Accuracy Calculation for the Checkit® Go

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The Checkit® Go is a disposable, single-use cartridge for measuring the volume dispensed by automated liquid handling systems (ALHS) and pipettes, including multi-channel pipettes. The 8 channels are spaced 9 mm apart, the same spacing as for 96 well microplates. They work by capillary action, whereby the liquid sample is dispensed into the wells in the flip tab, the flip tab is flipped up, and then capillary action draws the liquid up the capillary. The height of the liquid sample is compared against graduations, analogous to a bulb thermometer.

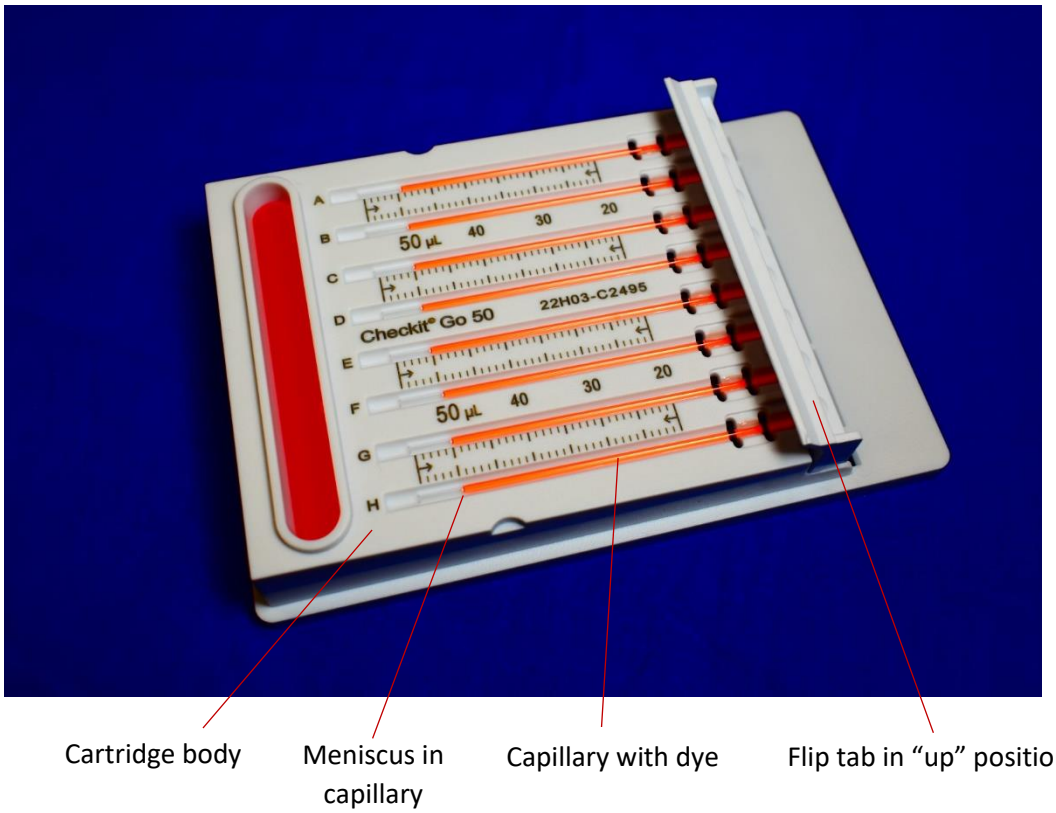


***Figure 1**. A Checkit Go cartridge with the basic parts labeled.*

In **Figure 2**, the cartridge has graduations from 4 µL to 12 µL, with each minor graduation representing an increment of 0.2 µL and each major graduation representing an increment of 1.0 µL. In this figure, the flip tab is "down" with the dispense well exposed and ready to accept liquid. Note the right end of the capillary, where the liquid enters, hangs off the cartridge body, extending about 13 mm to the right of the location where it is bonded to the cartridge body.

The cartridges are typically accurate to better than 1% and are guaranteed to be within 2%. The accuracy is the sum of several factors, including component accuracy, manufacturing accuracy, differences in environmental conditions between the factory and the place of use, and the shape of the meniscus. The contribution of each factor is described in the following sections. Table 1 displays information abou the range and graduations for each Checkit Go model.

