

# Survey Study on Dimensions of 96-well Assay Plates Relevant to HTS Applications

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## Abstract

For effective operation of automated high throughput screening (HTS) systems in drug discovery, the assay plate external footprint and well location dimensions must be both reproducible and consistent with the capabilities of available robotic handling equipment. The latter requirement has led to the development of the Society for Biomolecular Screening (SBS) MP96 standard for assay plate design, as an unbiased effort to ensure that microplate and robot manufacturers may achieve user-transparent interfacing of their respective products. Other features of assay plates, such as plate stiffness vs. flexing under robotic arm pressure, certainly affect robotics friendliness. However, the obvious starting point for assessing robotics friendliness is to survey the key external footprint and well location dimensions of 96-well plates relative to each other, and to the SBS MP96 standard. Such a survey study was conducted on an industry-wide sampling of commercially available 96-well polystyrene and polypropylene assay plates. This poster presentation summarizes the results of the study, thus providing a current snapshot of the variation among assay plates, and compliance to the SBS standard across the microplate industry.

## Materials and Methods

Samples of polystyrene (PS) and polypropylene (PP) 96 well microplates from an industry-wide cross-section of plate manufacturers were obtained commercially. The selected plates were subjected to dimensional analysis using standard metrology techniques. For PS plates, dimensional data were generated using manual measurements only, with the aid of calibrated measurement devices (e.g., micrometer, feeler gauge, T-square and/or right angle) on a stable flat tabletop surface. Three plates of each type were randomly selected from the unit package, then each plate was measured according to a defined protocol. While the PS plates tested were primarily clear with flat-bottoms (63% of total), some black (9%), white (16%), black-clear bottom (3%) and white-clear bottom (9%) plates (all, flat-bottom wells) were also tested. A total of 32 varieties of PS plates from eleven different manufacturers (including BD Labware's FALCON® brand) were tested. Care was taken to ensure that minimum necessary force was used, to avoid plate deformation during measurements. Whenever feasible, measurements were made with plate lying as flat as possible under no stress deformation, while supported by a flat planar marble work table.

PP plate data were generated by an independent metrology laboratory, Quality Plus, Inc. (170 Grove Street, Chicopee, MA, USA). Quality Plus used a variety of calibrated measurement devices, including a machine vision system where appropriate, to obtain dimensional data according to a design protocol provided by BD Labware. Five replicate PP plates from each of six different vendors (again, including BD Labware's FALCON® brand) were tested according to the defined protocol. One plate type was manufactured from a PP copolymer blend, rather than PP. Another plate type was available in V-bottom format only, whereas all others tested were flat-bottom format.

All original dimensional data were transferred into Microsoft® Excel spreadsheets, where mean value, standard deviation, and %CV results were calculated for each parameter tested. The rejection of outlier data point(s) was not allowed in any calculation. Where the Society for Biomolecular Screening MP96 Standard (T. Astle, J. Biomol. Screening 1(4):163 (1996)) provided a specification for the dimensional parameter measured, the extent of compliance of the tested plates vs. the SBS-defined specification was assessed.

## Results

For the PS plates as a group, dimensional differences between plate vendors were noted but such differences were relatively small in most cases. Also, compliance to even the least restrictive MP96-3 standard (i.e., a subset of MP96-1, including sections 1-3 only of the complete MP96-1 specification) parameters was incomplete for most or all plate types, particularly because of the noncumulative center-to-center well spacing and superposition specifications (sections 2.12 and 3.3 of MP96). The above conclusion is exemplified by the plate length, width and height data shown in Figures 1-3, respectively. Only 41% of PS plates tested met all length, width and height specifications. In many cases (e.g., FALCON® PS plates) the plates tested do not reflect any redesign effort directed toward meeting the SBS MP96 standard. Nonetheless, it appears that the majority of 96 well PS plates are substantially usable with robotic plate handling equipment amenable to HTS. However, some customization of robot software parameters may be required to allow optimal use with acceptably low failure rate.

In contrast to the corresponding PS plate data, the PP plate types tested exhibited a relatively wide range of variation on all parameters measured. For several dimensional parameters, substantial variation was observed within one or more plate types. This is illustrated by Figures 4 and 5, which show the length and width data, respectively, for PP plates. Only 2 PP plate types of 6 tested, PP-C and PP-D, were within the MP96 standard length and width specifications when measured at the top and bottom of the plate. However, 3 of the 4 corresponding sets of values measured at the middle of these plates (i.e., through the plate center as opposed to along an edge) were out of specification (both for PP-C, 1 of 2 for PP-D). Of the 6 PP plate types tested, the most nearly rectangular and least warped ones were the FALCON® plates.

