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Sample quality enhancement by using the ASKION C-line® system

Controlled freezing processes and long-term storage below the recrystallization temperature

White Paper

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Content

1. Biological background on freezing and storage of cells

1.1. Freezing

The survival rate of cells after a freezing process and thawing depends crucially on the used cooling rates. The optimal cooling rates vary from cell type to cell type considerably (Mullen und Critser 2007; Mazur u. a. 2008). Therefore, it is necessary to use an optimized freezing protocol for the cells and to apply this as accurately as possible (Figure 1).

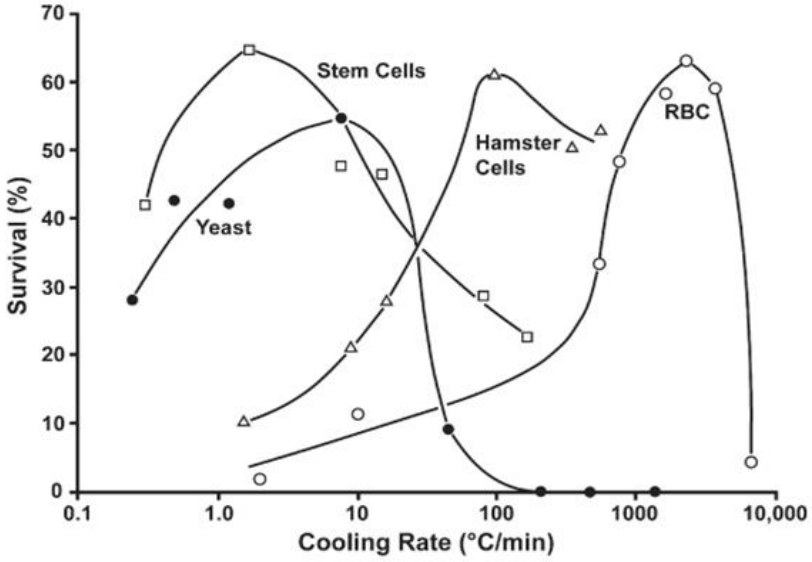

Figure 1: Relationship between the survival rate and the used cooling rate. The respective optima vary significantly from cell type to cell type (Mazur u. a. 2008). RBC – red blood cells

Only then it's possible to get a maximum of viable cells after thawing and continue work without loss of time. Another important point when freezing cells is the prevention of supercooling of the cell suspension until the beginning of crystallization. As a result of supercooling the chosen cooling rate isn't be met, because at the time of crystallization latent heat is released resulting in massive changes in the temperature gradient (Figure 2). Frequent and significant changes in the gradient have a negative effect on the survival rate of the cells. In order to prevent supercooling of the cell suspension a seeding which is a specific induction of crystallization in the range of the predetermined solidification temperature of the cell suspension is performed. This can be done in various ways, such as a rapid drop in temperature and a slight concussion of the samples near the solidification temperature.

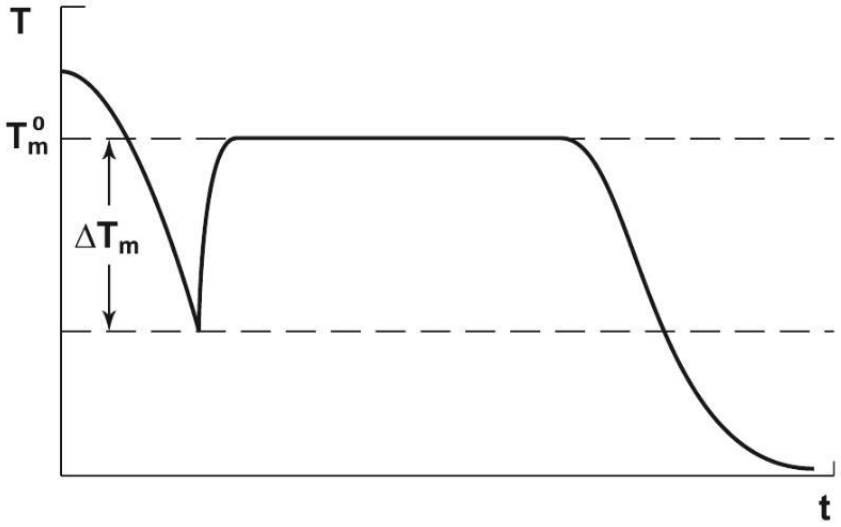

Figure 2: Temperature profile during the freezing of pure water as a model to illustrate the processes (Zhmakin 2009). Freezing cell suspensions, the graphs will vary depending on the composition of the liquid. T_m - solidification temperature; ΔT_m - supercooling of the liquid

