Testing the precision and accuracy of the VolumeCheck 384 for standard labware

Overview

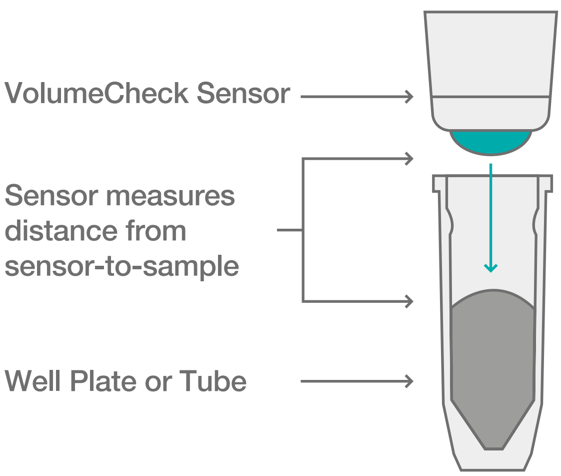
The following experiments were designed to capture the precision and accuracy of SPT Labtech's BioMicroLab VC384 instrument across various commonly used labware. As there are seemingly endless labware types, formats, and variations, the subset selected for testing was chosen as a good representative for the population. The data generated for the testing aims to provide researchers with a better understanding of where the VC384 instrument fits within various workflows and help them determine if the precision and accuracy of the instrument meet their needs at a given volume range.

Introduction

SPT Labtech's BioMicroLab VolumeCheck instruments (VC100 and VC384) are benchtop devices that connects to a Windows 7/8/10 PC via a USB connection. A control program running on the PC manages the instrument and organizes data collected by the VolumeCheck sensor.

VolumeCheck sensor is a non-contact ultrasonic sensor to measure the distance from the sensor to the surface of the sample liquid in a tube or well. Using a calibration table of distance measurements vs. known volumes, the VolumeCheck sensor can be used to measure unknown sample volumes in well plates and test tubes.

Figure A (below). To determine sample volume, a calibration plot of sensor-to-liquid distance and sample volume is required. Data for this calibration plot is obtained by incrementally dispensing and measuring the sample volume. The calibration plot data is stored in a calibration file that can be used for future volume measurements. The calibration file is specific to the selected labware and sample type.



key findings

- The VolumeCheck sensor maintains high-quality precision and accuracy across the labware cohort – more variation was found near the bottom and transition point of a tube or well.
- Properly characterizing the selected labware with a fitting calibration curve is paramount to getting accurate readings during sample runs. It is important to know what the intended volumes, labware, and liquid types are when creating calibration curves.
- The VC384 was found to consistently detect down to 500nL of sample volume in a specific labware – an impressive resolution for potential workflow applications.

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The VolumeCheck sensor can resolve small distances in sample height. However, the system's specification is influenced by variations in labware and samples. Samples that are 'well behaved', (i.e. that have no air bubbles in the liquid and no droplets on the inside wall of the tube) should provide sensor-to-liquid distance data that can be interpreted as sample volume.

VolumeCheck sensor resolution may be influenced by:

- Differences in labware, e.g. conical, V-shaped, or flat-bottom well plates and tubes
- Droplets on the wall of a tube or well
- Air bubbles in sample
- Shape of the sample's meniscus (uniform surface provides best measurement) (see Figure B)
- Plate-to-plate (or tube-to-tube) dimensional variations

Within the labware and sample limitations described above, the SPT Labtech's BioMicroLab VolumeCheck instruments can provide a highly consistent automated method for inspecting well plates and tube racks to verify the sample volume.

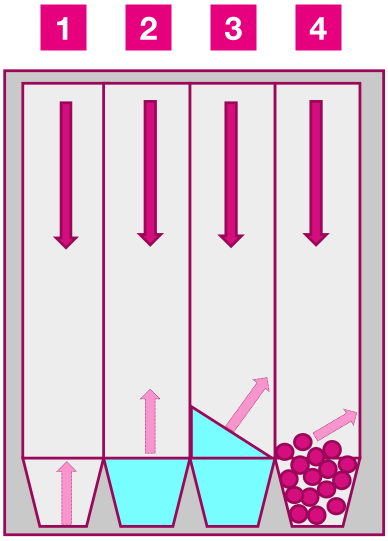

Figure B (right): This figure illustrates how the ultrasonic VolumeCheck sensor behaves under various conditions. Lane 1 and 2 show the expected return wave (light pink arrow) received from an empty well (lane 1) and from a relatively flat liquid meniscus (lane 2). Lane 3 contains a liquid with a highly skewed meniscus, which can happen with small volumes that do not fully cover the bottom of a well or liquids on either extreme of the viscosity spectrum. Lane 4 shows beads or a dry sample in a well and how the unevenness of the surface can greatly impact the distance measurement from the VolumeCheck sensor.

