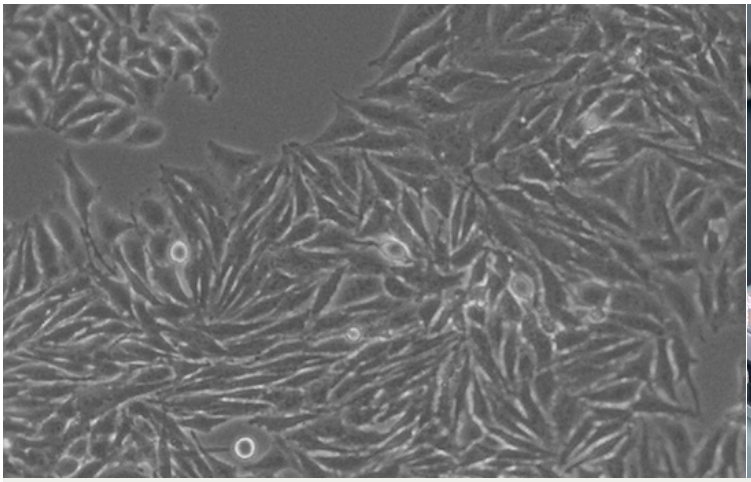


# Optimized cell culture

Establishing protocols for automated harvesting of various cell lines from multiwell plates

## Introduction

Culturing of various stable cell lines, including bacterial, yeast, hybridoma, insect and mammalian lines, and also stem cells, is an important task in the pharmaceutical industry. The cells need to be maintained and expanded for use in cell-based assays, such as siRNA screening, ADME and high content screening, as well as for clone selection. In addition to cell culture flasks (T-Flask, RoboFlask™), cells may be grown in multiwell plates – such as 6-well, 12-well and 24-well plates. This application note describes an automated procedure for harvesting commonly used human and animal cell lines that have been cultivated in multiwell plates, relieving laboratory personnel from tedious cell harvesting work and providing more time for other valuable tasks.

## Material and Methods

**Robotic Equipment**

Experiments were conducted under sterile conditions; the workstation was housed in a biological safety cabinet and the liquid system thoroughly sterilized. Automated harvesting was performed using a Freedom EVO® workstation (Figure 1), equipped with a liquid handling (LiHa) arm with fixed tips, a robotic manipulator (RoMa) arm and a bespoke tilting rack with a Thermo Scientific Variomag® Teleshake. The tilting rack allows complete liquid uptake from large diameter wells, for example from a 6-well plate, as well as shaking for mixing and cell detachment during cell dissociation (Figure 2).


*Figure 1 System overview; 1. Biosafety cabinet, 2. LiHa, 3. Te-Shake™ with chamber slides, 4. Reagent troughs and LiHa wash station, 5. Sterile system liquid, 6. RoMa, 7. Flask Flipper, 8. Te-Shake with tubes, 9. Tilting rack, 10. Centrifuge, 11. Liquid waste. Not all components are required for this application.*

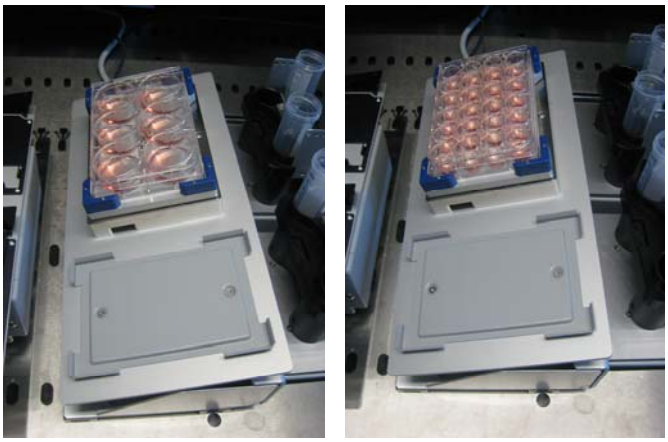


*Figure 2 Tilting rack with shaker, 6-well and 24-well plates.*

**Cell lines**

Four cell lines – HeLa, SaOs, CHO and HEK – with various adherence properties were selected (Table 1 and Figure 3).

| Cell line | Definition | Adherence property |
|---|---|---|
| **HeLa** | Derived from human cervical cancer (Henrietta Lacks, 1951) | Medium adherence<br>Tend to grow irregularly |
| **SaOs** | Sarcoma osteogenic cells | Strong adherence<br>Regular growth |
| **CHO** | Chinese hamster ovary cells. Widely used for protein production | Low adherence<br>Can grow in suspension |
| **HEK** | Human Embryonic Kidney cells. Transformed cells | Low adherence |

*Table 1 Cell lines used for experiments*.