In the case of freezing larger quantities of samples, it is furthermore important that all samples are subjected to the same temperature profile as uniformly as possible. The temperature profile should be reproducible over several freezing processes in order to guarantee a comparable quality of all samples. The final temperature of a freezing protocol should be as low as possible as well as the long term storage temperature (Hubel u. a. 2011; Sun u. a. 2002).

1.2. Storage

Storage in the gas phase of liquid nitrogen below -150°C is the most appropriate option, because the sample temperature is safely below the recrystallization temperature (Figure 3). Storage in liquid nitrogen would indeed allow even lower temperatures, however no sample vials are available at present which prevent that liquid nitrogen penetrates in these conditions. Lack of tightness can lead to cross-contamination during storage and bursting during thawing of the vials. The entire further handling of the frozen samples should occur in lowest possible temperatures to avoid recrystallization of the solidified suspension.

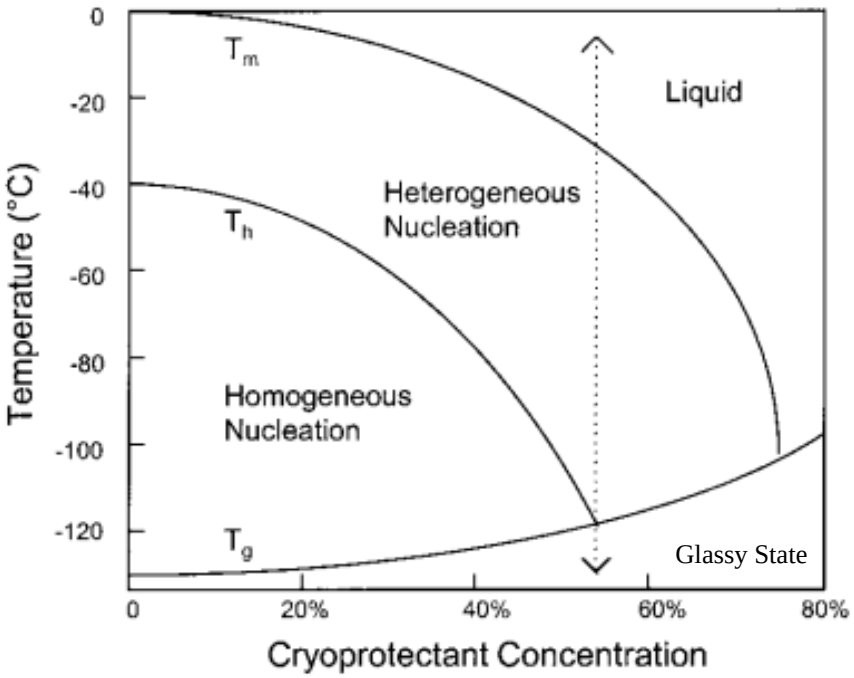

Figure 3: Phase diagram of an aqueous solution of a cryoprotectant. Depending on the concentration of the cryoprotectant, the transition temperature into the glassy phase is about -130°C. Above this temperature a cell-damaging ice crystal growth occurs (Wowk u. a. 2000).

To verify the above mentioned requirements and their benefits on the survival rate of frozen cells ASKION had carried out investigations at the Institute for Multiphase Processes at the University of Hannover. For this purpose the WB220 work bench from the Askion C-line® system was used (Figure 4). The device operates with two elevator freezers above liquid nitrogen. This unique setup results in very little turbulence and creates an almost homogeneous temperature distribution over the horizontal cross section of the freezer shaft. Thus allows applying freezing curves very accurately[1].

