Application Forum

Improved thermal management of microtiter plates using the BioCision CoolSink

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Introduction

There is a tacit assumption that any object in direct contact with crushed ice must rapidly equilibrate near 0°C. Yet target temperature and well-to-well temperature consistency are difficult to obtain by placing microtiter plates directly on crushed ice. In addition, arranging plates directly on ice presents a number of physical drawbacks including plate instability, variation in the distribution of the plate contact, potential for ice contamination of the well contents, degradation of support integrity with time, and difficulty in leveling the plate.

This study examines the thermal shift profile and equilibrium temperature properties of a room-temperature 96-well microtiter plate placed directly on ice or on a precision-machined thermoconductive interface. We demonstrate that the desired temperature management can easily be achieved through use of the BioCision CoolSink plate adaptor. Plates equilibrate more reproducibly, and at a temperature closer to that of the thermal sink. Performance can be further improved through the use of a gap-filling aqueous thermoconductive medium.

Materials and Methods

The Biocision CoolSink 96F is a plate adaptor tool constructed from a thermoconductive material and features a pedestal stage that contacts the entire undersurface of the well area of the plate.

The 96-well plate was either placed directly on finely crushed ice, directly mounted on the CoolSink, or mounted with ~2 mL aqueous thermoconductive medium (ACM) between the plate and the CoolSink. In the latter two cases, the CoolSink:plate assembly (PCS-ACM) was placed directly on the crushed ice.

One hundred microliters of water was introduced into each well of a representative 96-well flat-bottom culture plate. A thermocouple probe was placed with the bead submerged in well D6. Following initial equilibrium at 21.5°C, the plate (or CoolSink:plate assembly) was placed directly onto leveled, freshly prepared crushed ice and pressed down until the bottom surface uniformly contacted the crushed ice. The temperature shift profile was recorded by a data logger at 1-s intervals until the plate temperature reached equilibrium. During one of the lower temperature equilibrium plateaus, a second thermocouple with 0.1°C resolution was used to measure individual well temperatures. Experiments were repeated in quintuplicate.

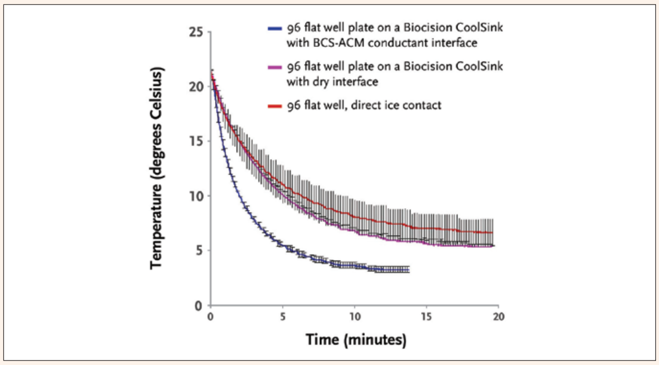

Figure 1. Temperature shift profiles for 96-well flat-bottom plate in direct contact with cold sink. Plates are resting on crushed ice (red), a CoolSink with dry interface (magenta), or a CoolSink with aqueous conductive medium at the interface (blue). Plots shown represent the average of 5 repeats. Data points were taken at 1 sec intervals. The error bars (shown for every tenth data point) represent one standard deviation.

To determine the variance in height of the 96-well flat-bottom plate undersurface, a ball probe on a precision mechanical dial indicator was applied to the surface following a grid path that traced through the center of each well. The same grid pattern was used for the CoolSink surface. Data points were collected at each well position.

Results & Discussion

Thermal shift profiles. We measured the temperature change of a representative well of a room-temperature 96-well plate as it equilibrated with a 0°C thermal mass. Plates placed directly on crushed ice reached an average final equilibrium temperature of 6.7°C, with a standard deviation of 1.25°C (Figure 1, red). Those placed on the CoolSink with dry interface (magenta) exhibited a highly reproducible thermal profile, with an average final equilibrium temperature of 5.5°C and standard deviation of 0.25°C. Average final equilibrium temperature was achieved most rapidly when ACM was used between the plate and the CoolSink (blue), and was reduced to 3.3°C with a standard deviation of 0.27°C. The plates equilibrate to the atmospheric temperature and the cold sink. Since the contact between the plate and the atmosphere (which has a relatively low thermal conductivity) is a constant in all the experiments, the main variable is the contact between the plate and the cold sink.

