

TECHNICAL NOTE

Precision of the CyBi®-Well vario 96/25 µL Head, Example Data of Different Liquids, Pipetting Modes and Disposable Tips with Fluorescence Readout

Katrin Undisz PhD, Heidi Prüfer, and Hanno Hermann PhD, CyBio AG

Key words

precision, fluorescence, disposable tips, low volume handling, DMSO, buffer, wet and dry pipetting

Summary

In this study the CyBi®-Well vario 96/25 µL pipetting head was used to determine the liquid handling precision with a fluorescence readout over a broad volume range using different pipetting modes, different disposable tips and liquids with different surface tensions. The data show the excellent pipetting performance of the CyBi®-Well vario 96/25 µL pipetting head under various conditions and provide a tool that supports the choice of the right experimental settings and disposable tips for a broad range of customer specific applications.

Introduction

The CyBi®-Well vario is well known for fast and precise simultaneous pipetting. Six* pipetting heads allow the reliable handling of different liquids over a broad volume range.

CyBio's in house specification check and quality control is performed with a standardized absorption method (p-Nitrophenol as dye solved in 0.1N NaOH) that is described in detail in every CyBi®-Well or CyBi®-Well vario manual and that is also used to determine the official specification values.

In many laboratories fluorescein solutions are used to validate liquid handling devices, because this fluorescent dye is very cost effective, stable for several months and safe to handle due to low toxicity (1).

This technical note intends to complete the absorption data by fluorescence data generated with a simple fluorescence intensity measurement. However, it is important to mention, that typically the precision of absorption measurements yields a better resolution than the precision of fluorescence measurements (difference approximately 1%) and that non normalized simple fluorescence measurements are influenced by a bundle of parameters outside the liquid handling device (2).

In this technical data sheet we show precision data of the CyBi®-Well vario with the 96/25 µL head using different volumes, different tips, dye solutions with different surface tensions, and different pipetting modes that were determined by a simple fluorescence intensity measurement. These data are example data from typical routine work in our application lab. The data also cover the low volume range below the specification limit and can only be reproduced when considering all methodical details that are described below.

References:

1. Harris D.L., and Mutz M., "Debunking the Myth: Validation of Fluorescein for Testing the Precision of Nanoiter Dispensing", JALA August 2006, pp. 233-239.
2. Rhode H., Schulze M., Renard S., Zimmermann P., Moore T., Cumme G. A., and Horn A., "An improved Method for Checking HTS/uHTS Liquid-Handling Systems", J Biomol Screening December 2004, 9(8), pp. 726 - 733.

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Materials and Reagents

- CyBi®-Well vario with 96/25 µL head (for dye solution) and 96/250 µL head (for buffer)
 - 250 µL shallow well tips (CyBio # OL 2001-25-300)
 - 250 µL deep well tips (CyBio # OL 2001-25-350)
 - 25 µL tips (CyBio # OL 2001-25-250)
 - 10 µL tips (CyBio # OL 2001-25-230)
- 96 well plates PS black (Greiner bio-one # 655 076)
- OmniTrays (Nunc # 140156) as disposable reservoirs
- Fluorescein-Sodium (Standard Fluka # 46960)
- Fluorescein (Reference standard Molecular Probes # F1300)
- PBS (Sigma # P3813)
- DMSO (SeccoSolv Merck Darmstadt # 1.02931.1000)
- Adhesive foil (Nunc # 236269)
- PolarStar (BMG Labtechnologies) with filter set 485nm (excitation wavelength) and 520nm (emission wavelength)

Methods

The precision test was performed in black 96 well plates with a final volume of 200 µL and a final dye concentration of 300 nM. The experimental settings for the different test volumes are described in Tab.1.

test volume [µL]	buffer volume [µL]	fluorescein working solution [µM]	final fluorescein concentration [nM]
20	180.0	3	300
10	190.0	6	300
5	195.0	12	300
2	198.0	30	300
1	199.0	60	300
0.5	199.5	120	300
0.2	199.8	300	300

Tab.1: Experimental settings to measure the precision of the CyBi®-Well vario 96/25 µL head

To obtain a test solution with low surface tension Fluorescein was dissolved in DMSO, for a test solution with high surface tension Fluorescein-Sodium was dissolved in PBS buffer. The working solutions with the different concentrations were prepared by diluting the dye solution with the highest concentration. All solutions were filtrated before use.