[1] For further informations please visit www.askion.com

Figure 4: ASKION C-line® WB220 (top) with detailed view of the two elevator freezers (below) and both the ambient temperature sensor and the reference sensor for temperature measurement.

2. Technical realization of homogenous and reproducible freezing processes

To achieve a comparable quality of each sample during controlled freezing of a large number of samples, it must be ensured that all samples of a batch follow the same temperature profile and that this profile is reproducible for different batches. To meet these requirements of homogeneity and reproducibility, the used freezer must have special properties. The decisive factor here is the presence of a homogeneous temperature stratification surrounding all samples evenly without turbulences. The setup of the freezer shaft in the WB220 ensures that temperature stratification. Thereby all samples of a freezing process are subjected to the same freezing curve and the process is precisely reproducible (Figure 6).

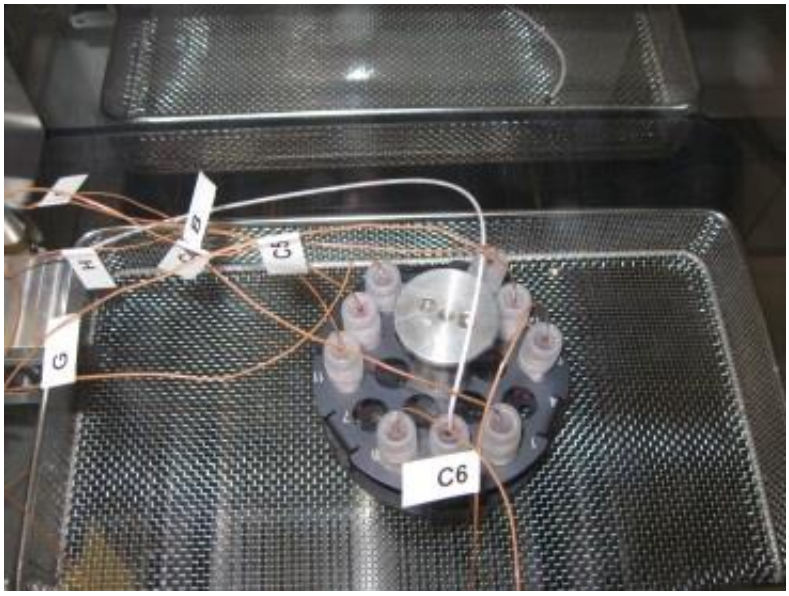

Figure 5: Eight samples were provided with a temperature sensor and frozen with a cooling rate of 1 K/min. Another vial served as a receptacle for the reference sensor.