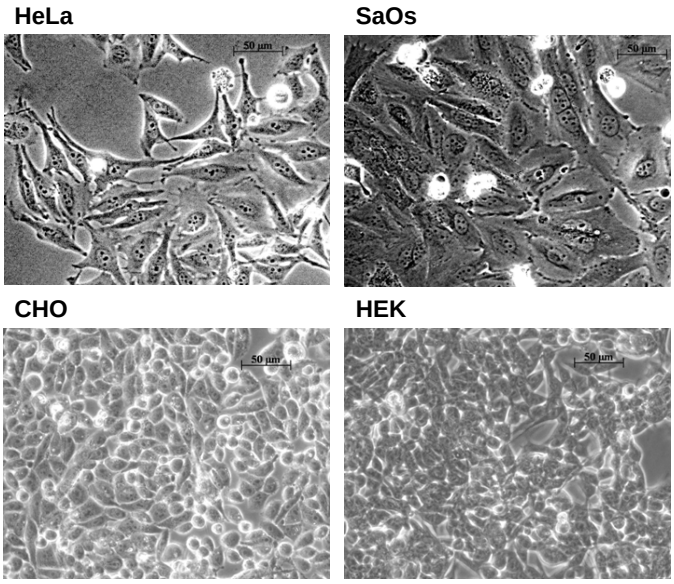



*Figure 3 Microscopy images of used cell lines, x40.*

**Experimental conditions**

Cells were grown in standard 6-well and/or 24-well plates (growth area 57 and 45.6 $cm^2$ respectively) until approximately 70 % confluency was reached. At this confluency cells are in the exponential growth phase and may be split or used for assays.

Optimal harvesting parameters, such as reagent volume, incubation time and mixing, were initially determined manually for each cell type. The protocol was subsequently transferred to the Freedom EVO liquid handling platform and the automated procedure optimized (Table 2).

Experiments were performed in parallel by both automatic and manual procedures, using three to six microplates. Cells from all the wells on a plate were combined and the overall cell number, viability and aggregation rate determined using a Cedex® (Roche Innovatis) cell counter. After harvesting, plates were checked under a microscope to confirm that all cells had been detached and collected.

| | **SaOs** | **Hela** | **Hela** | **CHO** | **HEK** | **HEK** |
|---|---|---|---|---|---|---|
| Plate format | 6 well | 6 well | 24 well | 6 well | 6 well | 24 well |
| Growth medium with FCS 10%; P/S 1% | DMEM/F-12; 5 mM Glu | DMEM/F-12 | DMEM/F-12 | Ham's F-12, W 1.176 g/l, NaHCO3 | DMEM/F-12 | DMEM/F-12 |
| Medium volume (ml) | 2.0 | 2.0 | 0.8 | 3.0 | 2 | 0.8 |
| PBS rinse (ml) | 1.8 | 1.8 | 0.8 | 1.8 | 1.8 | 0.8 |
| Volume Trypsin (ml) | 1.2 | 1.2 | 0.5 | 1.0 | 1.0 | 0.4 |
| Incubation (min) | I) 15, II) 15 | 15 | 16 | 5 | 3 | 5 |
| Shaking (rpm) | 1200 | 1200 | 1600 | 1200 | 1600 | 1200 |
| Medium stop (ml) | 1.2 | 1.2 | 0.6 | 1.2 | 1.6 | 0.6 |
| Mix with LiHa (ml) | 2.5 | 2.5 | 1.5 | 2.4 | 3 | 1.4 |
| Medium rinse (ml) | 1.5 | 1.5 | 0.6 | 1.2 | 1.5 | 0.6 |
| Duration (min) | 44 | 30 | 35 | 20 | 17 | 26 |

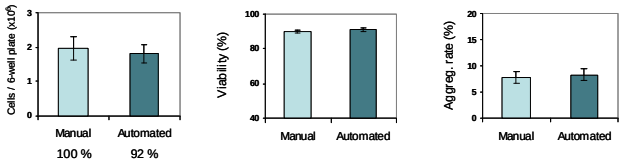
*Table 2 Parameters for harvesting of cell lines in multiwell plates.*

## Results and Discussion

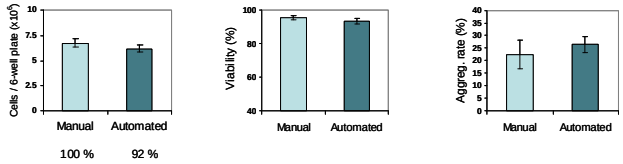
Automated harvesting was comparable to manual harvesting with regard to cell count, viability and aggregation rate (Figure 4).