A somewhat different picture was observed for PP plate height, as shown in Figure 6. In this case, 4 of 6 plate types met the MP96 standard when measured at all 4 corners, with one type showing borderline compliance at 2 of 4 corners. Plate type PP-A consistently failed to meet the MP96 plate height specification at one corner only, whereas plate type PP-D failed to meet specification at all 4 corners. The latter failure is perhaps more notable, in view of the good MP96 compliance shown by the PP-D plates with regard to length and width dimensions. It appears that the height differences observed would most likely result in operational problems in automated HTS use due to potentially poor stackability of plate types PP-A and PP-B, which were observed to be substantially different in height between the top vs. bottom edges.

An alternative view of the PP plate height data is shown in Figure 7, which presents the distribution of plate height results measured at each corner of all plates tested. It is of interest to note that the height region of highest frequency for all PP plates corresponds closely with the range of results actually exhibited by the FALCON® plates.

Another SBS parameter highly relevant to the automation friendliness of microplates is referred to herein as Well Height Above Resting Plane (WHARP), the height of each well bottom (top, fluid contact surface, not bottom side) above the resting plane on which the plate resides. In this experiment, the WHARP values were measured at the four "Outer" wells (A1, A12, H1 and H12 corners) as well as the four "Inner" wells (D6, D7, E6 and E7). WHARP data for PP plates are shown in Figure 8. Only 2 of 6 plate types (PP-B and FALCON®) exhibited nearly uniform WHARP between Inner and Outer wells, with mean  $\delta$ (WHARP) of 0.00579 and 0.00833 inches, respectively. The other 4 PP plate types showed substantial systematic differences between Inner vs. Outer well groups. In the worst case (plate type PP-D), the mean  $\delta$ (WHARP) was 0.02935 inches (0.745 mm). High  $\delta$ (WHARP) values suggest possible problems during automated operation. These include fluid delivery probe bottoming out on the plate (with probable catastrophic failure of the automated run!), or simply excessive reagent waste due to the inability to remove most of the contents from all wells.

Minimization of sidewall warpage in microplates allows more secure gripping by robotic arms, thereby improving automation friendliness. Sidewall warpage is also often referred to as runout, or TIR (Total Indicator Runout). All PP plates tested were within the MP96 specification of plate sides and ends being straight within  $\pm 0.020$  inches (0.50 mm). However, there was a wide range of variation within this allowed range, as seen in Figure 9. The lowest mean runout (0.00548 inches, 0.139 mm) was observed with FALCON® PP plates, whereas the highest mean runout (0.01362 inches, 0.346 mm) was observed with PP-A plates.

Accurate robotic fluid delivery to specific target wells, as well as obtaining unbiased detection results in the plate reader, are critically dependent upon precise location of the appropriate target well by the robot or detector element, respectively. The two parameters most closely related to this function include center-to-center distance (see Figure 10) and center-to-center superposition (see Figure 11). Accordingly, the MP96 Standard attempts to control these parameters as tightly as possible (for PS plates).

In fact, many or most PS plates have difficulty meeting the center-to-center distance specification consistently, due to its noncumulative aspect. For the PP plates, 4 of 6 types completely failed to meet specification. Of the other two types, PP-C and PP-D plates, 3 of 4 values only met the MP96 specification. Thus, none of the plate types tested were completely within the specification. A different view of this need suggests that in addition to nominal compliance with the specification, maintaining a good approximation of a rectangular (as opposed to trapezoidal) plate footprint is of significant value. In this regard, the criterion becomes one of having the corresponding long (A1-A12 and H1-H12) and short (A1-H1 and A12-H12) dimension pairs as equal to each other as possible.