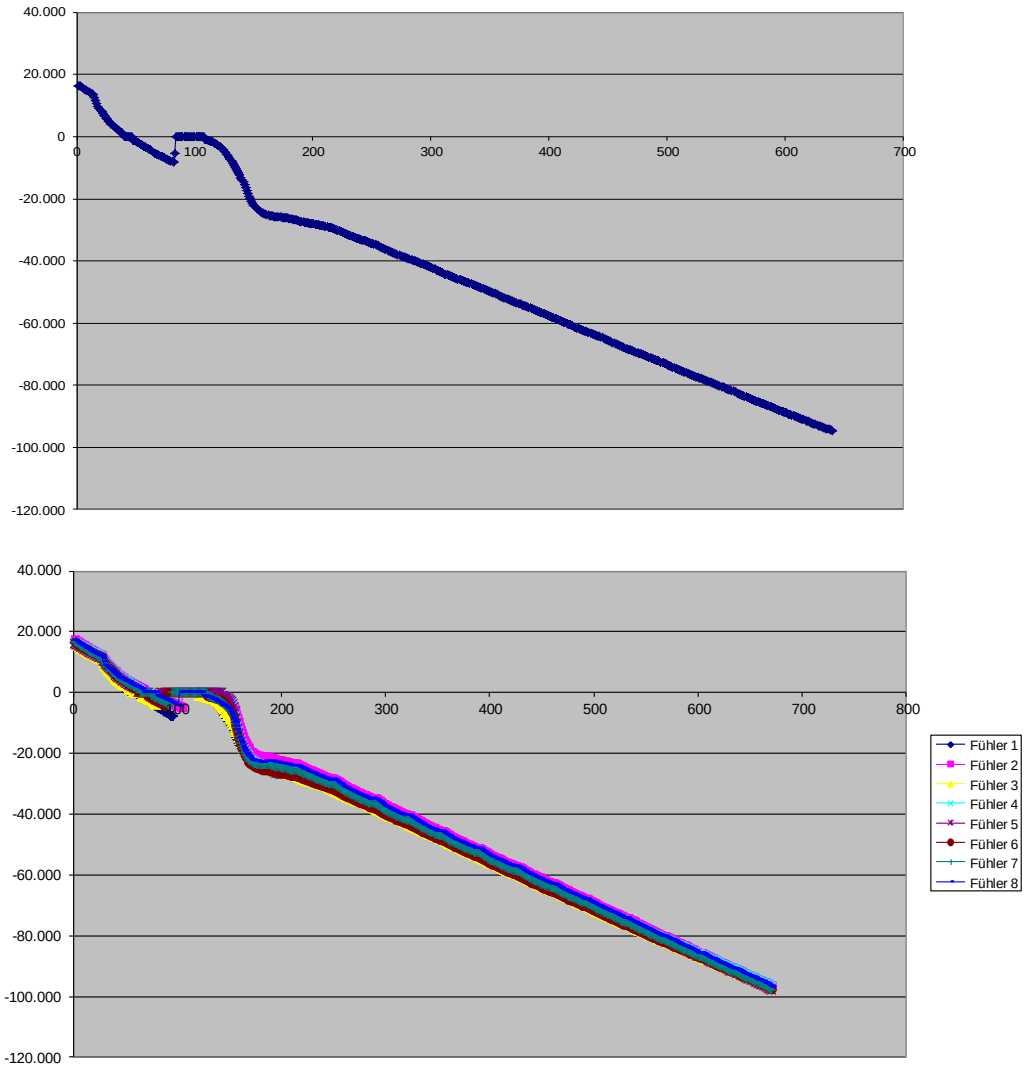

Figure 6: Temperature measurement values of the samples of Figure 5. The freezing process was controlled by the ambient temperature sensor located at the freezer basket. Because the selected freezing protocol includes no seeding the supercooling of the suspension and the subsequent plateau phase can be clearly seen (top diagram). The lower diagram shows the homogeneity of the controlled freezing process for 8 samples (Hofmann 2013).

3. Control of the freezing process as a function of the sample temperature

To apply an appropriate freezing protocol optimized for the particular sample type, it is necessary to measure the real temperature prevailing in the sample and to regulate on this measure. This ensures that the determined optimal temperature gradients are applied to the sample, actually. For this purpose, when using the WB220/230 a sensor can be placed in a reference sample and the entire freezing process is controlled in accordance with this sensor (Figure 5). Thus, it is possible to apply the selected freezing curve very accurately (Figure 7).

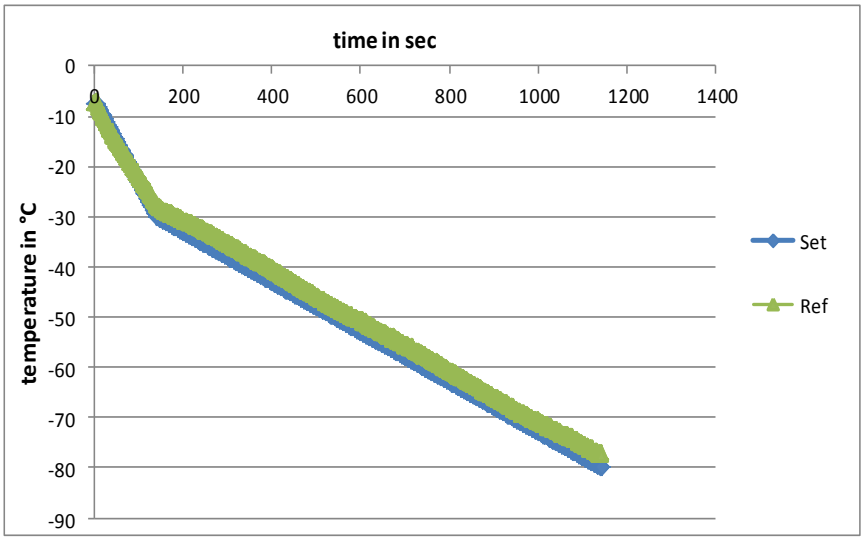

Figure 7: Freezing process of 250 µl straws with 2x10^5 cells controlled in accordance with the reference sensor, which was placed in one of the straws. The cooling rate was 10 K/min to -30°C then 3 K/min -80°C (Hofmann 2013).

