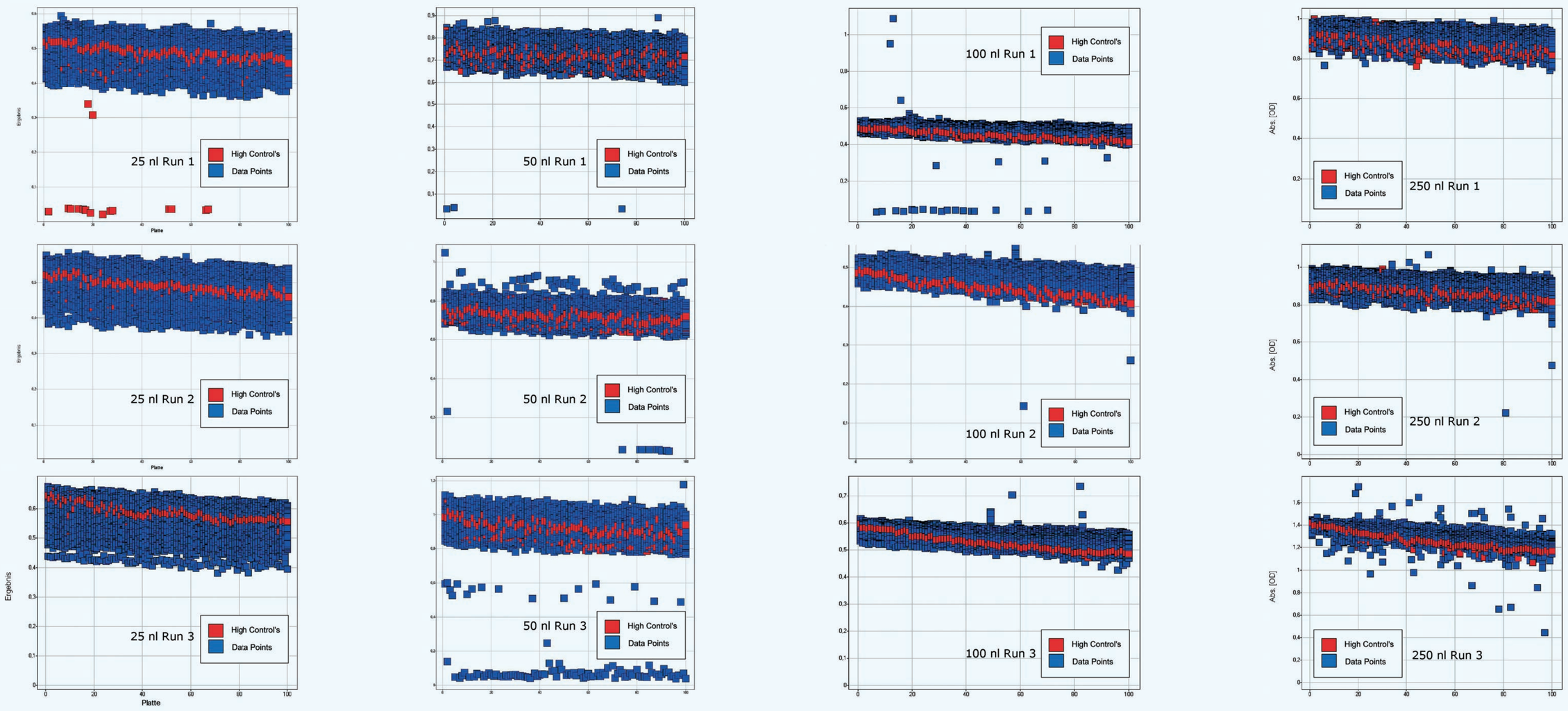
The precision of the Capillary Head is much better then the specification of 10%. The accuracy data from the 25 nl cassette were higher then 10%, but acceptable. The accuracy from the 50 nl, 100 nl and 250 nl cassette are below 10%. No outliers.

Table 2:
This table gives an overview about the performed runs. To reduce the number of plates and to demonstrate the multi use of compound plates, a set of 20 compound storage plates were reused 5 times as source plate for aspiration. Dispensing was done into 100 empty assay plates. This procedure was done with the 4 capillary cassettes and with 3 repetitions/ runs:

Run No.	Cassette Type	Number of CP	Number of AP	Transferred Compounds
1	25nl	20	100	36800
2	25nl	20	100	36800
3	25nl	20	100	36800
1	50nl	20	100	36800
2	50nl	20	100	36800
3	50nl	20	100	36800

Run No.	Cassette Type	Number of CP	Number of AP	Transferred Compounds
1	100nl	20	100	36800
2	100nl	20	100	36800
3	100nl	20	100	36800
1	250nl	20	100	36800
2	250nl	20	100	36800
3	250nl	20	100	36800

Graph 1-12: The measured signal (OD492 nm) 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. Pipetting error can be easily seen as blue or red spots below the main body of data.



Run No.	Transfer Volume	Transferred Wells	Outliers	Remarks
1	25 nl	36.800	14	14 outliers because of short capillary B23, adjustment of B23 height after run (a)
2	25 nl	36.800	0	No outlier
3	25 nl	36.800	0	No outlier
1	50 nl	36.800	3	Capillary G4 was to short, height adjustment after experiment (a)
2	50 nl	36.800	11	10 outliers because of short capillary G4, one outlier from capillary J13 (a)
3	50 nl	36.800	101	Several outliers because of short capillary G4. Capillary was not fixed properly in shaft. Could have been fixed easily by changing this capillary. (a)
1	100 nl	36.800	17	Capillary A17 defect, exchanged after run.
2	100 nl	36.800	2	Lower signal caused from diluted source well O20 through droplets from capillary wash. A optimization of the washing station to avoid droplets is already addressed by CyBio AG.
3	100 nl	36.800	0	No outlier
1	250 nl	36.800	0	No outlier
2	250 nl	36.800	0	No outlier, reduced volume capillary L5 and N3
3	250 nl	36.800	0	No outlier but increased scattering. Routine maintenance of capillaries by the CyBio cleaning procedure (citric acid / sodium hydroxide) will help to avoid these effects.

Table 3: Overview of pipetting errors (outliers)

Results

The most outliers were caused by bad height adjustment of single capillaries (a). We think this can be avoided in the future simply by a quality control, based on the transfer test protocol from a source volume of 2 µl, to detect capillaries which are not proper adjusted. A decrease of the OD signal from plate one to plate one hundred was observed in each run. This might be caused by the water absorption of the DMSO, which ends in a dilution of the source plates. These source plates were used 5 fold in each run, so the dye concentration decreased with the water absorption of the DMSO solvent.

Conclusion

The precision of the Capillary Head is much better then the specification and the accuracy data are in an acceptable range. The results from the reliability test demonstrate the robust transfer from a source volume of 1-2 µl, which is a far low dead volume range. The key learning's are, that the height adjustment of the capillaries is very important for direct compound transfer from only 1-2 µl compound solution. Therefore we believe that good quality control of the cassettes will solve this problem. Furthermore the optimized washing (now developed by the vendor), intermeditate function tests and cleaning routines by user, will all help to support the successful use of this technology in drug discovery.

Contact

Robert Ries, Boehringer Ingelheim Pharma GmbH & Co. KG, Dept. of Lead Discovery/ High Throughput Screening Group, Phone: +49 (7351) 54-4253, Email: robert.ries@boehringer-ingelheim.com

This poster has been presented at MIPTEC 2009, 13./ 14. October 2009, Basel (Switzerland)