Center-to-center superposition is important for several reasons. A skewed well array will have some or many wells in inappropriate locations, which may be inaccessible to robots and/or appear "different" than other wells to detection apparatus. Also, robots are incapable of determining which orientation (A1 at top left, or at bottom right) a microplate was actually loaded into the stacker or hotel equipment, and on occasion operators do not always place all plates in the same orientation. Thus it is useful if 180 degrees plate rotation does not substantially change the well array location. Section 3.3 of MP96 implies zero tolerance: "The centerline of the outside footprint dimensions shall be coincident with the centerline of the well to well spacing." However, perhaps a more reasonable interpretation would be to align the center-to-center superposition tolerance with the allowed noncumulative tolerance for center-to-center distance, which is specified as  $\pm 0.003$  inches ( $\pm 0.08$  mm). By the latter interpretation, all PP plate types except PP-A would meet specification on average, although due to high %CV some PP-D plates would exceed this specification in the lengthwise dimension.

From both types of center-to-center measurements (as shown in Figures 10 and 11), the FALCON® PP plates exhibit the most internally consistent well array distance and array vs. plate footprint superposition dimensions. While the uniform systematic deviation of the FALCON® PP plates from the MP96 center-to-center distance specification is easily eliminated as a potential performance issue by appropriate programming of array dimensions (a one-time operation), it is impossible with software adjustment to eliminate similar effects which are due to plate-to-plate variation.

Another measure of plate performance that relates to plate stability and stackability is flatness, as measured by determining whether feeler gauge of defined thickness can be slipped between the test plate and a flat resting surface. This parameter is not addressed in the MP96 specification. Nonetheless, it is advantageous if all points on the plate footprint ideally contact the resting plane surface. Accordingly, plate flatness was examined at corners and edge midpoints. All midpoints were found to touch the resting surface. However, in 4 of 6 PP plate types deviations from flatness of at least 0.010 inches were noted as shown in Figure 12. The FALCON® PP plates exhibited the smallest deviations (mean value of 0.0014 inches), with the PP-C plates nearly as low (mean of 0.0026 inches). The worst-performing plates in this regard were of PP-A type, with all 4 corner deviations  $> 0.010$  inches (0.25 mm).

## Conclusions

The SBS MP96 Standard is a welcome, rational effort to help plate manufacturers, HTS equipment manufacturers and HTS scientists coexist productively. The value of such an open standard in leveling the playing field among vendors is real, but may be overrated in the sense that significant performance differences remain between nominally "interchangeable" competitive microplates. Such differences are probably inevitable, and do not mean that the standard is wrong or irrelevant.

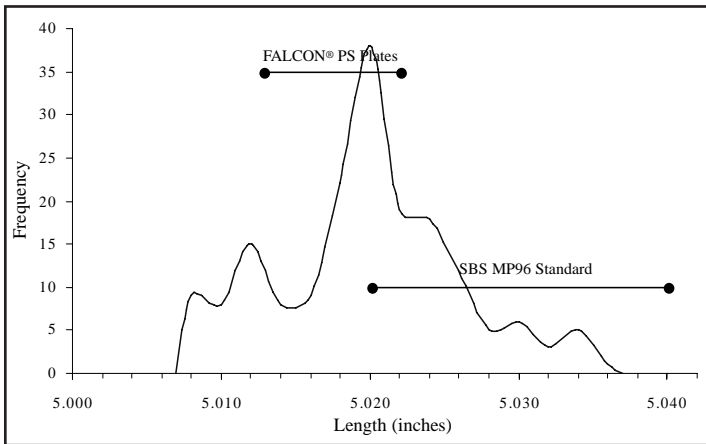
The extension of MP96 beyond the well-behaved PS plates to the less dimensionally controllable PP plates has resulted in a heterogeneous mix of commercial products that are not interchangeable in practice. This problem is partly one of limited experience with PP as an injection molding raw material, in the case of many microplate vendors. Given the dimensional variation evident in most of the existing 96-well PP plates, the design of 384-well and higher-density format PP plates would appear to represent a more daunting engineering challenge than was previously apparent.

With the development and elaboration of any open interdisciplinary standard such as the SBS MP96 Standard, there is an ongoing dynamic. In this case, it is the microplate and automation equipment manufacturers who bear the burden of determining how best to meet end-user assay needs and to approach or achieve user-transparency of plate-machine interfacing. As seen with PP plates, to the extent that the applicable standard (i.e., the current SBS MP96) precludes the ability of plate manufacturers to meet that standard, it also precludes them from obtaining economic benefit from addressing the standard. Thus, many will conclude that it is not worth attempting to meet the standard as currently defined. In retrospect, the above problem is due in large measure to the fact that the MP96 standard was derived primarily from consideration of PS material properties rather than those of PP. However, to relax specifications for PP plates by establishing a separate standard, for example, may work at the 96-well plate level, but is unlikely to prove acceptable at any higher density such as the 384-well plate format. To move forward productively is to continue the three-way dialog between microplate and automation equipment manufacturers with HTS end users.

