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# Evaporation from Cell Culture Plates

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When cells are cultured in plates with loose-fitting lids, the growth media will always lose water to the plate surroundings by evaporation, i.e. by water vapor escape. This may cause culture problems, since the medium components may reach concentrations which are harmful to the cells.

The evaporation is due to the fact that the air just above the surface of the medium is always saturated with water vapor, whereas the surrounding air under normal incubation conditions will never be completely saturated. Thus, water molecules will constantly escape along a concentration gradient from the surface to the surroundings by diffusion.

This article discusses the various factors that affect evaporation.

## Lid gap

The rate of evaporation is proportional to the area of the space between the lid and the plate edge separating the plate lumen from the surroundings. Hence, if the slit width is the same for all plates, the speed of evaporation will be proportional to the plate's total edge length, i.e. its circumference.

Further, the time it takes before the evaporation becomes critical will be proportional to the volume of liquid in the plate. If the depth of liquid is the same for all plates, the volume of liquid will in turn be proportional to the surface area of the liquid in the plate.

**Table 1**

Evaporation indices and other figures for Nunc cell culture plates with loose fitting lids. The indices, which are the circumferences of the plates divided by the respective surface areas of liquid, can be regarded as the relative speeds by which the water losses become "critical" provided that the lid gab widths and the depths of liquid are equal for all the plates. Thus, to obtain actual, relative figures, the indices should be multiplied by the respective actual slid widths and divided by the actual medium depths. See text for further explanation.

| Product | Evaporation index cm/cm² | Total liquid area cm² | Commonly used Medium vol. mL/well | Commonly used Med. depth mm |
|---|---|---|---|---|
| Dish 35 | 1.2 | 9 | 3 | 3.3 |
| Dish 60 | 0.8 | 21 | 5 | 2.4 |
| Dish 100 | 0.5 | 58 | 15 | 2.6 |
| Dish 140 | 0.3 | 146 | 35 | 2.4 |
| Dish 245 square | 0.2 | 510 | 135 | 2.6 |
| OmniTray | 0.5 | 87 | 25 | 2.9 |
| Multidish 4 rectangular | 0.4 | 94 | 6 | 2.6 |
| Multidish 8 rectangular | 0.4 | 92 | 3 | 2.6 |
| Multidish 4 square | 3.2 | 8 | 1 | 5 |
| | 1.6* | 16 | | |
| Multidish 6 | 0.7 | 58 | 3 | 3.1 |
| | 0.6* | 67 | | |
| Multidish 12 | 1.0 | 42 | 2 | 5.7 |
| | 0.6* | 67 | | |
| Multidish 24 | 0.9 | 45 | 1 | 5.3 |
| | 0.6* | 64 | | |
| Multidish 48 | 0.7 | 54 | 0.5 | 4.4 |
| MicroWell 96 | 1.3 | 32 | 0.2 | 5.5 |
| MicroWell 96 Edge | 0.7 | 59 | | |

*Indices when liquid has also been added to the spaces between the wells

Therefore, the various cell culture plates with loose-fitting lids can be characterized as more or less prone to critical evaporation according to their specific ratio: circumference/medium surface area, which may be denoted "evaporation index" (Table 1). In principle, the larger the index is for a product, the more prone it is to critical evaporation. Therefore, the tendency should be to use greater depths of medium (i.e. larger volumes of medium per square cm), the larger the indices are (Fig. 3).

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Also, as seen from the asterisked figures in Table 1, critical evaporation can be postponed by adding medium (or water) to the spaces, if present, between the wells in Thermo Scientific Nunc Multidishes and, in particular, in the Thermo Scientific Nunc MicroWell Edge Plate reservoir.

Because of the relatively high evaporation index for standard Nunc™ MicroWell™ plates, the plate lid has been equipped with an edge-rib for narrowing the gap width and thus diminishing the total area. For the same reason the lid has been given a ring shaped rib above each well, as any narrowing in the vapor path from the medium to the surroundings will add to the evaporation delay. These modifications, which are now standard, have been shown to reduce the evaporation by about 50%.

