Increased reliability of cell counting systems by using coated sample cups

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1.) Problem statement: Adsorption of cells to sample cups

Adsorption of cells was observed to a greater or lesser extent

- with different defined media
- with different cell lines
- with different sample cup material
  - styrene acrylonitrile (Cedex® cups, see figure 1)
  - polystyrene
  - polypropylene
  - Eppendorf® cups and sample tubes

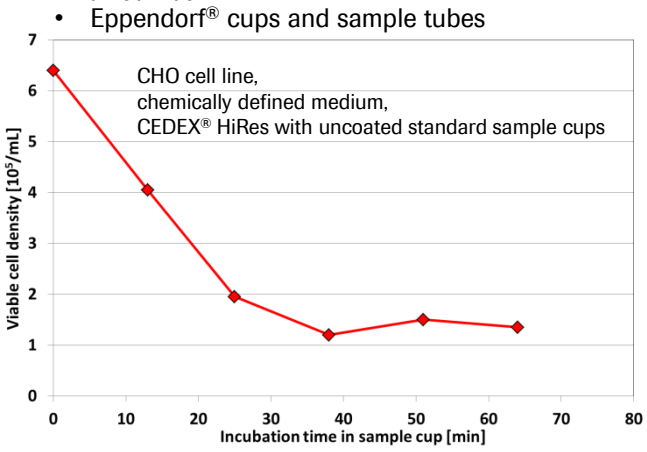

Figure 1: Adsorption of cells to standard sample cup

Adsorption follows certain rules:

- Classical adsorption kinetics: dependent on concentration, saturation, volume / surface ratio
- Adsorption can be inhibited by adsorptive substances: proteins (e.g. BSA), complex cell culture media components (e.g. hydrolysates)
- Adsorption of cells is reversible: desorption by adding trypsine; spontaneous desorption may occur after long incubation time (> 120 min)

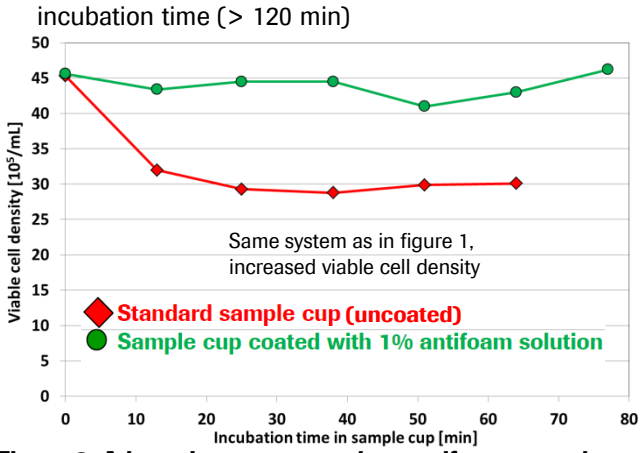

Figure 2: Adsorption to uncoated vs. antifoam coated cups

Adsorption of cells can be avoided by

- coating with BSA or FCS
- coating with anti-foam solution (see figure 2)
- once at a time measurement without autosampler
- …other creative lab solutions

Disadvantages of these approaches:

- Coating is not stable, debris may block measurement chamber of the device
- Once at a time measurement does not support high throughput
- BSA or FCS coating is not suitable for GMP environment
- Hidden expenses:
  - Additional workload for staff (overnight incubation in coating solution and subsequent drying by manual operation)
  - Increased device maintenance cost
  - Follow-up costs of erroneous measurements

2.) Prevention of adsorption by low fouling coating

Challenge:

Anti-fouling or ultra-low-binding surface coatings are common for multi-well plates. Common prices:

- multi-well plate: > 10 €
- sample cup: < 0.1 €

→ Finding a vendor for coated sample cups offering competitive pricing

Solution:

Development of inexpensive low fouling sample cups together with PolyAn GmbH

Advantages:

- Reliable prevention of cell adsorption
- Coating is covalently bound to cup surface → no spalling
- No hidden expenses

Disadvantage:

- Increased cost → coated sample cup ~ 1 €

Experimental setup to test coating success:

- Worst case system: media and cell line with strongest adsorption in previous experiments
- Two uncoated and ten coated cups were filled with sample of cell suspension at once
- Incubation for 30 minutes at room temperature
- Successive measurement as pictured in figure 3

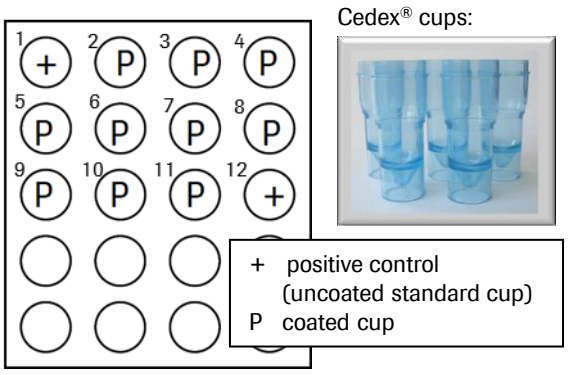

Figure 3: autosampler tray

Results:

→ No adsorption of the cells to the coated sample cups (see figure 4)