## Acknowledgments

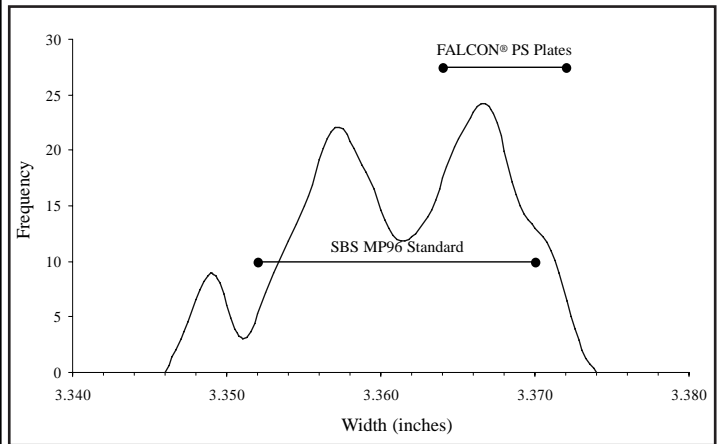
The authors wish to express their thanks to Douglas Henderson, BD Labware Design Engineer, for his significant contributions to the design of the FALCON® PP Multiwell™ Library Storage Plate.

### PS Plate Length Distribution: All Plates



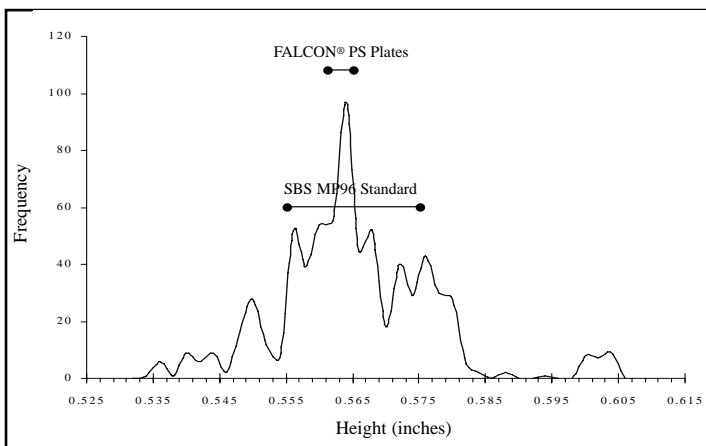
**Figure 1:** PS Plate Length Distribution Histogram — A total of 32 varieties of PS plates from 11 different manufacturers (including BD Labware's FALCON® Pro-Bind™ and TC-treated plates) were tested. Three plates of each type were measured for length at the *top* (A1 to A12) and *bottom* (H1 to H12) edges of the plate. The distribution of all length measurements is shown (not the mean values). While the PS plates tested were primarily clear with flat-bottoms (63% of total), some black (9%), white (16%), black-clear bottom (3%) and white-clear bottom (9%) plates (all, flat-bottom wells) were also tested. The lower horizontal bar indicates the range of plate length allowed by the SBS MP96 Standard. The upper horizontal bar indicates the range of length values measured for the FALCON plates tested.