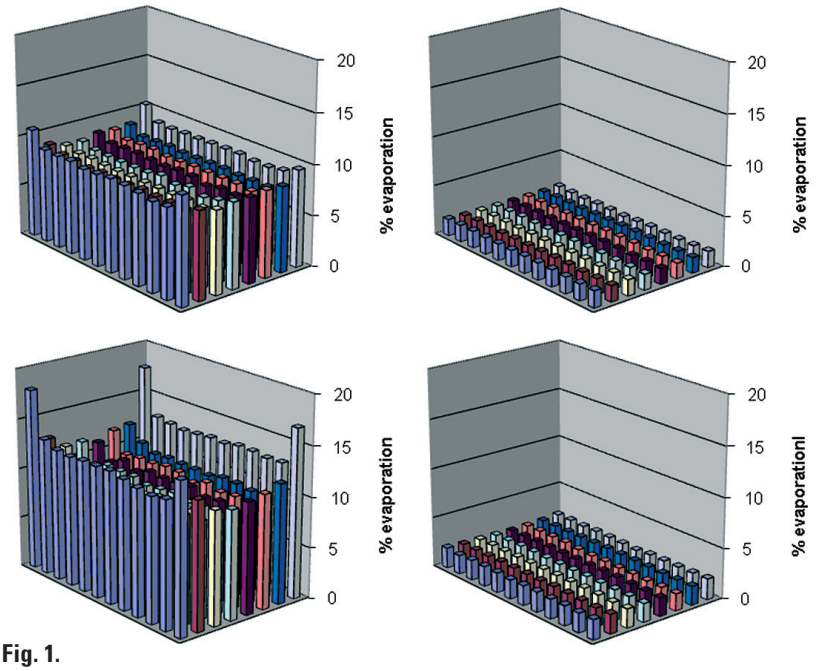


**Fig. 1.**
Evaporation percentages from the individual wells filled with 200 µL liquid in standard 96F plates (left) and in Edge plates (right) after incubation for 7 days at 37°C and 95% relative humidity (above) or 80% relative humidity (below). This clearly demonstrates the substantial evaporation reduction and equalization across the well matrix in Edge plates. See text for further explanation.

## Edge effect

In multiwell plates where all the wells are not positioned identically in relation to the edge of the plate, i.e. MicroWell plates and Multidishes except Multidish 4 (square), a larger evaporation will be seen in the peripheral wells than in the central ones. This is especially so in the corner wells because they are more exposed to the surroundings.

Therefore, users of MicroWell standard plates often avoid culturing in the peripheral wells, which are just filled with pure medium or water as reservoir for evaporation. This implies the drawback of missing fully 37.5% of the plate capacity, which can however be avoided by using instead the Edge plate, where the reservoir is established as peripheral moats around the 96 wells.

Fig. 1 shows the reduction and equalization of evaporation from the wells across the plate at the expense of evaporation from the reservoir in Edge plates compared with standard plates incubated on the middle shelf (of three shelves) in two incubators with different humidity. The plates were filled with 200 µL 0.002% aqueous crystal violet solution per well and weighed, and the Edge plates subsequently filled with 1.75 mL water per reservoir compartment. After 7 days' incubation, the Edge plate residual reservoirs were emptied, and all the plates were weighed again in order to determine the total evaporation from the wells. For distribution of the total evaporation between the wells, aliquots from the wells were transferred to new plates and the relative crystal violet concentrations were measured by OD at 590 nm. These figures were converted to the wells' individual evaporation percent by the following approximation, assuming proportionality between the OD and the evaporation percent:

$$\% \text{ well evaporation} = \frac{\text{g total water loss from wells}}{\text{g total water added to wells}} \times \frac{OD_{well}}{OD_{average}} \times 100$$

## Environmental humidity

To avoid significant evaporation from culture plates with loose-fitting lids, the plates should ideally be incubated at 100% relative humidity. However, the highest obtainable humidity in practice would normally be about 95%. A small water vapor gradient is therefore still operating causing a water loss which may become critical during long term culture. One reason for the inability to keep the humidity at 100% is that the incubator is normally opened frequently during incubation (see below).

The evaporation is also dependent on the plate position in the incubator, an upper position being more susceptible than a lower position consistent with the fact that the humidity is normally established from a water basin in the bottom of the incubator.

Fig. 2 shows the average plate evaporations obtained from plates positioned on the top, middle or bottom shelf at 95% or 80% humidity incubation. The evaporation is almost twice as large at 80% as at 95% humidity for both standard and Edge plates.

Fig. 3 shows that with halved volumes (i.e. halved liquid depths) in MicroWell plates, the evaporation percent increases in a proportionally shorter time, which demonstrates the significance of using Edge plates instead of standard plates, especially when smaller medium volumes are for some reason preferred.