This type of control has a positive effect on the survival rate of cells. In the present experiments HPME-cells with an initial cell densitiy of 8x10^5/ml were used (Mackay u. a. 2013). Both the membrane integrity and the vitality of the cells during freezing according to the reference sensor are significantly higher than for a regulation of the process according to the ambient temperature (Figure 8).

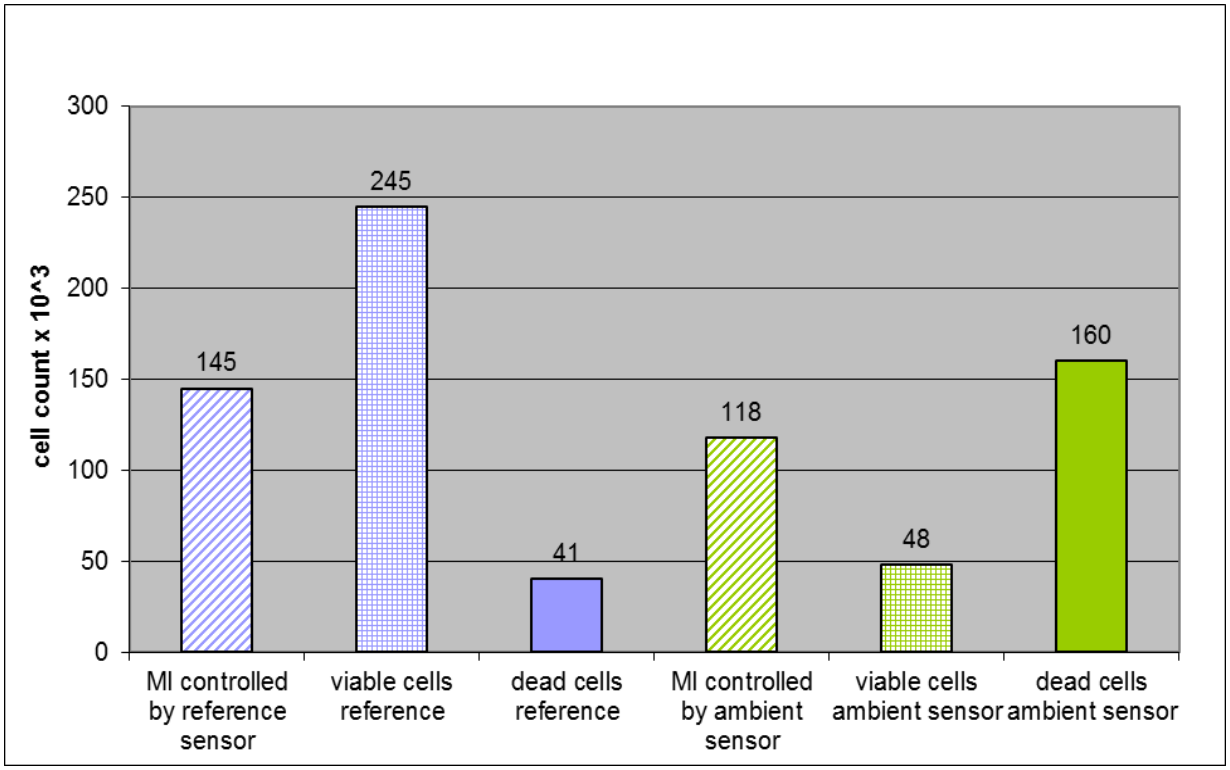

Figure 8: Controlling the freezing process in accordance with the reference sensor increases the number of viable cells (HPMEC) relative to a control according to the temperature of the surrounding gas. As viable cells were those ones classified, which adhered to the support within 24 hours after thawing and started to proliferate. Number of cells before freezing: $2x10^5$, MI - membrane integrity; (Hofmann 2013)