Materials and methods

Labware tested:

Labware description	Product number	Test liquid
Greiner-384 107µL	784201	aqueous (water)
Greiner-384 131µL (Flat Bottom)	781201	aqueous (water)
Greiner-96 Deep Well Plate U-Bottom1200µL	780215	aqueous (water)
Micronic-96 V-Bottom 0.75mL SC	MP3211-Z20	aqueous (water)
Sarstedt-24 (6x4) BML Rack 2mL	72-694-406	aqueous (water)
Eppendorf twin.tec 384 PCR	951020702	aqueous (water)
Eppendorf twin.tec 96 PCR	951020401	aqueous (water)
Greiner-384 Deep Well V-Bottom	781271	aqueous (water)

- The selection of labware chosen represents the diversity of types of labware used by researchers but is not exhaustive.
- Only new labware was used for the experiment.
- The testing was conducted with water only. The dispensing of the water was carried out with SPT Labtech's dragonfly and apricot S2 (for the dispensing into deep-well-format plates and tubes) platforms.
- All labware was centrifuged after dispensing.
- The testing was conducted at room temperature (~23C).

Calibration curves used:

Labware Description	Min Working Vol.	Max Working Vol.	Calibration Volumes
Greiner-384 107µL	1	90	0, 5, 10, 20, 40, 60, 80, 100
Greiner-384 131µL (Flat Bottom)	15	145	0, 10, 20, 30, 40, 50, 70, 95, 120, 145
Greiner-96 Deep Well Plate U-Bottom1200µL	50	1100	0, 40, 80, 115, 150, 200, 300, 500, 700, 900, 1100
Micronic-96 V-Bottom 0.75mL SC	10	600	0, 10, 20, 40, 60, 80, 100, 150, 200, 300, 500
Sarstedt-24 (6x4) BML Rack 2mL	10	2000	0, 10, 25, 50, 75, 100, 150, 200, 300, 450, 600, 1000
Eppendorf twin.tec 384 PCR	NA	45	0,2,4,6,10,15,20,25,30, 35, 40
Eppendorf twin.tec 96 PCR	NA	150	0, 5, 10, 15, 25, 40, 60, 80, 100, 120, 140
Greiner-384 Deep Well V-Bottom	20	225	0, 20, 40, 60, 80, 100, 125, 150, 175, 200, 225

- Each item of labware was calibrated using a volume range based on the manufacturer's maximum working volume and minimum working volume, where applicable.
- Calibration curves were carefully generated by the VolumeCheck software to reflect the shape of each tube and well. Note that some calibration curves are relatively straightforward (Image A) and some require a divider and two calibration lines (Image B).

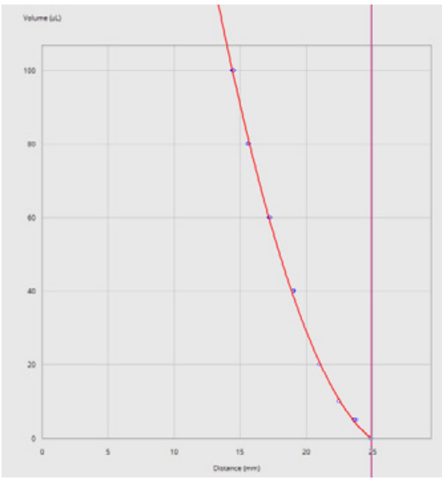

Image A

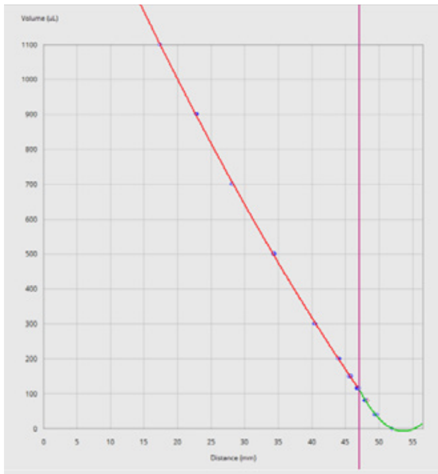

Image B

Sample plates used:

Labware Description	Scans per well	Wells per volume	Test Volumes (uL):
Greiner-384 107µL	5	8	0, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100
Greiner-384 131µL (Flat Bottom)	5	8	0, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 115, 130, 145
Greiner-96 Deep Well Plate U-Bottom1200µL	5	8	0, 30, 50, 75, 100, 150, 200, 300, 400, 500, 750, 1000
Micronic-96 V-Bottom 0.75mL SC	5	8	0, 10, 20, 30, 40, 50, 75, 100, 150, 200, 300, 500
Sarstedt-24 (6x4) BML Rack 2mL	5	4	0, 10, 25, 50, 75, 100, 125, 250, 450, 650, 900, 1200, 1500
Eppendorf twin.tec 384 PCR	5	8	0, 0.5, 1, 2, 3, 5, 7.5, 10,12.5, 15, 17.5, 20, 25, 30, 35, 40
Eppendorf twin.tec 96 PCR	5	8	0, 2.5, 5, 10, 15, 20, 25, 40, 60, 80, 100, 120
Greiner-384 Deep Well V-Bottom	5	8	0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220

Number of data points: 8* wells of each volume per plate, 2 plates per labware type

Total number of data points for each volume tested: 5 scans per well x 8 wells per plate x 2 plates = 80 data points.