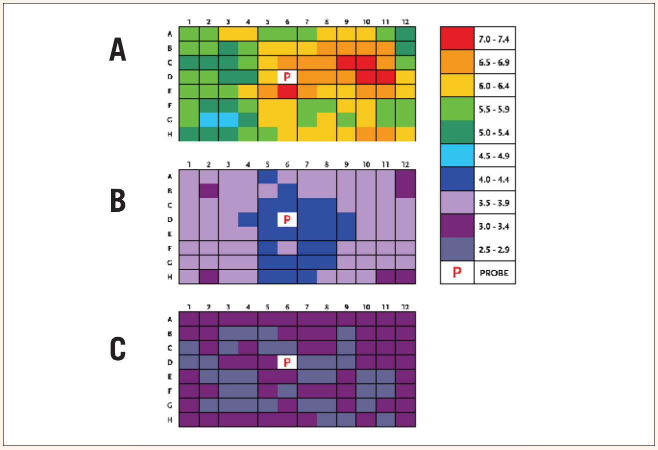

Figure 2. Final equilibrium well temperature for a 96-well flat bottom plate in direct contact (A) with crushed ice, (B) with a CoolSink with a dry interface, and (C) with a CoolSink with aqueous conductive medium in the interface. Colors represent 0.5°C temperature intervals of the corresponding plate wells. The white cell represents the well that was fitted with the thermocouple probe.

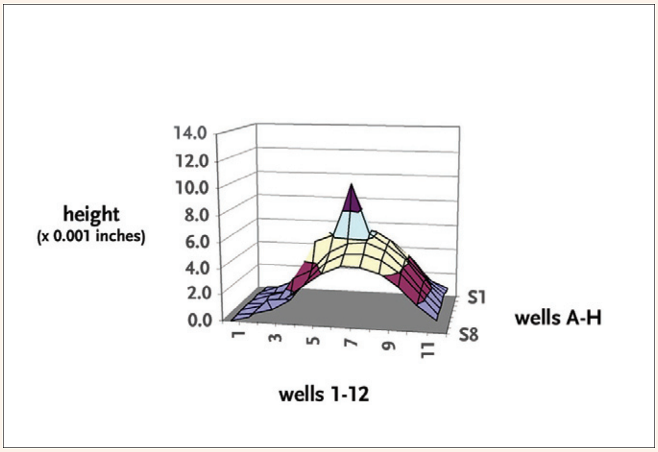

Figure 3. Variance in height of undersurface of 96-well flat-bottom plate. The x and y axes represent the well number and letter, and the z axis represents the relative deviation of the surface in units of 0.001 in. Data was collected using a ball dial indicator with a resolution of 0.0001 in.

Equilibrium well temperature distribution. We sought to define the variance among wells of a 96-well plate at equilibrium with a 0°C thermal mass (Figure 2; each color represents a 0.5°C range). The direct plate to ice interface (Figure 2A) shows a well-to-well temperature range of 3°C, with two higher temperature zones centered on E6 and C10. This is likely due to the plate flexing as it is pressed into the ice, leaving an insulating air gap as it springs back to its original shape. The dry plate to CoolSink interface (Figure 2B) exhibits a range of 1.5°C with a higher temperature zone at the center of the plate and four lower temperature wells corresponding to the four corners of the plate. The ACM plate to CoolSink interface (Figure 2C) displays a range of <1°C with an even distribution of the two temperature intervals.

Interface surface variance. We reasoned that some variance in well-to-well equilibrium temperature variation may be due to a non-uniformity in the relative surface height of the 96-well plate wells, leading to a differential in the insulating air gap. We measured the air gap between the 96-well plate and the CoolSink surface over the interface area. While the surface variation of the CoolSink contact pedestal was constant ±0.0001 in (data not shown), the relative surface height of the plate undersurface showed a variation of 0.006 in with one well position showing a relative height of 0.010 in. The surface height measurements showed a distinct dome shape to the plate undersurface (Figure 3), which correlates with the well temperature pattern observed in Figure 2B in which the 96-well plate is in contact with the CoolSink surface with a dry interface.

Conclusion

Low temperature control for culture plates can be a critical feature for a multitude of experimental and clinical situations. When manipulation of well contents is performed in the open-air room temperature condition, the final temperature is dependent upon a dynamic thermal equilibrium. As a result, an even temperature across the plate wells can be achieved only if the rate of thermal energy flow is uniform. The experimental results indicate that uniform and repeatable energy flow is very difficult to achieve using direct plate-to-ice contact as the unstable nature of the crushed ice allows insulating air gaps to form in an unpredictable manner.

Even when the undersurface of the plate deviates from flatness, a thermoconductive CoolSink interface between the plate and the cold sink can improve well-to-well temperature uniformity and the rate of temperature shift. This can also greatly reduce the risk of ice fragment contamination of the plate wells.

Even greater uniformity in well-to-well temperature values is observed when the air in the gap between the CoolSink surface and the plate undersurface is replaced with an aqueous conductive medium. A similar result can be achieved temporarily by allowing water to fill the gap; however, the low viscosity of water allows air to refill the gap after a brief interval. ACM can be quickly removed with a moist sponge followed by a dry tissue if it is likely to interfere with downstream steps.

As the influences of the physical dimension variance in microplates likely apply to all target temperatures, it can be assumed the application of thermoconductive interfaces would show equal value for these purposes. This is currently under investigation.

Reference

Information about the BioCision CoolSink 96F and related products can be found at www.biocision.com.

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