

MYCOPLASMA TEST

An untested cell line must be considered as potentially contaminated and must be cultured in dedicated spaces. Mycoplasma infection can have marked effects on gene expression and cell behaviour and work done with mycoplasma-infected cells cannot be regarded as valid (J.R.W. Masters, 2000).

Mycoplasma detection test is performed by Cell Culture Facility at least once a month on the mostly used and newly coming cell lines. Two kind of test are currently performed: PCR based and biochemical test.

One incubator and one hood are dedicated to untested cell lines (cell culture room build.9). Every line coming from the outside of IFOM will be split under the assigned hood and placed in the assigned incubator until checked for mycoplasma presence. No exception can be made, not even if the cell line has just been tested in the lab where it came from.

In order to increase the quality of the cell culture work, it has been recently introduced the *extra mycoplasma testing* which is randomly performed on all cell lines present in all users incubators.

In case of contaminated cells two policies will be adopted:

1. Cell lines of common use: will be immediately discarded and previous passages thawed and checked.
2. Irreplaceable clones: we'll try to clean them with appropriate drug.

WHAT YOU HAVE TO DO:

1. Do not use any cell line out of mycoplasma spaces until it has been tested.
2. Contact CCS to know when next mycoplasma test will be performed.
3. Give to techs a frozen vial at least 10 days before the test date and fill the apposite form (in absence of filled form, cells will be not tested).
4. INFECTED (retro, lenti, adeno) and HUMAN PRIMARY CELLS: only growing cells, kept **by users** at least 10 days without antibiotics will be tested (PCR test only).

WHAT CELL CULTURE STAFF DOES:

1. CCS thaws and grows cells for at least 1 week without antibiotics (Pen/Strep, Hygro, G418, Puro, etc.).
2. It performs PCR and biochemical test on medium and cells.
3. In case of doubtful result, test is repeated.
4. Positive results are communicated by E-mail.