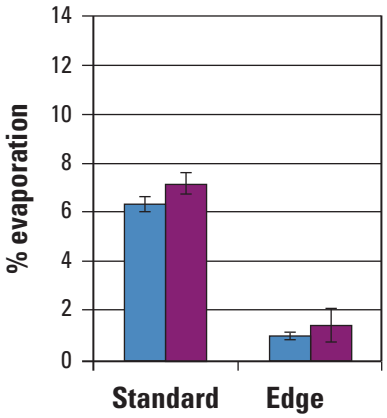


**Fig. 3.**
Total evaporation percentages from the wells filled with 100 µL liquid in standard and Edge plates after incubation on the middle incubator shelf for 4 days at 37°C and 95% relative humidity (violet columns) compared with the data from Fig. 2 for 200 µL liquid per well and incubation for 7 days under similar conditions (blue columns). This demonstrates that the evaporation percent increases in proportionally shorter time with decreased liquid volume.
See text for further explanation.

## Temperature

Animal cell cultures are normally incubated at 37°C, at which temperature the pressure of saturated water vapor is about 2.5 times higher than at room temperature (20°C). In addition, the surrounding humidity is normally far lower outside the incubator than inside. Therefore, the evaporation gradient is greatly increased when the plates are removed from the incubator. On the other hand, the evaporation will decrease with falling temperature due to the pressure decrease, and because air can contain about 2.5 times more water at 37°C than at 20°C, some of the water vapor will be trapped by condensation. This is observed by dew formation on the inside of the lid soon after removal of the plate from the incubator. However, the overall conclusion would be to keep the number of plate removals from the incubator at a minimum during culture.

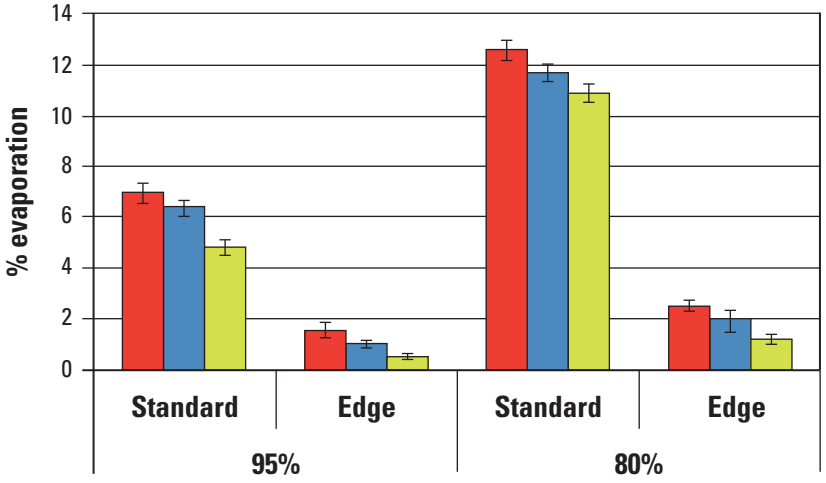


**Fig. 2.**
Total evaporation percentages from the wells filled with 200 µL liquid in standard 96F and Edge plates after incubation on the incubator top shelf (red), middle shelf (blue) and bottom shelf (green) for 7 days at 37°C and 95% or 80% relative humidity. Beside the overall evaporation reduction in Edge plates, this demonstrates the evaporation's dependence on the surrounding humidity and on the plate position in the incubator.
See text for further explanation.

## Air circulation

If the plates are exposed to "draught", the evaporation will be more rapid than if only pure diffusion forces are operating. There will always be some draught, even inside a closed incubator due to its air circulating fan, but when the incubator is opened, or when the plates are moved outside the incubator, a significant draught may be experienced. Therefore, the incubator should only be opened when absolutely necessary, and the plates should be moved slowly and