All CyBi®-Well vario pipetting methods were set up in CyBio Control 3.40 according to the following rules:

- one set of new tips per volume and pipetting mode
- piston speed: 100 rpm (characterizes the flow rate of the liquids during aspiration/dispensation; = 41µL/s with a 250µL head and 4 µL/s with a 25 µL head at 100 rpm, respectively)
- stage speed: 50 rpm
- priming of tips: at least 1x with highest tip volume
- break of 1s after every aspiration and dispensing step
- immersion depth 1 - 2 mm

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- final volume 200 μL per well
- final dye concentration 300 nM
- aspiration always with overstroke
- the first pipetting cycle was always dispensed back into the reservoir
- pipetting of the desired volume as part of the total volume into the test plate
- **dry pipetting** means dispensing the desired volume of the dye solution about 1 mm above the bottom of the dry plate and then touching the droplet by moving the stage up, PBS buffer is used to fill the wells up to the final volume of 200 μL
- **wet pipetting** means dispensing the desired volume with tips immersed for about 1 mm into the provided PBS buffer liquid
- eject the residual volume with maximum overstroke back into the source reservoir or waste
- immediate sealing of the plates
- shaking of the plates for at least 10 minutes, waiting for at least 30 minutes
- centrifugation of the plates for 2 minutes at 2000 rpm
- readout was performed not earlier than two hours after finishing the pipetting procedure

Results and Discussion

In Tab.2 the precision data of the CyBi®-Well vario 96/25 μL head using different volumes, different tips, dye solutions with different surface tensions and different pipetting modes are summarized. The precision was calculated as percentage standard deviation (coefficient of variation = CV in %) over a 96-well microplate. Three microplates were prepared per volume and the results were averaged.

Tips	Test volume	DMSO dry [%CV]	DMSO wet [%CV]	Buffer dry [%CV]	Buffer wet [%CV]
25 μL tips					
	20 μL	1.6	1.9	1.9	1.7
	10 μL	1.8	1.6	1.9	1.7
	5 μL	2.1	1.5	2.2	1.8
	2 μL	2.0	2.0	2.8	1.6
	1 μL	1.9	1.7	3.2	1.6
	0.5 μL	3.1	2.2	7.1	2.3
	0.2 μL	6.1	3.0	(n. r.)	11.5
10 μL tips					
	10 μL	1.3	1.5	1.5	1.7
	5 μL	1.6	1.8	1.6	1.6
	2 μL	1.7	1.6	2.4	1.8
	1 μL	1.8	1.9	3.6	2.0
	0.5 μL	1.9	1.7	12.8	2.5
	0.2 μL	3.3	2.5	(n. r.)	2.8

Tab.2: Overview of precision data (fluorescence readout) that were obtained with the CyBi®-Well vario 96/25 μL head for various volumes with various tips, various liquids and various pipetting modes ($n=3$), constellations that result in CV values higher than 15% are not recommended (n. r.).

The results indicate, that the CyBi®-Well vario 96/25 μL head allows highly precise high and low volume handling in both, wet to wet and wet to dry pipetting modes.

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As expected, the precision data are somewhat better in the wet mode than in the dry mode (see Fig.1). Because of the lower internal air volume between liquid surface and pistons the results with the 10 μL tips, in most cases, are better than those obtained with the 25 μL tips (see Fig.2). In the wet pipetting mode and also while dry pipetting of DMSO solutions, the precision is excellent down to volumes as low as 0.2 μL . This strongly indicates the special suitability of the 10 μL tips for compound handling.

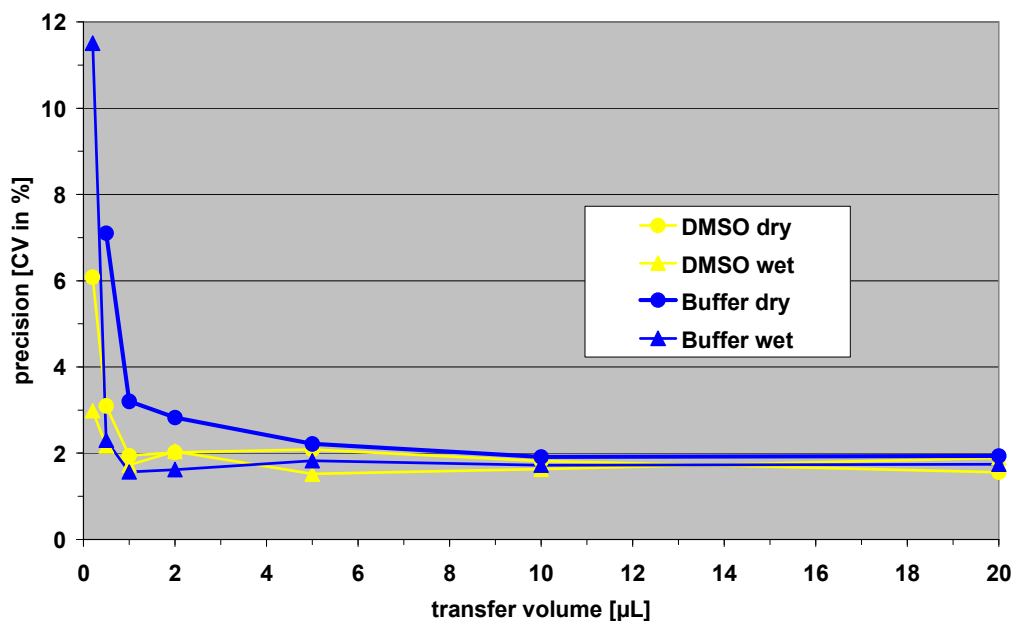


Fig.1: Precision data (fluorescence readout) obtained with the CyBi®-Well vario 96/25 μL head, comparison of pipetting DMSO and buffer in the dry and wet mode with 25 μL tips.

