

Introduction:

Modern drug discovery and research labs are utilizing complex automation systems to assist in high throughput screening of novel drug candidates. A large portion of these laboratories are using ANSI-SBS-standard 96- and 384-well plates to achieve the necessary throughput. The volume of sample transferred into and out of these plates can be critical to the success of assays or validation of procedures. One specific example is in the preparation of mother/daughter plates from compounds pulled from library stores. It is not uncommon to find a compound storage tube containing less volume than expected, which in turn may result in incorrect volumes delivered to mother plates to be used for screening tests. A simple approach to determining volumes in these prepared mother plates is needed.

Using a new pressure-based volume measurement technology, it is now possible to make direct measurements of volumes in microplates. This new measurement technology is found in the Artel SDS, which provides precise and accurate volume measurements in 96- and 384-well microplates. The SDS technology determines well volume by sealing and pressurizing individual wells and is able to accurately and repeatedly measure the contents of a well without regard to the shape of the well, type of plastic, color of material, or type of sample.

Using four common plate types and a variety of precision metal cylinders of known volume, the basic operational principles of this volume measurement technology is demonstrated herein.

Operating Principle:

The Artel SDS technology is based upon measurement of pressure in a confined microplate well, when a known volume of air is pumped into that well. Simply stated, the technology is based upon the ideal gas law:

$$PV = nRT \quad (1)$$

where P is the pressure of a system confined to volume V , n is the moles of gas in the confined system, R is the ideal gas constant, and T is the temperature of the gas in the system. Equation (1) states that if a confined, constant number of gas molecules, held at constant temperature is compressed (i.e., volume is decreased), then the pressure will increase in direct proportion to the decrease in volume:

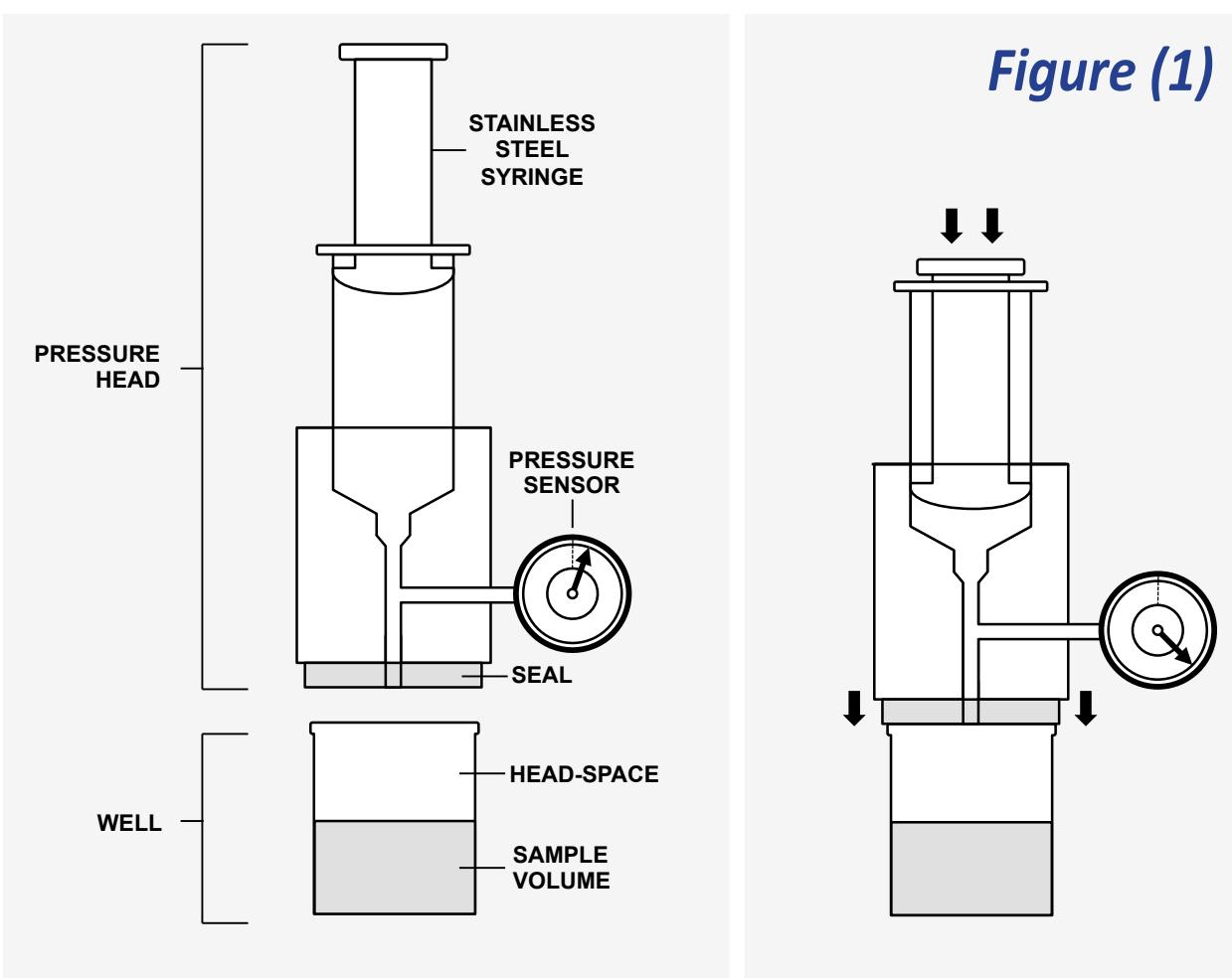


Figure (1)

$$P \propto 1/V \quad (2)$$

Equation (2) is more commonly known as Boyle's law, and can be used to compare the state of a gas under two different sets of conditions, as expressed in equation (3):