### PS Plate Width Distribution: All Plates



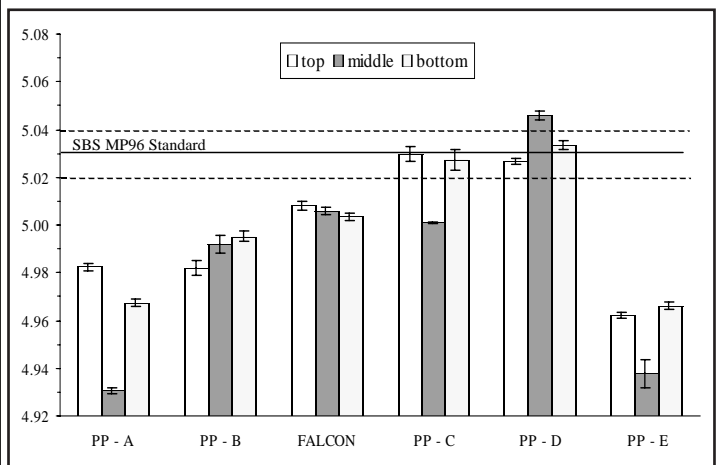
**Figure 2:** PS Plate Width Distribution Histogram — A total of 32 varieties of PS plates from 11 different manufacturers (including BD Labware's FALCON Pro-Bind and TC-treated plates) were tested. Three plates of each type were measured for width at the *left* (A1 to H1) and *right* (A12 to H12) edges of the plate. The distribution of all width measurements is shown (not the mean values). While the PS plates tested were primarily clear with flat-bottoms (63% of total), some black (9%), white (16%), black-clear bottom (3%) and white-clear bottom (9%) plates (all, flat-bottom wells) were also tested. The lower horizontal bar indicates the range of plate width allowed by the SBS MP96 Standard. The upper horizontal bar indicates the range of width values measured for the FALCON plates tested.

### PS Plate Height Distribution: All Plates



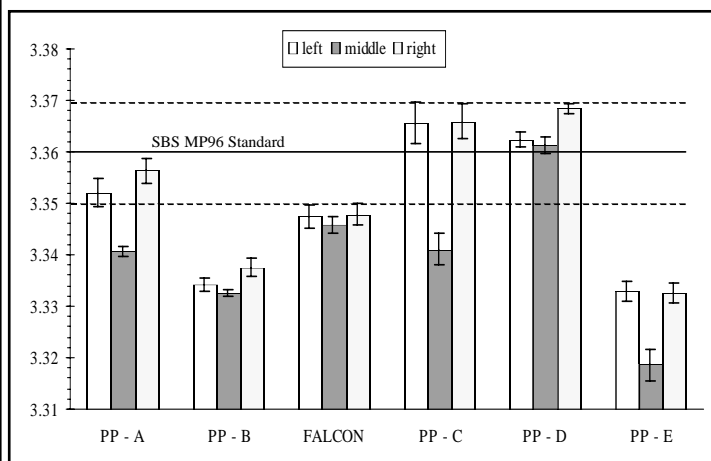
**Figure 3:** PS Plate Height Distribution Histogram — A total of 32 varieties of PS plates from 11 different manufacturers (including BD Labware's FALCON Pro-Bind and TC-treated plates) were tested. Three plates of each type were measured for height at the *top* (A1 to A12) and *bottom* (H1 to H12) edges of the plate. The distribution of all height measurements is shown (not the mean values). While the PS plates tested were primarily clear with flat-bottoms (63% of total), some black (9%), white (16%), black-clear bottom (3%) and white-clear bottom (9%) plates (all, flat-bottom wells) were also tested. The lower horizontal bar indicates the range of plate height allowed by the SBS MP96 Standard. The upper horizontal bar indicates the range of height values measured for the FALCON plates tested in this study.

### PP Plate Length Distribution: All Plates



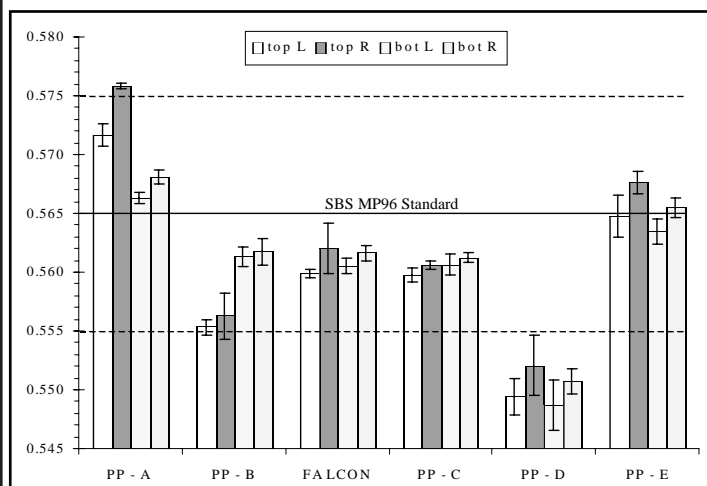
**Figure 4:** PP Plate Length Results — PP plates from 6 different manufacturers (including BD Labware's FALCON HTS Multiwell™ Library Storage Plates) were tested. Five plates of each type were measured for length at the *top* (A1 to A12 edge), *middle* (A1-H1 midpoint to A12-H12 midpoint) and *bottom* (H1 to H12 edge) of the plate. The 3 mean length measurements are shown with the corresponding standard deviations, for each plate type. While the PP plates tested were primarily flat-bottomed (63% of total), one V-bottom type was included. One other type of plate was made from a PP copolymer, rather than PP only. The horizontal bars indicate the range of plate length allowed by the SBS MP96 Standard.