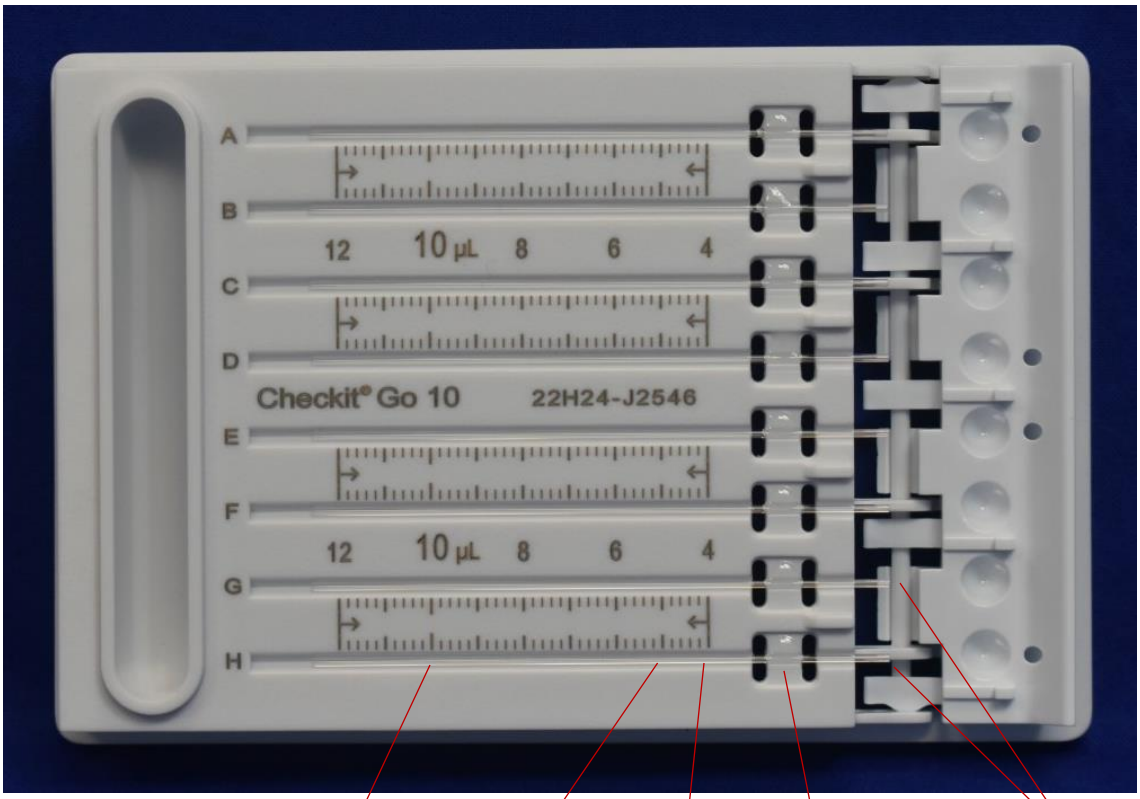


***Figure 2**. A Checkit Go cartridge showing the graduations. The dispense volume target range of the cartridge is from 50% of nominal to the nominal value, shown in a larger font. The range for this cartridge is 5 to 10 µL. The graduations extend below and above this range so that extent of the dispense error can be measured.*

Table 1. Range of graduations for each Checkit Go model.

	Checkit Go 5 µL	Checkit Go 10 µL	Checkit Go 20 µL	Checkit Go 50 µL
Range of graduations	2.0 to 6.0 µL	4.0 to 12 µL	8 to 22 µL	20 to 55 µL
Dispense volume target range (from 50% nominal to nominal value)	2.5 to 5.0 µL	5 to 10 µL	10 to 20 µL	25 to 50 µL
Minor graduation increments	0.1 µL	0.2 µL	0.5 µL	1 µL
50% of nominal graduation	2.5 µL	5 µL	10 µL	25 µL
Nominal graduation	5 µL	10 µL	20 µL	50 µL

Cartridge Inspection for Correct Assembly

Every Checkit Go cartridge is inspected using several cameras, a confocal sensor, and proprietary software. The image analysis and the confocal sensor systems ensure that the correct components are assembled on every cartridge, including that every cartridge has the correct capillaries, exactly one capillary, centered in each capillary trough, properly positioned axially, and properly bonded, and that every cartridge has the correct flip tab and that the flip tab is properly assembled.

Material Accuracy

There are two salient component features in the Checkit Go that affect measurement accuracy: the internal diameter of the glass capillary and the positioning of the graduations on the plastic cartridge body with respect to the capillary.

The internal volume of the glass capillaries is certified by their ISO9001 registered manufacturer to be accurate to within 0.5%. Each lot is also inspected by Next Advance using a 5-digit balance calibrated with NIST traceable weights.

Table 2. Accuracy of the capillaries for each Checkit Go model.

	Checkit Go 5 µL	Checkit Go 10 µL	Checkit Go 20 µL	Checkit Go 50 µL
Accuracy of capillary internal volume per unit length	±0.5%	±0.5%	±0.5%	±0.5%

We have independently verified their consistency of cross-sectional area along their length, cutting four representative capillary samples: corresponding to the four Checkit Go models, approximately every 10 mm. A third-party, ISO 17025 registered, metrology firm, used their digital comparator to measure the internal diameters of section of capillary. The measured values validate the expected inner diameters, and established their uniformity with radial variance of less than 2 µm.

Manufacturing Accuracy

The polystyrene Checkit Go bodies are laser marked by Next Advance. Placement of the graduations is critical for the Checkit's accuracy. The positions of the graduation marks is analyzed using a telecentric camera system and image analysis software developed in-house for this purpose. See Appendix A for an explanation of the system and its accuracy.