4. Advantages of a specific seeding

As already mentioned at the beginning, an avoiding of supercooling of the cell suspension during the freezing process is very important in order to achieve maximum survival rates of the cells. In the case of the WB220/230 this is realized by means of a purposeful seeding in terms of a rapid temperature drop. While following the freezing curve the sample basket of the elevator freezer is quickly driven down to a temperature range of approximately -160°C when reaching the range of the solidification temperature of the samples. The samples are held in that area parameterized as a function of sample volume. After that the elevator freezer moves back to the corresponding temperature of the freezing curve. This rapid drop in temperature causes the solidification of suspensions and reliably prevents supercooling. As a result, changes in the temperature gradient are prevented and the survival rate of the cells is increased (Figure 9).

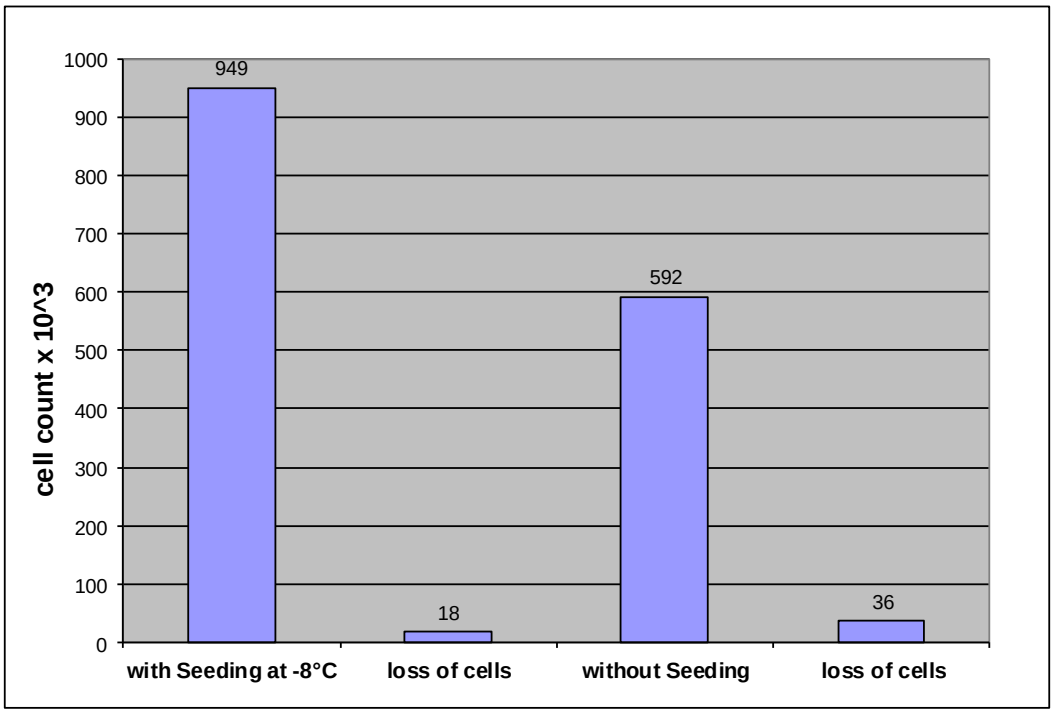

Figure 9: Effects of a specific seeding on the vitality of frozen cells (HPMEC). The number of viable cells and their vitality is significantly increased by seeding. Freezing protocol used: 10 K/min to -30°C, 3 K/min to -80°C, seeding at -8°C, determination of number of cells after 24 h of recultivation (Hofmann 2013)