### PP Plate Width Distribution: All Plates



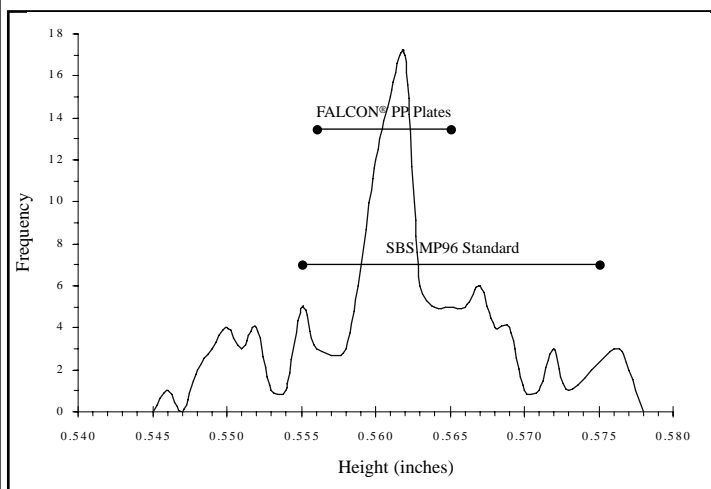
**Figure 5:** PP Plate Width Results — PP plates from 6 different manufacturers (including BD Labware's FALCON® HTS Multiwell™ Library Storage Plates) were tested. Five plates of each type were measured for width at the *left* (A1 to H1 edge), *middle* (A1-A12 midpoint to H1-H12 midpoint) and *right* (A12 to H12 edge) of the plate. The 3 mean width measurements are shown with the corresponding standard deviations, for each plate type. While the PP plates tested were primarily flat-bottomed (63% of total), one V-bottom type was included. One other type of plate was made from a PP copolymer, rather than PP only. The horizontal bars indicate the range of plate width allowed by the SBS MP96 Standard.

### PP Plate Height Distribution: All Plates



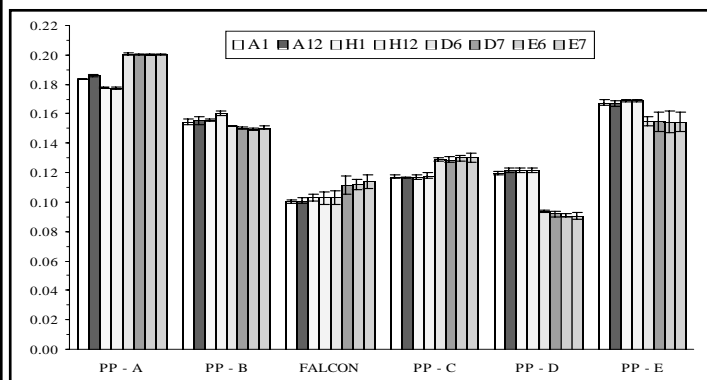
**Figure 6:** PP Plate Height Results — PP plates from 6 different manufacturers (including BD Labware's FALCON HTS Multiwell Library Storage Plates) were tested. Five plates of each type were measured for height at the *top left* (A1), *top right* (A12), *bottom left* (H1) and *bottom right* (H12) corners of the plate. The 4 mean height measurements are shown with the corresponding standard deviations, for each plate type. The horizontal bars indicate the range of plate height allowed by the SBS MP96 Standard.