Next, at the start of every production run, several cartridges are manufactured and the position of several nominal graduations and several randomly selected graduations, with respect to the entrance end of the corresponding capillary, are measured. The position of the array of graduations is adjusted and new measurements taken. Our criterion is that all markings must be within 0.18 mm with respect to the entrance end of the capillaries. Considering that our digital calipers are accurate to within 0.02

mm, that leaves a maximum permissible error of 0.20 mm. During several production runs, all of the graduations on all of the cartridges were measured with the telecentric camera system. All laser markings were positioned correctly, confirming that there is negligible positional variation of the markings within a production run. In addition, every cartridge is inspected by a second telecentric camera system which measures the distance from the entrance end of all 8 capillaries to the corresponding first graduation on every cartridge that we release. The second telecentric camera system is calibrated using NIST traceable standards and has an accuracy of ± 21.6 µm.

Table 3. Positional accuracy of the graduations with respect to the corresponding capillaries.

	Checkit Go 5 µL	Checkit Go 10 µL	Checkit Go 20 µL	Checkit Go 50 µL
Positional accuracy of the graduations	± 0.20 mm	± 0.20 mm	± 0.20 mm	± 0.20 mm
Length of capillary for 50% of nominal volume	27.5 mm	27.5 mm	32.0 mm	32.5 mm
Accuracy at 50% of nominal graduation	± 0.73%	± 0.73%	± 0.63%	± 0.62%
Length of capillary for nominal volume	55.0 mm	55.0 mm	64.0 mm	65.0 mm
Accuracy at nominal graduation	± 0.36%	± 0.36%	± 0.31%	± 0.31%

Environmental Conditions

Environmental conditions include air pressure and temperature.

Air Pressure Effects. The effect of air pressure on the liquid being measured is irrelevant and negligible. They are irrelevant since we are directly measuring volume, not density. They are negligible because in the most extreme case, such as the change in air pressure from the Next Advance factory at 180 meters above sea level to an altitude of 2500 meters, reduces the density of water by 0.0003%. or, since we are measuring volume, not density, this change in density is irrelevant, not to mention so slight as to be negligible. The effect of the change in air pressure on the cartridge, composed of solid materials: the plastic cartridge body and the glass capillaries, is far less, and thus extremely negligible.

Temperature Effects. There may be a difference in temperature from where the Checkit Go is manufactured and the laboratory in which the Checkit go is used. There are two factors to consider: (i) changes to the internal cross-sectional area of the glass capillaries, and (ii) changes in the distance from the capillary entrance to the markings. If the laboratory is warmer than the manufacturing facility, (i) the glass capillaries will have a slightly larger interior volume per unit length and (ii) the entrance end of the glass capillary up to the adhesive bond and the plastic housing will have expanded such that the graduations are further from the entrance end of the capillary, thus the dye will not extend as far with respect to the markings, leading to slightly lower readings. If the laboratory is

colder, than the effects will be the reverse: (i) the interior of the capillaries will be smaller and (ii) the entrance end of the glass capillary up to the adhesive bond and the plastic will have contracted slightly, so the dye will extend further with respect to the markings and readings will be slightly higher. The Checkit Go cartridges are manufactured, inspected, and tested at 22 to 25°C (71.6 to 77°F). To be conservative, these values are padded, in that production actually occurs in a tighter temperature range. Factoring in the accuracy NIST traceable thermometer, which is accurate to within 0.4°C, we say the temperature range for production is 21.6 to 25.4°C. Assuming that laboratories are kept at 18 to 29°C, the laboratories are at a maximum difference of 7.4 degrees colder or 7.4 degrees hotter.

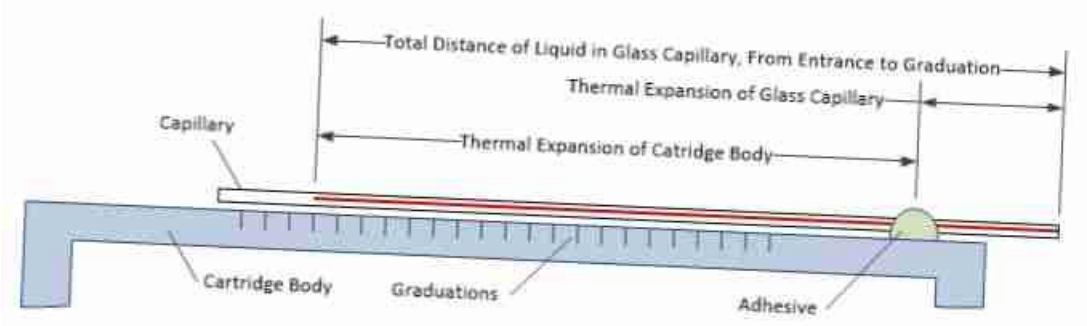


***Figure 3**. Schematic of the Checkit Go cartridge for purposes of showing how thermal expansion affects the distance from the capillary entrance to the graduations.*

Refer to Figure 3 The linear coefficient of thermal expansion (CTE) for the borosilicate glass capillaries is 3.3 x 10^{-6} per degree C. The volume per unit length is equivalent to the internal cross-sectional area of the capillaries, which is proportional to the radius squared.

$$Change\ in\ Area = \frac{\pi(r + \Delta r)^2 - \pi r^2}{\pi r^2}$$

where $\Delta r = r \times \Delta temperature \times CTE_{capillary}$

The volume per unit length can be up to 0.0049% lesser, in laboratories colder than the factory, leading to liquid aliquots that fill slightly more capillary length, or up to 0.0049% greater, in laboratories warmer than the factory, leading to liquid aliquots that fill slightly less capillary length and slightly lower volumetric readings.

