Development of a new storage plate for sample solutions reducing compound consumption and supporting assay miniaturization

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Introduction

384 well polypropylene microplates are frequently used for storage of compound solutions, patient samples or biomolecules.

Direct transfer of sample material from a storage into an assay plate is becoming more and more important in screening, diagnostic and research applications. For instance pre-dilution with aqueous buffers of DMSO compound solutions sometimes results in precipitation of samples which for highly active compounds may lead to false positive results if tips are not washed sufficiently.

Currently available polypropylene microplates do not fulfill all needs for direct sample transfer. Especially low sample volumes cannot be positioned exactly enough for reliable access by pin tools or pipette tips and valuable sample material can get lost.

In cooperation with the Compound Management and HTS groups of Boehringer Ingelheim (Biberach, Germany) a new well geometry has been developed which enables the perfect positioning of even small sample volumes and facilitates the direct transfer from storage into assay plates.

Requirements for the new well/plate design:

- The wells should have a maximal volume of approximately 100 µL in order to enable pre-dilution of samples.
- The positioning of small sample volumes must be absolutely focused in the well cone so that the sample can be easily transferred by pin tools or pipette tips.
- The plate should follow the most important ANSI recommendations (length, width, height, bowing) to be compatible with robotic liquid handling systems and common plate handling processes (e.g. heat sealing, piercing, stacking).

Development and Results

To determine the optimal well cone geometry a hybrid prototype microplate with three different cone geometries was designed (Fig. 1).

- Design 1: conical well with a round bottom
- Design 2: conical well with a flat bottom
- Design 3: blunt cone with a round bottom

Different Well Geometries of cones

Fig. 1

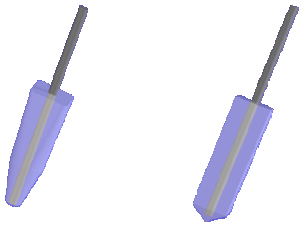
The resulting hybrid test plate was evaluated in empirical / practical tests. The wells were tested with 50 nL and 100 nL pin tools by Boehringer Ingelheim according to the following procedure:

- Filling of the plate with 1 µL and 2 µL DMSO Orange G solution, respectively (Flexdrop PE)
- Visual control and centrifugation
- Transfer with 50 nL and 100 nL pin tools into an assay plate (20/40 µl aqueous buffer solution, V&P Scientific)
- Validation of the transfer precision by absorption measurement (TECAN Ultra)

Table 1: Pin tool liquid transfer from different wells

				Design 1	Design 2	Design 3
50 nl pin tool	from 1µl	into 20µl	CV [%]	6.410	2.982	4.480
			Min [OD]	0.384	0.528	0.487
			Max [OD]	0.618	0.616	0.601
			Vol. [nl]	53	53	51
	from 2µl	into 20µl	CV [%]	4.533	2.849	5.209
			Min [OD]	0.523	0.577	0.508
			Max [OD]	0.675	0.660	0.679
			Vol. [nl]	58	58	58
100 nl pin tool	from 2µl	into 40µl	CV [%]	3.075	3.895	3.310
			Min [OD]	1.088	1.095	1.025
			Max [OD]	1.246	1.253	1.211
			Vol. [nl]	106	108	101
	from 1µl	into 40µl	CV [%]	5.501	5.726	4.789
			Min [OD]	0.952	0.905	0.787
			Max [OD]	1.152	1.148	1.098
			Vol. [nl]	95	96	90

- Design 1 showed splashing with some pipetting devices.
- Pin tools can be damaged with design 3 if the pins touch the well wall.
- Design 2 showed the best performance in liquid transfer (Table 1).
- The flat well bottom of design 2 was easily accessible in contrast to the standard V-bottom design (Fig. 2).
- The flat bottom slightly reduced the liquid height for volumes below 1 µl and concentrated the sample in the well cone.



0.8 mm pin tool in the new small volume well

0.8 mm pin tool in the standard V-bottom well

Fig. 2: Positioning of pin tools

- To reach a total volume of 100 µL the well cone was combined with a square well geometry at the top (Fig. 3).
- The final working volume of the well is 1- 90 µL, the maximal volume is 107 µL. The outer dimensions and the microplates height (Fig. 4 and Fig. 5) is following the ANSI-recommendations.

Fig. 3: Shape of the new well design

- The shape of the well top and the height of the well allow easy piercing and reduce the risk of cross contamination by pierced sealing foil.

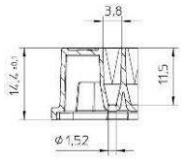


Fig. 4: New well design

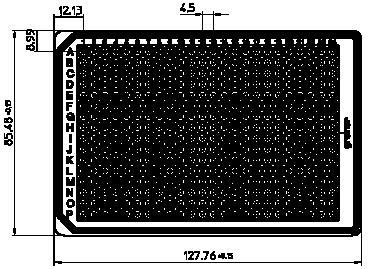


Fig. 5: Microplate dimensions

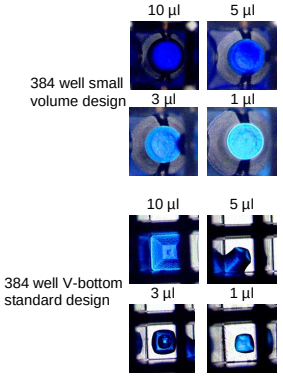


Fig. 6: Location of liquid at the bottom of the new well in comparison to a standard V-bottom well

Testing of the first molded samples

After the determination of the best well cone design the mold was constructed and first prototype samples manufactured.

Filling properties of the first prototypes were tested by dry transfer of aqueous Brilliant Blue solution (1, 2, 3, 5 and 10 µl) into the wells and visual examination of the location of the liquid (Fig. 6).

Results

As expected from the pre-tests the liquid distribution in the first test plates was perfectly focused in the middle of the wells and completely covered the well bottom. In comparison existing plates on the market show an uneven liquid distribution (Fig. 6).

Future perspectives

Acoustic liquid handling is becoming an increasingly accepted technology for the accurate transfer of sub microliter volumes. One of the most commonly used microplate in acoustic liquid handling is the standard 384 well flat bottom polypropylene microplate. Due to the advanced molding technology and the innovative tool design the new plate might be an interesting alternative. The flat and homogenous microplate bottom improves the performance of acoustic liquid handling and the conical well design results in a low dead volume.

Conclusion

A new 384 well polypropylene plate has been designed and developed that offers several advantages in comparison to existing plates:

- Low sample volumes are positioned precisely enough for reliable direct compound transfer by pin tools or pipette tips.
- The large total well volume allows substantial pre-dilution of compound solutions with aqueous buffers or DMSO when necessary.
- The plate follows the most important ANSI recommendations (length, width, height, bowing) and is compatible with robotic liquid handling systems and common plate handling processes (e.g. heat sealing, piercing, stacking).

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