### PP Plate Height Distribution: All Plates



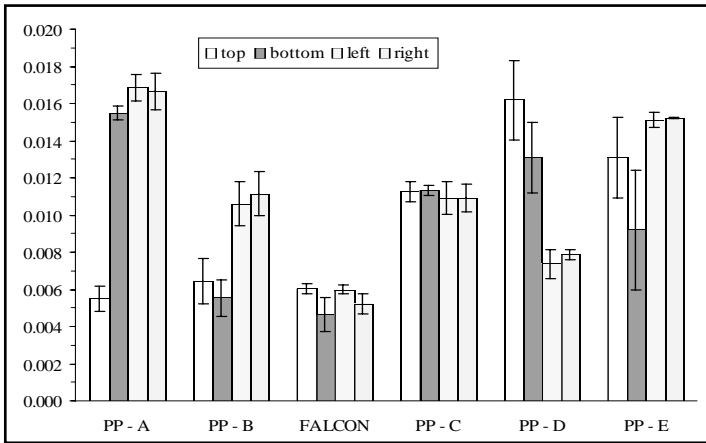
**Figure 7:** PP Plate Height Distribution Histogram — PP plates from 6 different manufacturers (including BD Labware's FALCON HTS Multiwell Library Storage Plates) were tested. Five plates of each type were measured for height at the *top left* (A1), *top right* (A12), *bottom left* (H1) and *bottom right* (H12) corners of the plate. The distribution of all height measurements is shown (not the mean values). The lower horizontal bar indicates the range of plate height allowed by the SBS MP96 Standard. The upper horizontal bar indicates the range of height values measured for the FALCON plates tested.

### PP Plate Well Height Above Resting Plane



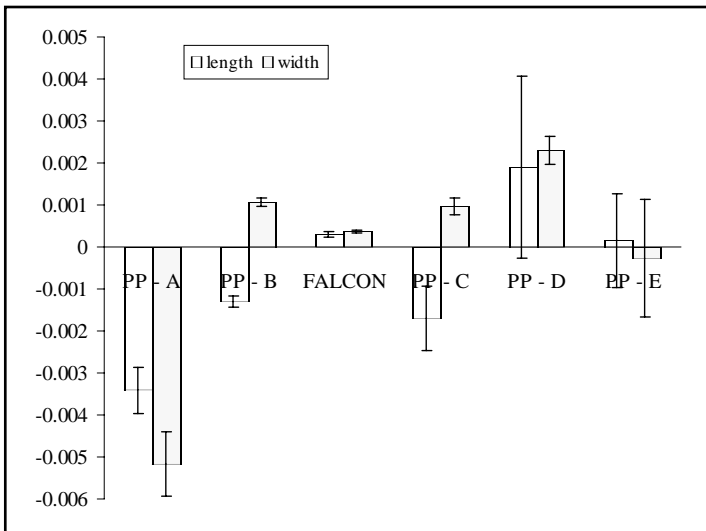
**Figure 8:** PP Plate Well Height Above Resting Plane (WHARP) Results — PP plates from 6 different manufacturers (including BD Labware's FALCON HTS Multiwell Library Storage Plates) were tested. Five plates of each type were measured for WHARP at the *OUTER* wells A1, A12, H1, H12, as well as the *INNER* wells D6, D7, E6 and E7, of the plate. **WHARP represents the height of the top, fluid-contact surface of a well bottom above the resting plane surface on which the plate resides.** For optimal fluidic access to wells, ideally all wells should have equivalent WHARP values. For non-ideal plates, the maximum within-plate WHARP difference is expected to be observed between the *INNER* and *OUTER* well groups defined above. The 8 mean WHARP measurements are shown with the corresponding standard deviations, for each plate type. The SBS MP96 Standard does not define any range for plate WHARP, although MP96 does provide a specification for the distance between the *bottom surface* of the well array and the resting plane (see section 7 of MP96 Standard).