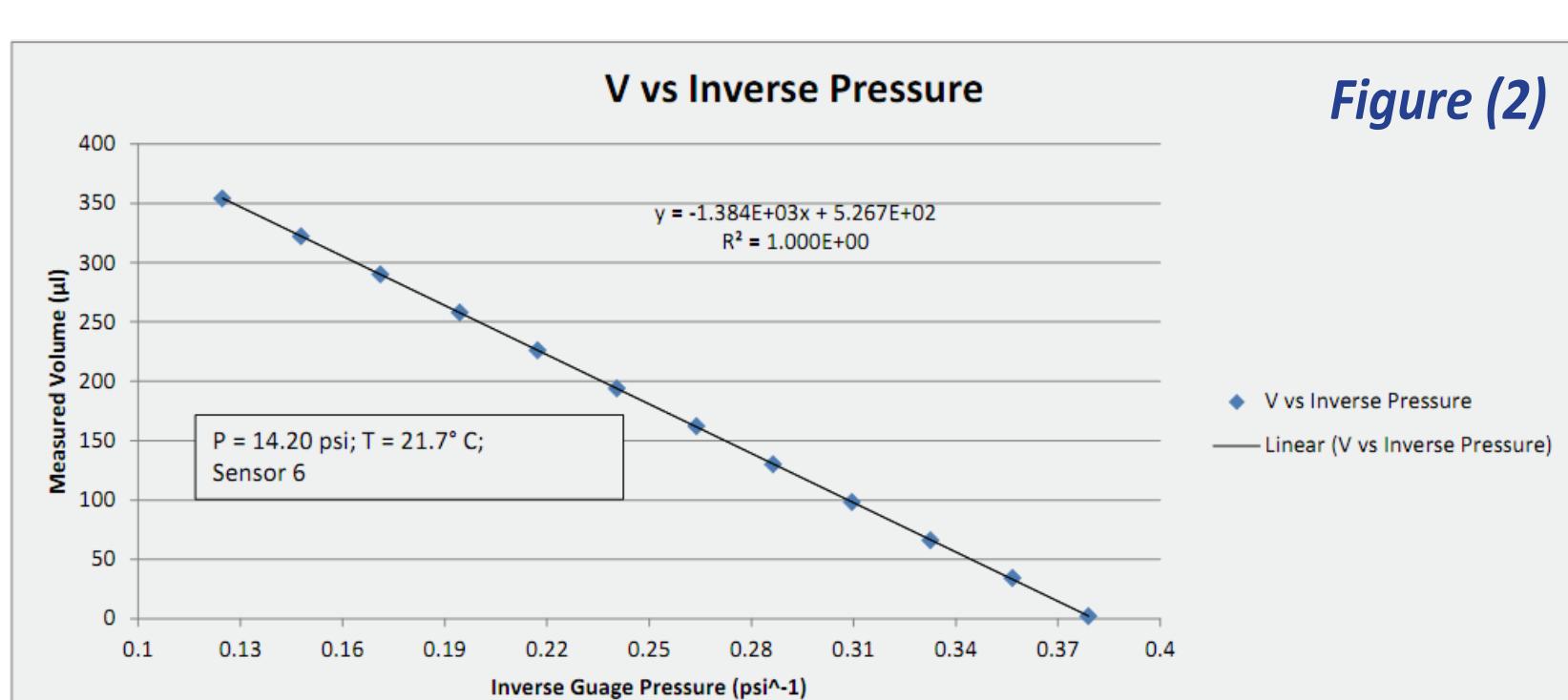


Figure (2)

$$P_1V_1 = P_2V_2 \quad (3)$$

This equation (3) describes the basic operational principle of the SDS technology. The pressure head at P_1 , shown in Figure (1), is pressed down onto the well of a microplate, the syringe piston pumps in a known volume of air V_1 , and the pressure P_2 is measured. Equation (3) is then used to calculate the volume of the well V_2 . This volume V_2 is either the volume of the well, if the well is empty, or the volume of the headspace above a sample in the well. Thus by knowing the volume of the empty well, one can calculate the volume occupied by a sample in that well.

Equation (3) demonstrates that the SDS technology can be used to measure the pressure of an empty well, followed by measurement of pressure when that well is filled with a known volume. Plotting the volume V_2 as a function of inverse pressure $1/P_2$ produces a line with slope P_1V_1 , as shown in Figure 2. Plotting this relationship produces a calibration curve that can be used to extrapolate unknown volumes from the pressure measured for these volumes, in that well type.

Method:

Instrument Calibration: Before collecting volume measurements of sample plates, a calibration of each plate type listed in the Materials section was established by measuring the wells of an empty plate several times, followed by measuring those same wells filled with metallic cylinders of known volumes. The empty well volumes and the filled well volumes were then used to establish a calibration curve that matches a known volume with a specific pressure for each plate type. This calibration curve was then used to accurately estimate the volume of any samples measured in all plates of the plate type of interest.

Experiment: The following experiment was performed for each of the plate types listed in the Materials section:

- An empty plate was measured three (3) times on the Artel SDS. The three reads per well were averaged and used as a "tare" value for each well in the plate. Although a calibration curve had already been established for the plate type, well specific tare values were used to significantly reduce the variation within the plate.
- A precision metal cylinder of known volume (Figure 3) was then placed in every well of the plate (Figure 4), and the plate was re-measured.
- The tare volumes collected in step 1 were then subtracted (per well), giving the volume of the metal cylinder in each well.

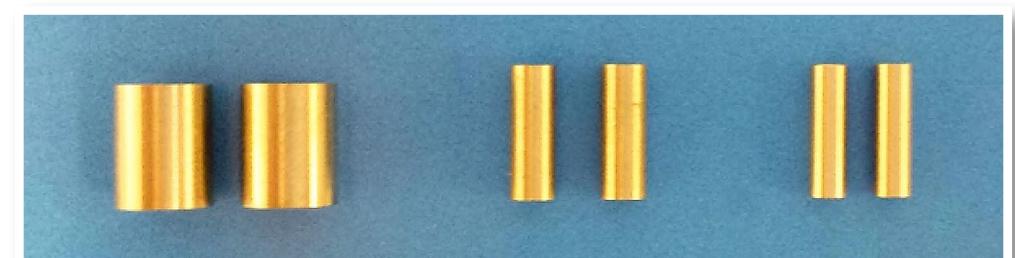


Figure 3: Photo of 242 μl, 67 μl and 43 μl high precision metal cylinders.