The glass capillaries are bonded to the cartridge body, with the entrance end of the glass capillaries extending about 13 mm beyond the location of the bond. Thus, the change in distance from the entrance of the capillary to the graduations must be examined in two parts, the change in distance from the entrance of the capillary to the bond due to thermal expansion (or contraction) of the glass and the distance from the bond to the graduations, due to thermal expansion (or contraction) of the polystyrene cartridge body. The linear coefficient of thermal expansion of the high impact polystyrene cartridge body is provided by the plastic manufacturer as 40 – 60 x 10^{-6} per degree C. To

be conservative, we assume it is at the maximum value of 60 x 10^{-6} per degree C. The change in distance from the capillary entrance to a particular graduation is the sum of the change in distance from the capillary entrance to the bond, $L_{entrance\text{-}bond}$ plus the change in distance from the bond to the graduation, $L_{bond\text{-}grad}$, where $L_{entrance\text{-}bond}$ and $L_{bond\text{-}grad}$ are given by,

$$\Delta Lentrance\_bond = Lentrance\_bond \times \Delta temperature \times CTEcapillary$$

and

$$\Delta Lbond\_grad = Lbond\_grad \times \Delta temperature \times CTEcartridge\ body$$

To calculate change in volume, ΔV from the capillary entrance to the graduation,

$$\Delta V = ((Area + \Delta Area)(L + \Delta L) - (Area \times L))/(Area \times L)$$

where L represents the length from the capillary entrance to the graduation

See the table below for the calculated values. Note that if the enclosed volume is smaller due to thermal contraction of the capillary and cartridge body, the meniscus in the liquid will travel further and lead to a positive volumetric measurement error.

Table 4. The effect of temperature change on the accuracy of the Checkit Go cartridges.

	Checkit Go 5 µL	Checkit Go 10 µL	Checkit Go 20 µL	Checkit Go 50 µL
Maximum change in volume per unit length of capillary	± 0.00488%	± 0.00488%	± 0.00488%	± 0.00488%
Distance from capillary entrance to bond	12.75 mm	12.75 mm	13.0 mm	13.5 mm
Distance from bond to 50% of nom. graduation	14.75 mm	14.75 mm	19.0 mm	19.0 mm
Maximum change in distance to 50% of nominal graduation	± 6.86 µm	± 6.86 µm	± 8.75 µm	± 8.77 µm
Distance from bond to nominal graduation	42.25 mm	42.25 mm	51.00 mm	51.50 mm
Maximum change in distance to nominal graduation	± 19.07 µm	± 19.07 µm	± 22.96 µm	± 23.20 µm
Maximum change in volumetric value at 50% of nominal graduation	± 0.030%	± 0.030%	± 0.032%	± 0.032%
Maximum change in volumetric value at nominal graduation	± 0.040%	± 0.040%	± 0.041%	± 0.041%

Boundary Conditions

In this section of boundary conditions, we analyze errors due to the shapes of the menisci and any possible residual dye left in the well.

The "entrance" meniscus is very nearly flat—a balance between the weight of the liquid column and surface tension. It does become concave as the liquid evaporates from the entrance region. However, being that this "entrance" meniscus is originally flat and that as it becomes concave it does not change the height of the liquid column, the shape of the "entrance" meniscus does not contribute to any significant measurement error. For example, if the meniscus depth is 0.02 mm, the volumetric error for the smallest measurement volume is about 0.015%.

In contrast, the "reading" meniscus, which is at the end of the liquid column inside each of the capillaries, is not flat. The "reading" meniscus, is concave, as shown in Figure 4 below. The liquid nearest the inner walls of the capillary is pulled further up the tube than the center of the liquid column.

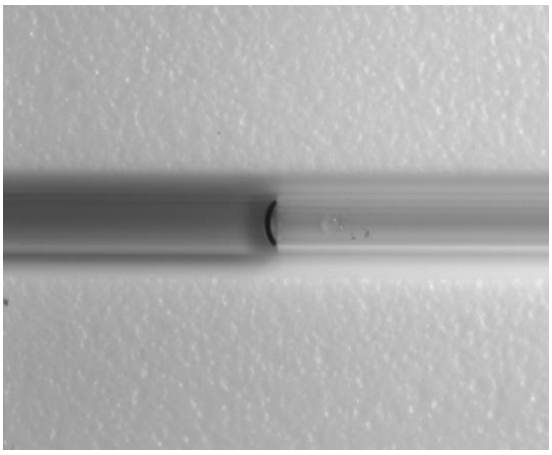


***Figure 4**. Photograph of dye in a capillary. Note the concave meniscus shape.*

The telecentric camera system, described earlier, recorded images of "reading" menisci for several liquids and capillaries. The various liquids included typical sample liquids: our supplied dye solution and solutions of ethanol, glycerol, and DMSO. The glass capillaries ranged in diameter from those used in our smallest volume Checkit Go model to our largest model.