Cell count for manual harvesting was standardized at 100 %, and results from the automated procedure measured relative to this value. Automated cell harvesting achieved cell counts ranging between 92-105 %, with viability between 80 % and 100 %. However, HeLa cells harvested from 24-well plates with a confluency of 90-100 % showed a viability of only 60 %. This low viability is probably the result of cells reaching the stationary stage due to nutrient depletion. Aggregation rate was highest for HEK cells, however the aggregation was not due to insufficient mixing as this cell type is known to build aggregates. Detailed results are shown in Figure 4.

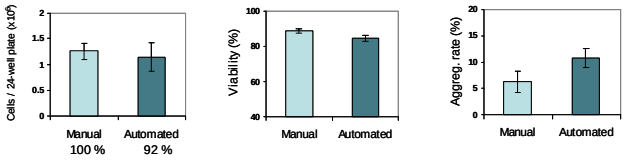
**SaOs (n = 4), 6-well plates**



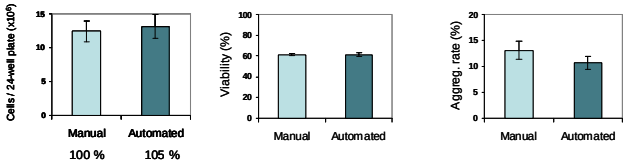

**HeLa (n = 6), 6-well plates**



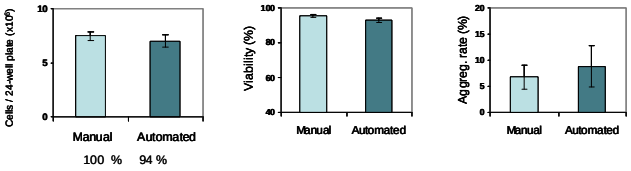

**HeLa (n = 4), 24-well plates, confluency 50-60 %**



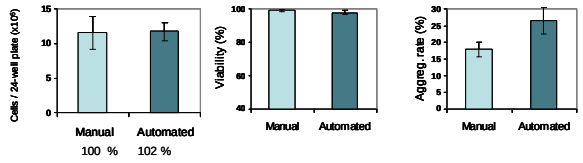

**HeLa (n = 3), 24-well plates, confluency 90-100 %**




**CHO (n = 4), 6-well plates**




**HEK (n = 4), 6-well plates**




**HEK (n = 5), 24-well plates**

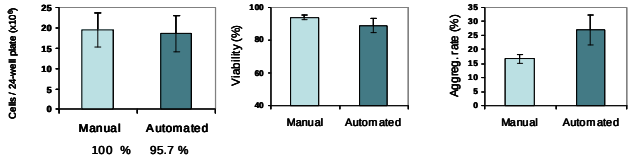



*Figure 4 Cell amount, cell viability and aggregation rate of manual and automated protocols in multiwell plates.*

The most critical parameters for a successful cell harvest with sufficient density and viability were found to be confluency, dissociation procedure and pipetting speed. Protocols were optimized for a confluency of about 70 %, unless otherwise stated. An automated confluency check can assist determination of optimal harvesting time, and Tecan has integrated the Cellavista® cell imaging system – which allows for non-invasive brightfield and fluorescence analysis of cell culture flasks and microplates – onto the Freedom EVO platform.

Tapping the microplate to release dissociated cells is another important consideration when transferring a manual procedure to an automated system. Although the Teleshake was able to suspend cells, parameters such as shaking frequency and time needed to be optimized for each cell line. The speed was adjusted so that the entire well surface was covered with reagent and agitated during incubation. The dissociation reaction was stopped by the addition of medium containing 10 % serum and subsequent shaking step increased harvesting rate significantly (data not shown). The pipetting speed of the cell suspension, and uptake of partition volume, was very important to obtaining comparable harvesting results. Pipetting speed must be moderate, even if this prolongs the procedure for large volumes, as high pipetting speeds and extensive mix cycles might lead to a lower number of cells harvested. Good maintenance practice must be observed, with regular thorough cleaning of the liquid system even if it has not been used, in order to obtain reproducible results.

## Conclusions

Comparable cell harvesting results were obtained for cell count, viability and aggregation rate for manual and automated procedures performed in multiwell plates. Individual protocols have been developed and automation parameters optimized for each of the four cell lines selected; this process must be repeated and adapted for additional cell lines. The Freedom EVOware scripts developed for this study, which are available from Tecan, provide a good starting point for protocols for new cell lines. The four cell lines studied were chosen for their wide range of characteristics, such as strength of adherence and aggregation rate, so the development of protocols for other cell lines with similar characteristics will be straightforward.

## Acknowledgements

We would like to thank Prof Dr Ursula Graf-Hausner and Dr Nicola Franscini, Institute of Chemistry and Biological Chemistry, Zurich University of Applied Sciences, Wädenswil, Switzerland, for the collaboration and for performing the experiments and establishing the protocols.

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396597 V1.0, 01-2011