### PP Plate Sidewall Warp/Runout



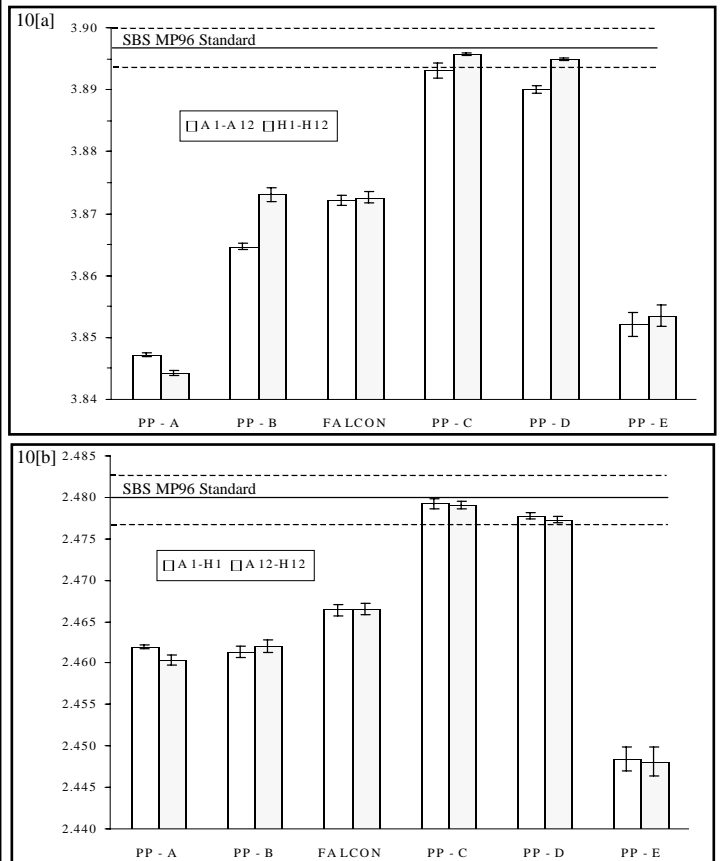
**Figure 9:** PP Plate Sidewall Warp/Runout Results — PP plates from 6 different manufacturers (including BD Labware's FALCON HTS Multiwell Library Storage Plates) were tested. Five plates of each type were measured for runout at the midpoint of the top, bottom, left and right edges of the plate. The 4 mean runout measurements are shown with the corresponding standard deviations, for each plate type. The plate runout allowed by the SBS MP96 Standard, section 3.2, is that within  $\pm 0.020$  inch ( $\pm 0.50$  mm) deviation from a straight line. All PP plates tested met this specification. However, only the FALCON plates exhibited runout values consistently lower than 0.007 inch (0.18 mm). For all other PP plates, 50-100% of runout values were  $> 0.010$  inch ( $> 0.25$  mm), ranging up to 0.019 inch.

### Center-to-Center Superposition: Well Array vs. PP Plate



**Figure 11:** PP Plate Well Center-to-Center Superposition Results — PP plates from 6 different manufacturers (including BD Labware's FALCON HTS Multiwell Library Storage Plates) were tested. Five plates of each type were measured for accuracy of center-to-center superposition between the well array and the plate footprint. This was measured in both the lengthwise and widthwise orthogonal dimensions. The 2 mean differential center-to-center superposition measurements are shown with the corresponding standard deviations, for each plate type. The well array vs. plate center-to-center superposition specification is absolute (i.e., allows zero tolerance) according to the SBS MP96 Standard, section 3.3. However, the more pragmatic approach adopted here allows an error limit of  $\pm 0.003$  inch ( $\pm 0.08$  mm), to be consistent with the noncumulative error limit set by MP96 for well center-to-center distance (see Figure 10). Even against this relaxed "specification" the PP-A plates failed to show well-behaved superposition. Plate type PP-E only exhibited a bimodal distribution in which the 2 modes were each significantly different from zero offset (i.e., showed poor superposition), as if 2 different molds (each with antipodal superposition offsets vs. the other one) were used. When the mean absolute values were calculated instead to reflect the true mean offsets from center, these values for the PP-E plate type were 0.00099 inch lengthwise and 0.00122 inch widthwise.

### PP Plate Well Center-to-Center Distance



**Figure 10:** PP Plate Well Center-to-Center Distance Results — PP plates from 6 different manufacturers (including BD Labware's FALCON HTS Multiwell Library Storage Plates) were tested. Five plates of each type were measured for center-to-center distance between the maximally separated wells on each edge. This was measured in both the *lengthwise* (A1 to A12, and H1 to H12; see Figure 10[a]) and *widthwise* (A1 to H1, and A12 to H12; see Figure 10[b]) dimensions. The 4 mean center-to-center distance measurements are shown with the corresponding standard deviations, for each plate type. The horizontal bars indicate the range of well center-to-center distance for the indicated well pairs allowed by the SBS MP96 Standard, sections 2.11 and 2.12, which specify that wells should be separated by multiples of 0.03543 inch (9.00 mm) with a noncumulative error limit of  $\pm 0.003$  inch ( $\pm 0.08$  mm).