To determine the maximum volumetric measurement error due to the concave shape of the menisci, we first determined that the dye solution we supply with the Checkit Go cartridges leads to deeper menisci than other typical solutions such as ethanol, glycerol and DMSO solutions. The maximum menisci depth for each size capillary was determined by measuring menisci depths in recently manufactured Checkit Go cartridges using the dye solution we supply with the Checkit Go cartridges. We observed 10, 19, 38 and 16 menisci in capillaries for 5, 10, 20, and 50 µL cartridges, respectively. Since the dye supplied with the Checkit Go exhibits the greatest surface tension of the tested liquids,

it has the deepest menisci. In all cases, the "reading" meniscus has the shape very nearly that of a section of a sphere, as shown in Figure 5.

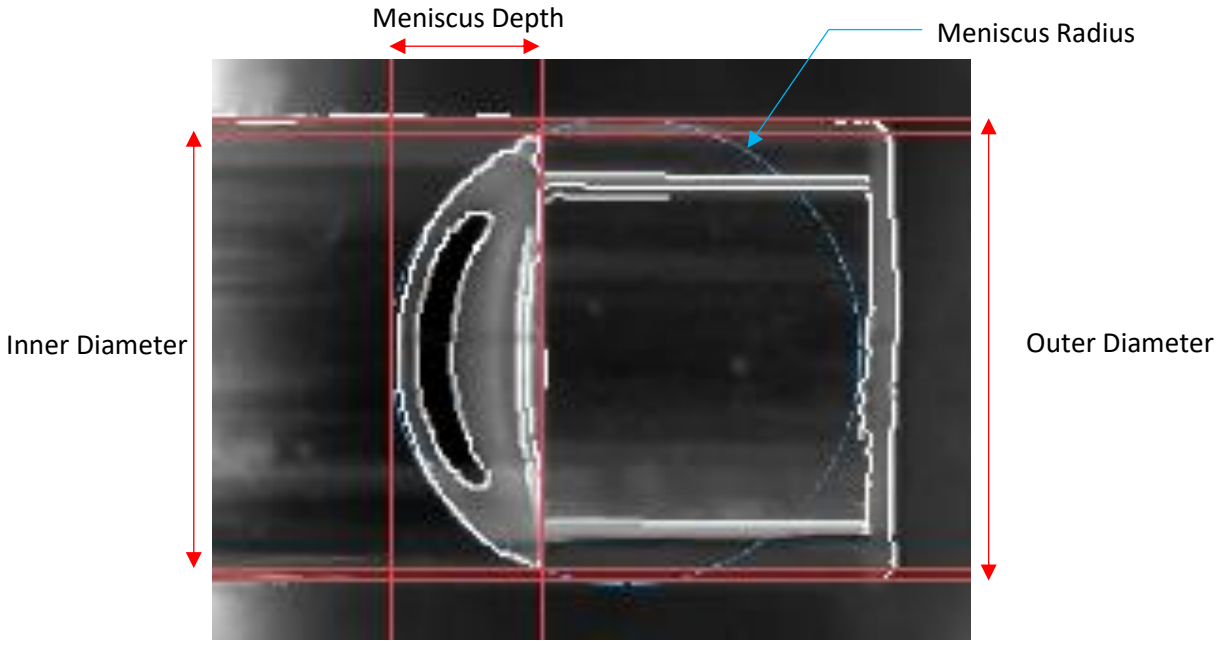
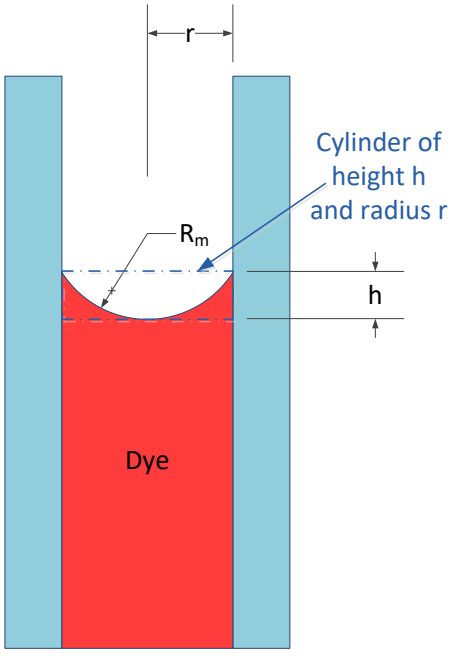
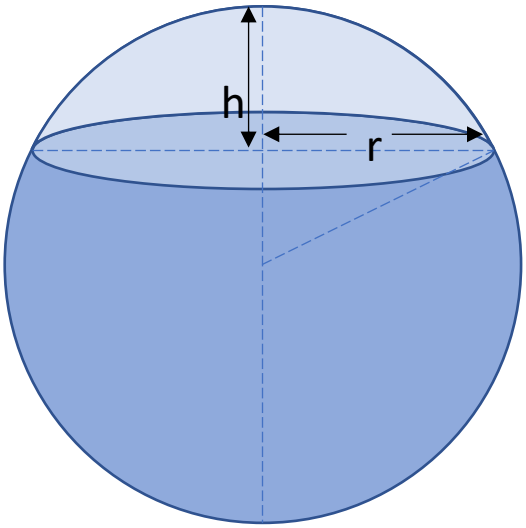