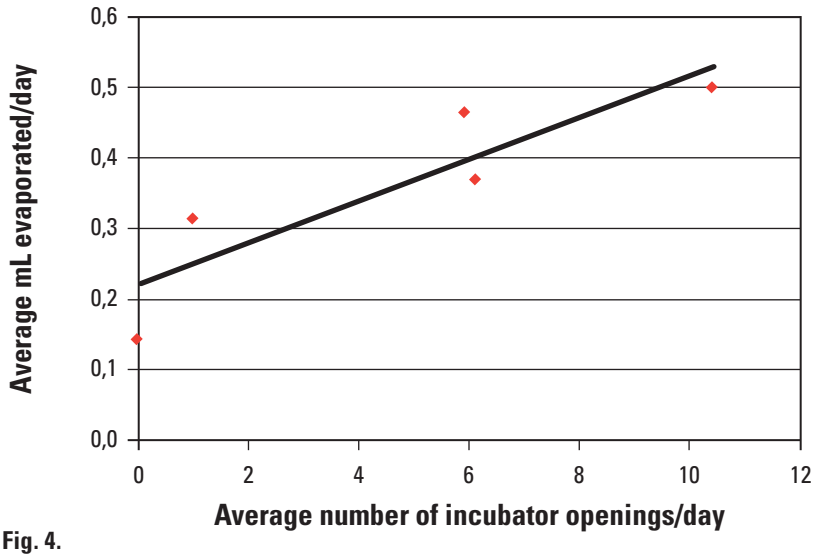


**Fig. 4.**
Evaporation from the reservoir in an Edge plate as a function of incubator door openings during incubation at 37°C and 95% relative humidity. This demonstrates that an additional evaporation occurs every time the incubator is opened. See text for further explanation.

only taken the shortest possible distance from the incubator for inspection.

Fig. 4 shows the evaporation from the reservoir in an Edge plate as a function of the frequency of incubator openings. This indicates that with the initial 1.75 mL per reservoir compartment, 7 mL in total, the evaporation protection may last for 14-28 days incubation at 95% humidity depending on incubator openings up to 10 times per day.

## Summary

Evaporation from cell culture plates may cause problems during longer term culture, i.e. 1 week or more, or when small medium volumes are applied, especially in MicroWell plates. However, the evaporation very much depends on the actual culture conditions, and the following precautions would help to reduce evaporation problems:

1. Use a humidity control that secures the highest possible relative humidity.
2. Limit the number of culture inspections outside the incubator.
3. Keep the culture inspection time outside the incubator as short as possible.
4. Take the plates the shortest possible distance from the incubator.
5. Move the plates slowly.
6. Do not open the incubator unnecessarily long or often.
7. If possible, add water or medium to the spaces between the wells.
8. Use larger depths of medium in plates having a large evaporation index (Table 1).
9. Use Edge plates instead of standard 96 well plates.

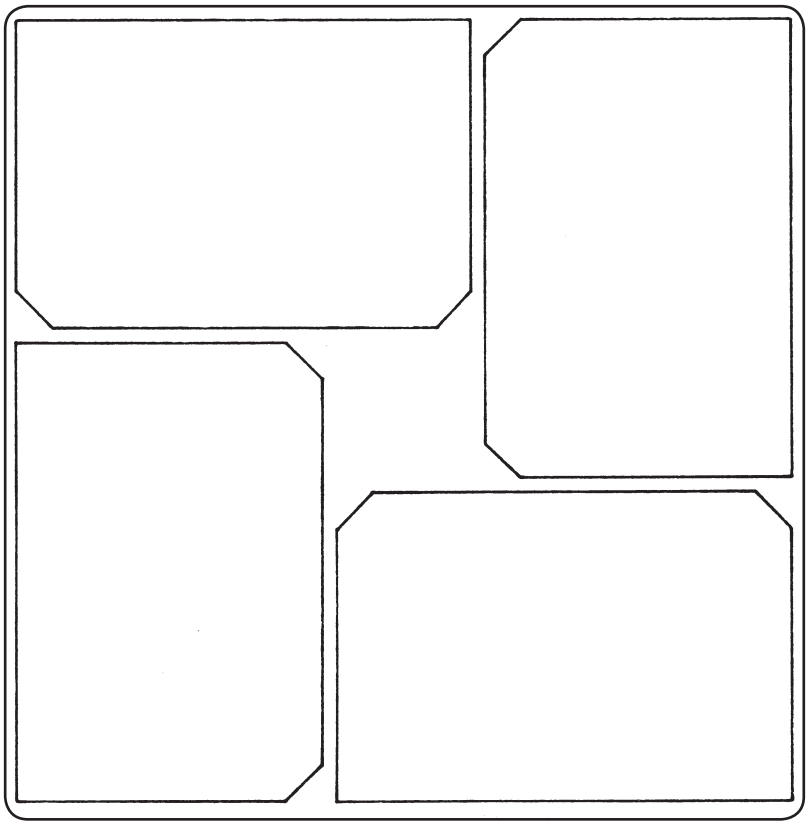

**Fig. 5.**
Position pattern of four MicroWell plates in a large Nunc square dish.

These precautions can to a large extent be substituted by holding the plates in a humidity chamber during incubation and transport. The large Thermo Scientific Nunc square dish equipped with a wet filter paper in the bottom is highly suitable for this purpose and will hold four MicroWell plates as illustrated in Fig. 5.


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