# BEBPA'S HALLOWEEN HORRORS

**Assays that have scared and scarred you!**

# Outliers suddenly appearing in a 96-well plate cell-based assay

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**Assay Type:** ☐ Binding ☒ Cell-Based ☐ Animal-Assay ☐ Other (Please fill in)

Assay for VEGF & inhibitors, alamarBlue reduction by HUVECs

Retrospective investigation of raw data: outliers due to very low reading in one well of triplicate. Occurring at low frequency, random positions on plates, in test samples, reference standard & control. Appearance coincided with new aliquot of cells, batches of assay medium, 96-well plates, tips.

Likely suspects & easy checks: plate reader? pipettes? tips? etc. . . . checked out OK.

Plan: run assay with 5 times number of plates (=15 plates), simplified layout, microscope examination of all wells at each step after cells + VEGF dispensed (normal procedure: a few wells checked at 48h).

After seeding with cells + VEGF, discoloration visible in well C9 of 1 plate. After 48h, no / very few adherent cells in wells C2 - C9 of this plate. alamarBlue added as usual. End readout C2-C9 replicated outlier problem.

…….. Start examining remaining unused plates (plate + lid packaged in individual wrappers)

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Then . .
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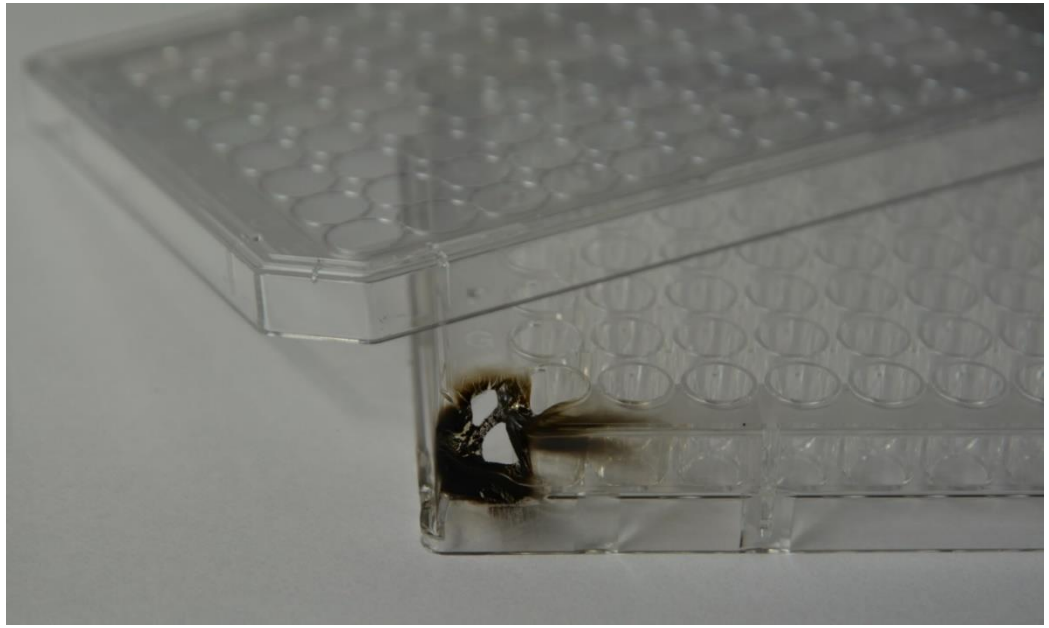

Lid & wrapper undamaged

Conclusions: The plates proved to be the cause of the problem. Trending assay results & ready availability of raw data (absorbance values with well position) proved their worth. Automatic AI-assisted microscopic examination of cell layer in each well at each step could have alerted to problem.

And – no component can be trusted!