***Figure 5**. Close up image of a meniscus in a capillary, illustrating that its shape is very nearly that of a section of a sphere. The meniscus depth and radius are used to determine the volume of air included in the concave shape. Note that the inner diameter is almost as large as the outer diameter because this glass capillary has a very thin wall.*

The liquid extending beyond the center (or "bottom") of the meniscus can lead to an error of less liquid being accounted for. That volume of liquid can range from zero if the meniscus is almost flat, to a significant amount if the meniscus is relatively deep. As shown in Figure 6, the volume of the excess liquid of that meniscus is equal to the volume of a cylinder of height h and radius r, the inside radius of the capillary, less the volume of the spherical cap of air, of height h and spherical radius R_m.



We confirmed that each meniscus is very nearly shaped as a spherical surface. The volume of the spherical cap shaped volume of air was calculated using the equation shown in Figure 7. These maximum volumetric measurement errors for each size capillary are presented in Table 5.



$$V = \frac{1}{6}\pi\, h(3r^2 + h^2)$$

where

$h = meniscus\ depth$

$r = \frac{1}{2}(inner\ diameter\ of\ capillary)$

Figure 7. The equation in this figure is used to calculate the volume of the spherical cap.

Table 5. The volumetric measurement error due to the shape of the "reading" meniscus.

	Checkit Go 5 µL	Checkit Go 10 µL	Checkit Go 20 µL	Checkit Go 50 µL
Maximum depth *h* of "reading" meniscus	0.093 mm	0.153 mm	0.205 mm	0.312 mm
Inner radius r of capillary	0.170 mm	0.241 mm	0.315 mm	0.495 mm
Volume of cylinder of height h and radius r	0.0084 µL	0.0278 µL	.0640 µL	0.2401 µL
Volume of air	0.0046 µL	0.0158 µL	0.0365 µL	0.1360 µL
Volume of liquid	0.0038 µL	0.0120 µL	0.0275 µL	0.1041 µL
Volume of liquid at 50% of nominal graduation	2.5 µL	5.0 µL	10 µL	25 µL
Maximum error at 50% of nominal graduation	-0.15%	-0.24%	-0.27%	-0.42%
Volume of liquid at nominal graduation	5.0 µL	10 µL	20 µL	50 µL
Maximum error at nominal graduation	-0.08%	-0.12%	-0.14%	-0.21%

Residual Liquid in Wells. While sample liquid remaining in the wells instead of being pulled into the capillary is rare, we ensure that the Checkit Go is still accurate when this occurs by accounting for this residual in our permissible error in our accuracy claim. We ensure that using the dye provided with the Checkit Go, is limited to a tiny droplet, shaped like a sphere with the bottom flattened to the shape of the well surface it is resting on. The maximum permissible sized sphere has a diameter less than one-half of the outer diameter of the capillary, i.e. its radius is less than half the outside radius

of the capillary. We use the outside diameter of the capillaries as a guideline because it affords a convenient method of comparison.

The volume of a (non-truncated) sphere is,

$$V_{sphere} = \frac{4}{3}\pi R^3 \sim 4.189\, R^3 \qquad (1)$$

However, the bottom of the sphere is relatively flat. The more hydrophobic the surface of the well tab is, the more the droplet beads up, leading to a greater contact angle, as shown in Figure 8 below. The more hydrophilic the surface is, the more the droplet spreads out. The more is spreads out, the the less its height in the center and the more the hypothetical sphere is truncated, thus the less its volume for a given radius. The least truncated / greatest beading up we noticed throughout our testing was a 96 degree contact angle. To determine this angle, we used a horizontally oriented microscope camera to observe many droplets on placed carefully cleaned surfaces.

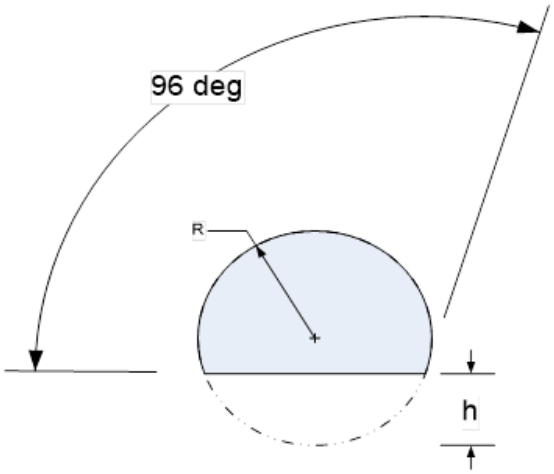


***Figure 8**. Diagram showing a droplet, its contact angle, and the truncated portion of a sphere, where it is resting on a flat surface.*

While we are assuming that the bottom of the droplet is flat, note that these droplets are quite small relative to the curvature of the wells they rest in. The additional volume is insignificant. Note that 96 degrees is the maximum angle. In most all cases, droplets of any consequence are more spread out, and thus represent less volume for their radius.