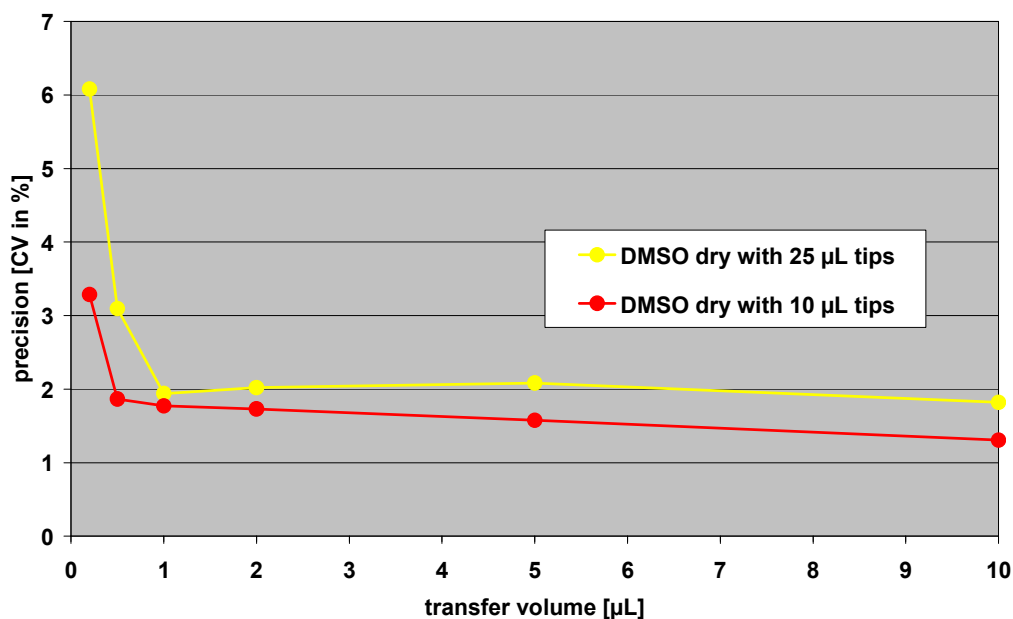


Fig.2: Precision data (fluorescence readout) obtained with the CyBi®-Well vario 96/25 μL head, comparison of pipetting DMSO in the dry mode with 25 μL and 10 μL tips.

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Buffer solutions can reliably be handled in the dry mode down to a volume of 1 μL . Precision errors are less than 5 % with both 25 μL tips and 10 μL tips. With further decreasing volumes the droplet formation at the tip orifice becomes more and more critical due to the high surface tension (that has to be overcome) and the precision error increases (for 0.5 μL to 7.1 % CV with 25 μL tips and to 12.8 % CV with 10 μL tips, see Tab.2).

Further, the quality of the microplates, especially the flatness of the plate bottom, is essential for the outcome of wet to dry liquid transfers. CyBi[®]-Well and CyBi[®]-Well vario allow a vertical adjustment of the stage height, in steps of 1/10 mm, for touching the tips to the plate bottom in order to reliably deposit a droplet. Often 1/10 mm stage height difference is enough to cross the rubicon between good precision data and not acceptable results.

Sometimes low volumes of compound solutions are stored in deep well plates that can not be handled with the 25 μL or 10 μL tips. In these cases 250 μL deep well tips of CyBio can be used also with the 96/25 μL pipetting head to prepare shallow well compound plates or to handle the compounds directly in the wet mode down to volumes as low as 1 μL (data see Tab. 3). The opportunity of the common usage of alternative disposable tips with a certain pipetting head is a general benefit of the CyBio equipment due to the special CyBio tip-sealing technology.

Tips	Test volume	DMSO dry [%CV]	DMSO wet [%CV]
250 μL deep well tips			
	5 μL	3.1	1.5
	2 μL	(n. r.)	1.6
	1 μL	(n. r.)	2.1

Tab.3: Precision example data (fluorescence readout) using the CyBi[®]-Well vario 96/25 μL head with 250 μL deep well tips for various volumes with various tips, various liquids and various pipetting modes (n=3), constellations that result in CV values higher than 15% are not recommended (n. r.).