→ Successful consistent low fouling coating of sample cups

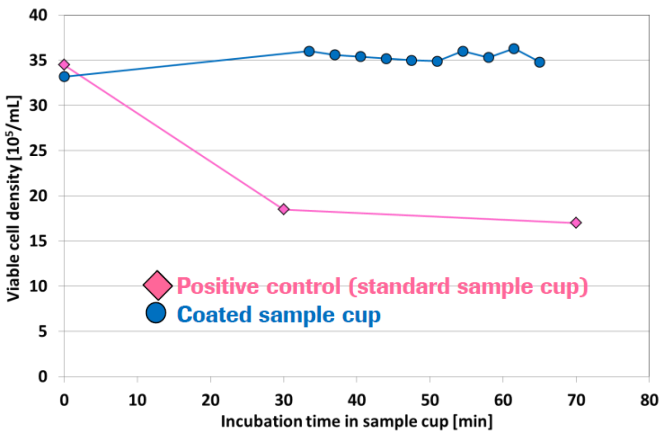

Figure 4: Adsorption of cells to uncoated vs. low fouling coated cups

3.) Quality control of coated sample cups

Challenge:

- Measurement results could be influenced by the quality of the coating
- Need of quality control when reliability of results is of high importance
  - → Decision making in GMP environment
  - → Process characterization
- Quality control (QC) further increases cost
- Identification of QC parameter:
  Cell culture testing is not suitable for QC on a regular basis, but was required for identification of
  - → Quality influencing parameters during coating process
  - → Quality describing physical attributes that can easily be measured for QC on a regular basis

→ **Test cups:** variation of potential quality influencing parameter

Experimental setup of cell culture testing:

- Worst case system (media & cell line)
- two uncoated, two standard coated & ten test cups were filled with sample of cell suspension at once
- incubation for 30 minutes at room temperature
- successive measurement as pictured in figure 5

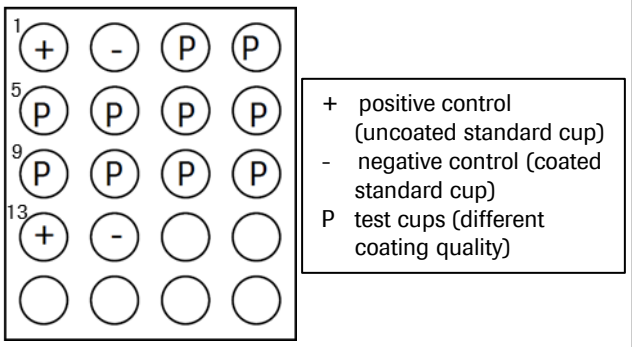

Figure 5: autosampler tray

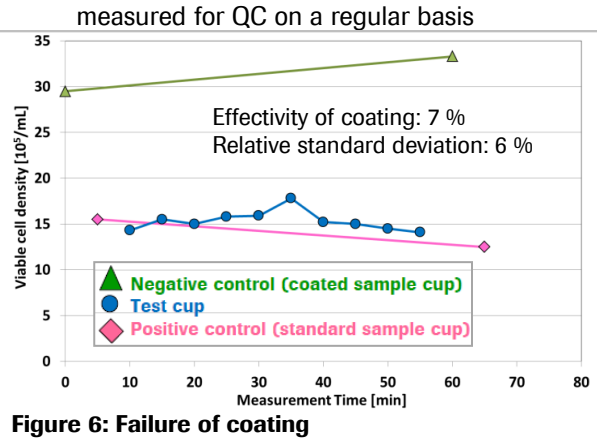

Figure 6: Failure of coating

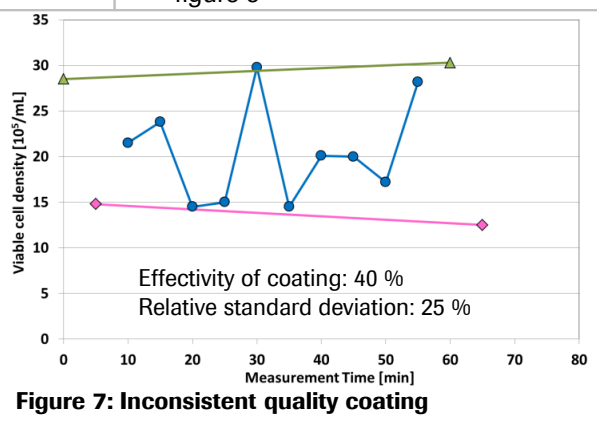

Figure 7: Inconsistent quality coating

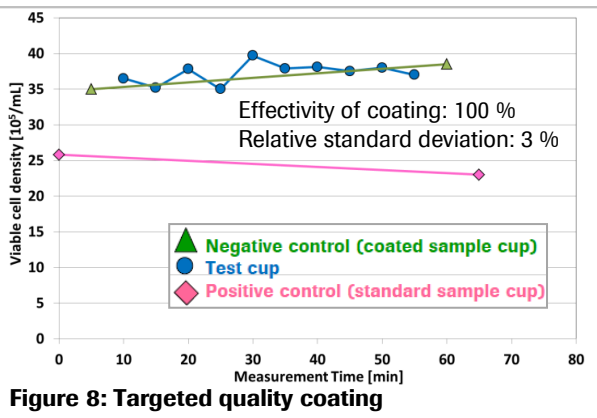

Figure 8: Targeted quality coating

4.) Summary

Quality controlled low fouling coated sample cups can be used to improve measurement reliability of cell counting systems where required