**The Sarstedt-24 tube rack was an exception: only 4 wells were used for each volume.*

Results and analysis

96 - Deep Well Block

Labware Description	Metric	25-50µL	50-100µL	100-300µL	300-500µL	500-1000µL	1000µL and Up
Greiner-96 Deep Well U-Bot 1200µL	% CV	10	5	2	1	<1	<1
	± µL	3.3	2.9	3.4	3.8	4.7	6.6

384 - Deep Well Block

Labware Description	Metric	10-20µL	20-40µL	40-80µL	80-125µL	125-250µL
Greiner-384 Deep Well Master Block	% CV	4	3	1	1	1
	± µL	2.2	2.6	3.1	3.3	4.9

Tube Labware

Labware Description	Metric	0-25µL	25-50µL	50-100µL	100-300µL	300-500µL	500-1000µL	1000µL and Up
Micronic-96 V-Bot 0.75mL Ext Thd	% CV	9	11	5	1	<1	<1	
	± µL	1.1	4.8	3.2	3.2	9.4	11.8	
Sarstedt 2mL Tube in 24 well BML rack	% CV			16	7	1	1	<1
	± µL			11.0	10.5	4.0	18.3	27.1

Assay Plates

Labware Description	Metric	0-3µL	3-5µL	5-10µL	10-20µL	20-40µL	40-80µL	80-125µL	125-250µL
Greiner-384 107µL	% CV	2	2	1	3	2	1	1	
	± µL	0.7	1.5	1.9	2.2	1.2	2.1	3.8	
Greiner-384 152µL Flat Bottom	% CV			7	6	4	1	<1	<1
	± µL			0.6	0.8	1.6	2.1	4.4	4.6

PCR Plates

Labware Description	Metric	0-1µL	1-3µL	3-5µL	5-10µL	10-20µL	20-40µL	40-80µL	80-125µL	125-250µL
Eppendorf-384 Twin Tec PCR	% CV	15	10	5	4	3	3	2		
	± µL	0.3	0.4	0.7	0.8	0.6	0.8	1.4		
Eppendorf-96 Twin Tec PCR	% CV		10	10	7	4	3	1	1	1
	± µL		1.4	1.3	1.2	1.4	1.7	2.5	2.7	2.8

Precision across the subset labware and volumes was relatively high: the vast majority of %CV was <= 5. The higher CVs are typically at very low volume ranges for the given labware. It is important to understand the actual workflow use case for volume verification as CVs may need to be tighter on average for inventory tracking or normalization, but looser for quality control checks and output volume checks. Accuracy follows the same trend for the most part with perhaps more sensitivity around the transition point of a tube or well. This is not unexpected since the VC384 is using a distance measurement and a small change in distance can correspond to a relatively large change in volume at these transition points (see Figure C).

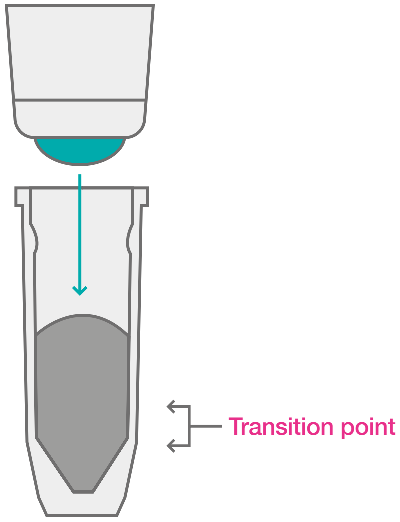

Figure C (right): Many tubes and plates have a transition point along the inner wall of the tube or well. These are most commonly caused by a V or U bottom that eventually hits an inflection point and continues smoothly for the rest of the tube or well length. For accurate readings with the VolumeCheck sensor, it is crucial to characterize this transition point thoroughly in the calibration curve because small changes in distance can correspond to large differences in volume.

It is also important to note that on the low end, the VC384 was able to detect down to 500nL in a 384-well Eppendorf PCR plate. At the higher end, precision and accuracy remain very high: exceeding 1000µL for various labware. This shows the VC384 can perform accurate volume verification at both ends of an extremely broad spectrum.

Conclusion

When measuring volumes using an ultrasonic sensor, it is important to ensure calibration is carefully considered to ensure accurate readings. Proper calibration methods take labware, liquid type, room temperature, and centrifugation into account. Ideally, a high-accuracy liquid dispensing method should be employed, such as provided by the positive-displacement dispensing technology of the SPT Labtech dragonfly.

Taking a particular labware item's transition point into account is important, so it can be characterized during calibration to provide a better understanding of where small changes in measurement may account for larger jumps in volume. If these changes are not well defined in the calibration curve, precision and accuracy will be much more variable.

When calibrated accurately and used within a given labware item's working volume specifications, the VC384 delivers precise and accurate volume data to a given workflow. This provides researchers with useful metadata to manage inventory, serve as a quality control checkpoint to reduce process risk, and verify that the various liquid handling instruments used in a workflow are dispensing as expected.