The volume of a spherical cap is $V_{cap} = \frac{1}{3}\pi\, h^2(3R - h)$. (2)

Using basic trigonometry, h = R cos Θ, where Θ = 6 degrees from vertical for the 96 degree contact angle. So, h = 0.9945R

Plugging in 0.9945R for h in equation 2 yields,

$$V_{cap} = 2.077\ R^3. \quad (3)$$

And the maximum volume of the residual droplet,

$$V_{droplet} = V_{sphere} - V_{cap} = 2.112R^3 \quad (4)$$

When a residual droplet is present in the well, there is slightly less dye in the capillary, so it will cause the readings to be slightly lower, never higher.

Table 6. The effect of residual dye in the well.

	Checkit Go 5 µL	Checkit Go 10 µL	Checkit Go 20 µL	Checkit Go 50 µL
Outside diameter of capillary (mm)	0.8636	0.9652	0.9017	1.4097
Allowable residual radius	0.2159	0.2413	0.2254	0.3524
Maximum acceptable residual (µL)	0.0213	0.0297	0.0242	0.0924
Residual error at 50% of nom. graduation (lower end of recommended range)	-0.85%	-0.59%	-0.24%	-0.37%
Residual error at nominal graduation (upper end of recommended range)	-0.43%	-0.30%	-0.12%	-0.18%

Interestingly, the walls of the 20 µL capillaries are so much thinner than the walls of the 10 µL capillaries that the outside diameter of the 20 µL capillaries is actually less than the outside diameter of the. 10 µL capillaries.

Adjustment of marks to partially center the error.

Table 7. Positional Adjustment of graduations.

	Checkit Go 5 µL	Checkit Go 10 µL	Checkit Go 20 µL	Checkit Go 50 µL
Adjustment (mm)	0.14	0.14	0.13	0.18
Capillary volume (µL per mm)	0.0909	0.1818	0.3125	0.7692
Adjustment (µL)	0.013	0.025	0.041	0.138
Adjustment (%) at 50% of Nominal	0.51%	0.51%	0.41%	0.55%
Adjustment (%) at Nominal	0.25%	0.25%	0.20%	0.28%

Note that meniscus and residuals can both lead to negative errors, while other factors can lead to positive and negative errors. Thus, errors have the potential to be greater in the negative direction -

a reading being too low, than in the positive direction. Since a curved meniscus will always be present, and to reduce the maximum potential error, we shifted the "zero" point slightly. See Table 7.

Accuracy of the Checkit Go Models

Since each effect is small, the combined effects can be added linearly, since the error compounded on an error is negligible. For example, if a value is 0.5% high, and another value is 0.5% high, the combination will lead to a value that is 1.0025% high. In this case, the difference between only adding the effects linearly, and accounting for the compounded effect is 0.0025% which is negligible.

Table 8 shows the total accuracy as a sum of the combined effects provided in Tables 2 through 6.

Confirmation Testing of Finished Product

As yet a further check for accuracy, we conducted a study comparing volumetric measurements using Checkit Go cartridges and measurements using a 5-digit balance. The density of our dye was determined using the balance and a volumetric flask. To confirm our process for determining the density of the dye, the same process was used to determine the density of Type 1 ASTM distilled water, and the result matched the published value. Next, aliquots of dye were dispensed into the flip tab of the checkits, and the mass of the aliquot was measured. The flip tab was then assembled onto a Checkit Go cartridge and the volume was measured using the Checkit go cartridge. The rate of evaporation was measured and we compensated for the evaporation loss during the handling from the balance measurement to the dispense into the Checkit. Several hundred Checkit Go measurements yielded a typical absolute error of less than 1% from the volume measured with the balance.

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Table 8. The Accuracy of the Checkit Go models.

	Checkit Go 5 µL		Checkit Go 10 µL		Checkit Go 20 µL		Checkit Go 50 µL	
Contributing Factor	50%	Nominal	50%	Nominal	50%	Nominal	50%	Nominal
Table 2. Capillary Inside Diameter Accuracy	±0.5%	±0.5%	±0.5%	±0.5%	±0.5%	±0.5%	±0.5%	±0.5%
Table 3. Graduation Positional Accuracy	± 0.73%	± 0.36%	± 0.73%	± 0.36%	± 0.63%	± 0.31%	± 0.62%	± 0.31%
Table 4. Temperature in Lab	± 0.030%	± 0.040%	± 0.030%	± 0.040%	± 0.032%	± 0.041%	± 0.032%	± 0.041%
Table 5. Meniscus Shape	-0.15/+0%	-0.08/+0%	-0.24/+0%	-0.12/+0%	-0.27/+0%	-0.14/+0%	-0.42/+0%	-0.21/+0%
Table 6. Residual in Well	-0.85/+0%	-0.43/+0%	-0.59/+0%	-0.30/+0%	-0.24/+0%	-0.12/+0%	-0.37/+0%	-0.18/+0%
Subtotal Potential Error	-2.26% / +1.26%	-1.40% / +0.90%	-2.09% / +1.26%	-1.32% / +0.90%	-1.67% / +1.16%	-1.11% / +0.85%	-1.93% / +1.15%	-1.24% / +0.85%
Table 7. Adjustment of Graduation Positions	+0.51%	+0.25%	+0.51%	+0.25%	+0.41%	+0.20%	+0.55%	+0.28%
Total Potential Error	-1.75% / -+1.77%	-1.15% / +1.16%	-1.58% / +1.77%	-1.07% / +1.16%	-1.27% / +1.56%	-0.91% / +1.06%	-1.38% / +1.70%	-0.96% / +1.13%