5. The benefit of a continuous cooling chain

After the freezing process, the further proper handling has also influence on the sample quality. A constant cooling of the samples below the recrystallization temperature in all steps of a procedure increases the survival of the cells significantly, especially in straws. Due to their low volume, this sample format is heated very quickly. A key aspect in the development of the entire C-line® system was the maintenance of a continuous cooling chain at temperatures of not more than -100°C. The entire handling of the samples in the storage system occurs at these temperatures whereas storage takes place in the gas phase of liquid nitrogen at -150°C. Therefore, the sample material isn't subjected to large temperature differences and the quality is maintained throughout the entire storage period. As an example to prove the influence of sample heating on the quality of the different sample formats (250 µl straws and 1.8 ml vials), both straws and vials were distributed among the two elevator freezers of the WB220. One freezer was held constantly at -100°C after the freezing process. The other freezer was moved five times within 10 minutes upwards to an ambient temperature of 8°C and downwards to -100°C (Figure 10). This cyclical heating of the samples resulted in temperature fluctuations in the range of -100°C to -70°C in straws.

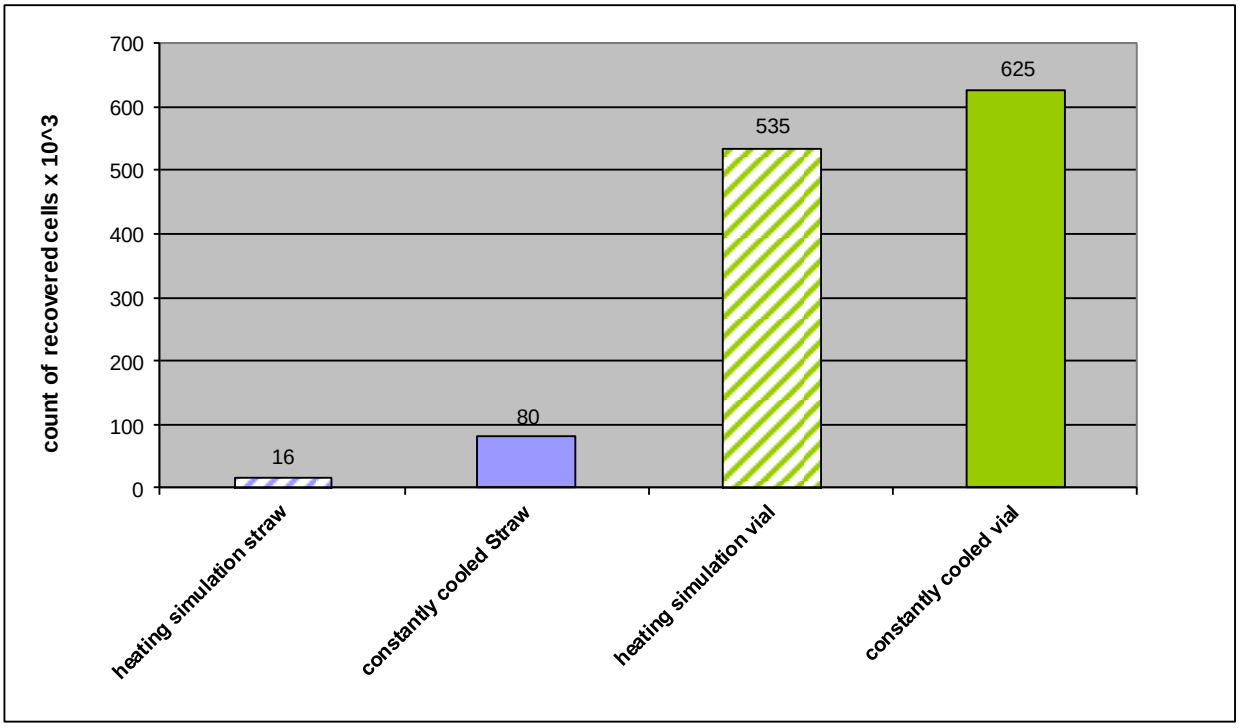

Figure 10: The temporary repeated heating of the sample leads especially in straws to a significant loss of cells. This effect can also be seen in vials (Hofmann 2013).

6. Synopsis

The results of the experiments at the Institute for Multiphase Processes of the University of Hannover consolidate the facts already known from the literature about the importance of accurate and reproducible freezing processes as well as the permanent storage of the material at the lowest possible temperature with minimal fluctuations. The Askion C-line® system offers all necessary components starting with an exact freezing up to reliable long term storage from one manufacturer.

References

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