# Passage numbers explained

## 1. What does 'passage number' mean?

The passage number of a cell culture is a record of the number of times the culture has been subcultured, i.e. harvested and reseeded into multiple 'daughter' cell culture flasks.

The question about whether thawing cells represents a passage or not is one that is asked frequently. When cells are trypsinized for freezing and then thawed and reseeded, this represents one passage, albeit with time out in the freezer. As a passage is recognised as the transfer of the cells to another culture dish, the passage number should be increased on reseeding, but not on freezing.

## 2. What does 'population doubling number' mean?

The population doubling (PD or pd) number is the approximate number of doublings that the cell population has undergone since isolation. This is a more meaningful estimate of the age of a finite cell line. When the split ratio used to passage cells is 1:2 the passage number is equal to the PD number; 1:4 would be 2 PDs and so on.

## 3. Why should 'passage number' be considered as a guide only?

Passage number does not consider the seeding densities used or cell numbers subsequently harvested and so typically gives little indication of the actual number of population doublings. For example, one cell culturist may split a cell culture noted to be at passage number 10 by 1:4 and another cell culturist could split the same culture at a ratio of 1:10. Both would label the new flasks with the same passage number i.e. 11.

However, the cells subject to the higher split ratio would undergo more rounds of cell division and subsequently include cells that are more generations away from the original cell culture isolate.

There is no direct test to determine the passage number or PD number of a cell culture. Therefore, proper records must be made at each subculture.

## 4. What is the significance of 'population doubling (PD or pd) number'?

The PD number provides a guide to the extent of proliferation left in a culture. Finite cell lines derived from primary cultures at the first subculture have a limited lifespan and after a certain number of cell divisions the cells will senesce (stop dividing), whereas immortalised, continuous cell lines will proliferate indefinitely.

PD number is often used with regard to finite cultures whereas passage number (usually from the last time the cells were thawed) is generally used for continuous cell lines.



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It is well established that all cell cultures have the potential to change with time due to selective pressures in the culture environment and, particularly with continuous cell lines, due to genetic instability. Cells may continue to proliferate but as the passage number increases their phenotype and genotype can change. More rapidly growing cells, better adapted to *in vitro* culture, over-grow the slower/non-proliferating cells.

Changes such as loss of differentiated properties or changed susceptibility to viral infection over time in culture have been observed.

Primary cell cultures most closely represent the tissue of origin. When subcultured they have a finite lifespan and are more prone to significant changes with increasing passage as they adapt to *in vitro* culture. This means the population doubling number should be carefully recorded. In comparison, a continuous cell line, e.g. derived from a human cancer, can be passaged an infinite number of times.

After prolonged passaging the difference in phenotype over 10 passages is likely to be much less than the difference between the first 10 passages of primary cells.

## 5. How many passages can a cell line be cultured for?

For the majority of cell cultures in the ECACC catalogues the limit of the number of passages they can undergo has not been determined. Finite cell lines derived from primary cells will senesce after a number of PDs characteristic of the cell type (e.g. for normal human fibroblasts this is around 60 PDs). Continuous lines have an unlimited lifespan. Although cells may continue to proliferate for an extended period, over time their phenotype and genotype can change.

Finite cell lines may lose their specialised phenotype and continuous cell lines may develop considerable genetic instability and resultant phenotypic heterogeneity. It is advisable for researchers to monitor characteristics of particular interest to ensure the cells remain fit for purpose.

## 6. What steps can I take to avoid problems associated with increasing passage number?

i. We recommend that when working with a cell line, researchers generate and freeze down stock of the cell culture so that they can keep returning to stock of the same history to repeat experiments. ECACC offers a custom banking service where we can generate a batch of ampoules specifically for a customer.

ii. Set limits when you work with a culture i.e. only perform a relatively low number of passages, e.g. 10 – 20, before returning to another ampoule of the same stock. With finite cell lines the number of passages the cells can undergo can be determined by



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passaging the cells until the onset of senescence.

iii. When not using a cell line it is preferable to stop active cultivation and to return to a cryopreserved bank when required. This reduces the potential for genotypic and phenotypic drift that could occur if the same culture was maintained and passaged continuously. This also saves on material and labour costs.

iv. Test the cells with regard to the work in question. If there are particular markers or receptors that are being studied establish base lines for their presence. Monitor these characteristics to obtain an idea of whether and how they may vary in relation to increasing passage no. It is important to minimise other variables, e.g. the maintenance regime and culture environment, so that they do not complicate the effects of passage number.

v. Changes in morphology can occur over time. It is relatively easy to check the visual appearance of cell cultures on an inverted microscope and it is worth routinely making a digital record of appearance of cells. It may be possible to use this as an indicator for the age of a culture.

vi. Determine growth curves for young and old cultures, with increasing passage proliferation rates will decrease, particularly for primary cultures with a finite lifespan as they approach senescence.

## 7. What does the plus (+) in front of the passage number mean?

Where the passage number is preceded by a plus (+), the passage number should not be regarded as a definite passage number for these cells. The plus (+) indicates the number of additional or plus passages which have been carried out on the cells whilst being cultured here at ECACC.



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