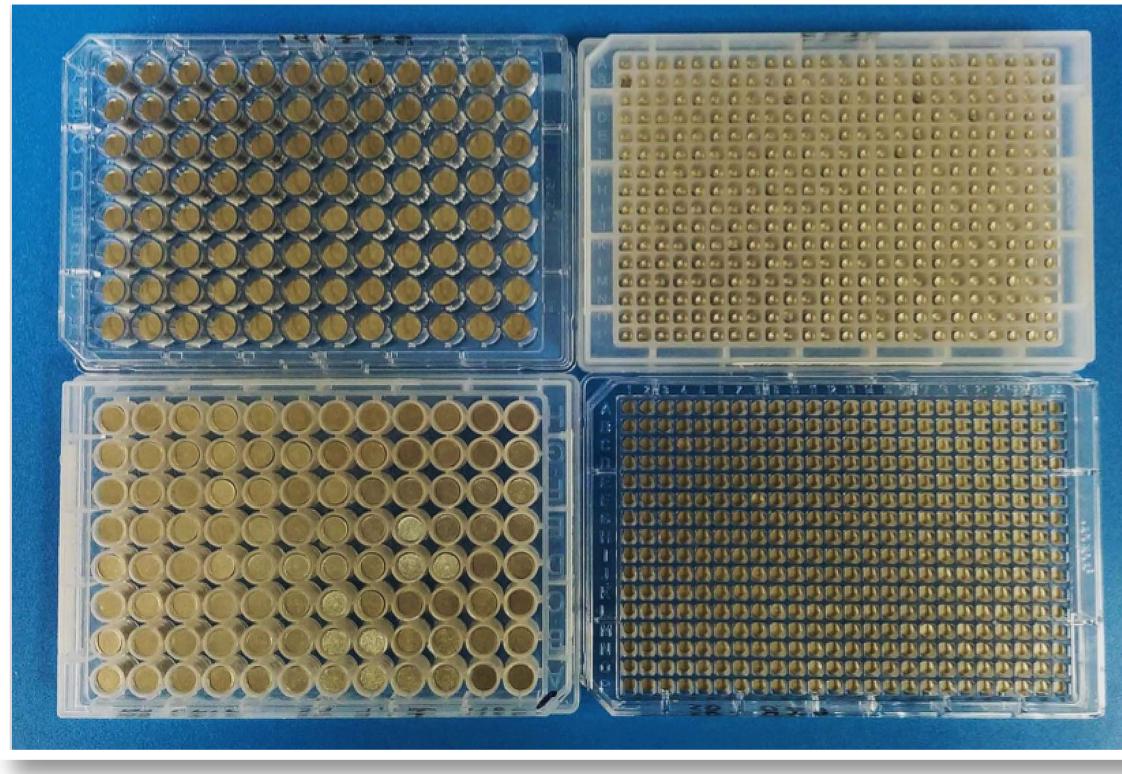


Figure 4: Photo of filled 96-well and 384-well plates

Materials:

- 96 Costar 3365 – Polypropylene – Round Bottom
- 96 Greiner 655101 – Polystyrene – Flat Bottom
- 384 Greiner 781101 – Polystyrene – Flat Bottom
- 384 Costar 3657 – Polypropylene – Round Bottom
- 43 μl, 67 μl, and 242 μl high precision metal volume simulating cylinders

Results:

Table 1: Greiner 384-well: Tare-subtracted measurement of 67 μl volume cylinders in a Greiner 384-well plate

	Average	STDEV	CV	% Error	Min	Max	Range
base	67.2	0.6	0.9%	0.3%	46.4	69.1	4.5
tared	66.3	0.2	0.3%	1.0%	59.5	67.1	1.1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	68.0	65.2	67.3	66.7	66.7	65.7	67.0	66.9	66.9	67.0	67.0	67.2	67.1	67.1	67.1	67.1	67.1	67.1	67.1	67.1	67.1	67.1	67.1	
B	67.0	67.4	67.4	66.5	67.3	66.1	67.1	67.1	67.4	66.6	67.1	67.2	67.1	67.1	67.1	67.1	67.1	67.1	67.1	67.1	67.1	67.1	67.1	
C	65.9	65.9	66.6	66.6	66.3	67.3	67.5	67.2	66.9	66.9	67.3	67.5	67.5	67.1	67.2	67.1	67.1	67.1	67.1	67.1	67.1	67.1	67.1	
D	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
E	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
F	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
G	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
H	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
I	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
J	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
K	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
L	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
M	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
N	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
O	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	
P	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	67.4	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	68.0	65.2	67.3	66.7	66.7	65.7	67.0	66.9	6															