Appendix A – telecentric camera system

Our imaging inspection station uses a monochromatic CMOS detector with pixels of size 2.4 µm x 2.4 µm, and a telecentric lens system with a magnification such that every pixel corresponds to 6.37 x 6.37 µm. The telecentric lens is designed to have very little distortion and even very little change in magnification within the working distance of the lens. The manufacturer states that the telecentric lens has less than 0.05% distortion and less than 0.1 degree of divergence (spreading of the light rays). To confirm that our imaging station is accurate, we characterized this system using images taken of a NIST traceable dot-pattern. The dot pattern is etched in glass with 0.25 mm diameter dots placed in a rectangular array at 0.50 mm spacing. The NIST calibration certificate states that the grid has a maximum deviation of 0.48 µm in the X-direction, a 1.02 µm in the Y- direction and a maximum positional error is ±1.83 µm.

In combination with the dot-pattern, the python library OpenCV was used to determine the distortion and rectify images taken with the system, to remove the distortion of the system. Figure A1 below illustrates these steps. The top left is an exaggerated distorted image. The top right is the inverse mapping of that same dot-pattern, and the red dots in the bottom image form the image corrected pattern.

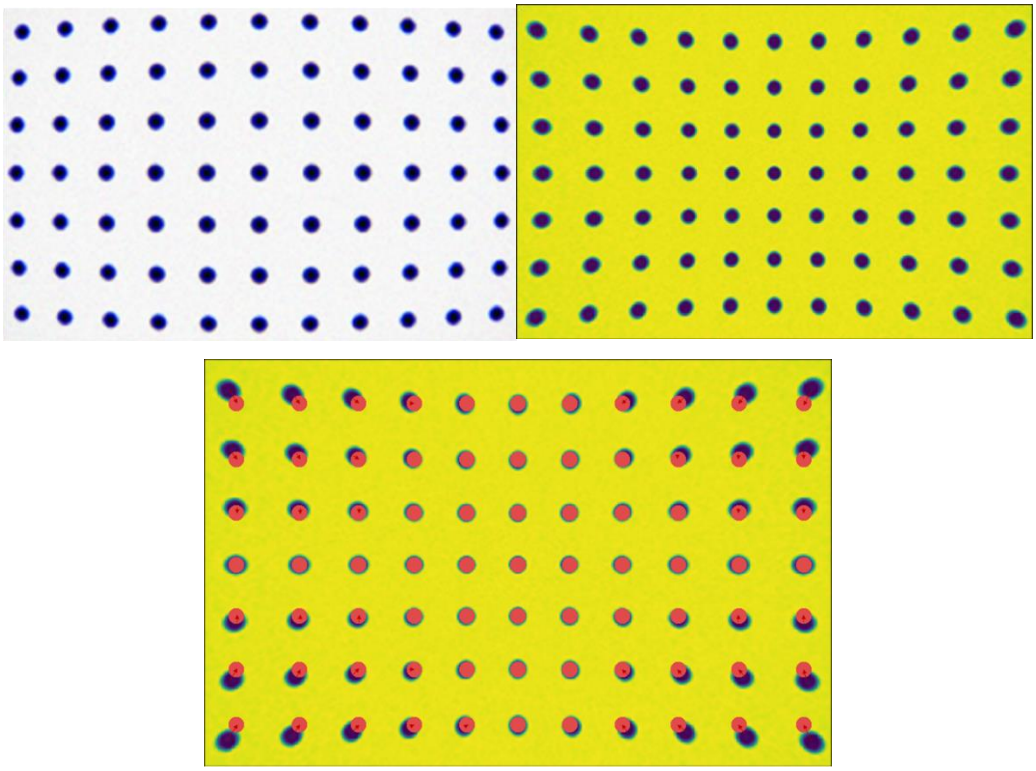

Figure A1*: The top left image taken of a dot grid, with the distortion exaggerated. The top right image shows the distortion correction using OpenCV. The bottom image illustrates the initial imaged dots in blue and the corrected pattern of dots in red.*

In our system the maximum correction distance was found to be only 2.1 pixels, with an average of 1.2 pixels. Adding a pixel for uncertainty in OpenCV's edge detection algorithm, the process leaves us with a positional error of up to 3.1 pixels at any point in the image. Accounting for the possible error in the dot pattern itself, our total error in these measurements comes to 3.1 x 6.37 µm + 1.83 µm = ±21.6 µm. Therefore, this imaging system is accurate enough to confirm the positions of the graduations with respect to the capillary, because it is an order of magnitude more accurate.