

No. 1: Golden Rules for Working with Cell Cultures



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Susi and Adi know from their own (sometimes bitter) experience the sort of things that cause problems for them and their colleagues in cell culture bottles in the lab. Susi and Adi have thus (not least in their own interest) tried to establish a few rules for successfully working with cell cultures in the lab. These rules are just as useful for laboratory old-timers as for greenhorns.

Some of these rules, however, may fall into the category of "old rope", so please bear with us. It is always useful to read over them again as they can be quite decisive in determining whether one is successful or unsuccessful when working with cell cultures.

- Always treat material as if it were infectious.
- Never use contaminated material within a sterile area.
- Use the correct sequence when working with different cell lines:
 - a) Diploid cells (primary cultures, lines for the production of vaccines, etc.)
 - b) Diploid cells (laboratory lines)
 - c) Continuous, slow-growing line.
 - d) Continuous, rapidly-growing lines.
 - e) New, uncontrolled lines.
 - f) Lines which may be contaminated.
 - g) Virus-producing lines.

If this sequence can not be adhered to, the laminar-flow cabinet must be cleaned between runs.

- If possible, keep cultures free of antibiotics in order to be able to recognise contamination in time.
- Keep lab protocols on all transfers, results and preparation of media.
- Never use the same media bottle for different cell lines. This is false economy: If caps are dropped or bottles unintentionally touched, replace them with new ones.
- Never pour medium out of the bottle.
- Always use pipetors or vacuum systems.
Disposable bottles made of plastic react sensitively to a Bunsen burner. The necks of glass bottles on the other hand prefer heat (at least 60 sec = > 200 °C).
- Maintain the sterile area and laminar flow cabinet in a clean and orderly condition. Only those instruments and accessories that are really required should be used here; and if so, they should only be used during the actual working process.
- Switch on the sterile laminar flow at least 20 minutes prior to starting work. The work bench should be cleaned with 70% alcohol before working with the cultures.
- Working areas and floors should be disinfected daily.
- Laminar flows should be regularly and properly maintained (class II vertical flow should be preferred). At least once per year, have INTEGRA Biosciences' Service carry out a particle count and flow rate.
- Ensure that the incubator is also serviced at least once per year with respect to CO₂ content and temperature. This should also be carried out by INTEGRA Biosciences' Service.
- Laboratory clothing should be particularly clean. In a sterile area, a lab coat should always be worn over normal clothing. Jewellery should never be worn. Long hair should be stacked and if possible covered with a cap. Hands and finger nails should be thoroughly scrubbed. Hand cream should be applied only after completing work.
- Never allow non-authorised persons to enter the sterile area.
- All stored cell lines should be checked once a month - or more often

if there is a danger of contamination - for the presence of mycoplasma.

- Cell cultures that are used frequently should be subcultured and stored as duplicate strains.
- Cell lines should be stored in duplicate in liquid nitrogen. If possible, use two separate nitrogen containers.
- Diploid cell lines or cell strains should always be stored and incubated separately. This helps to exclude cross contamination with continuous or transformed lines.

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