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Matrigel plug assay: evaluation of the angiogenic response by reverse transcription-quantitative PCR

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The subcutaneous Matrigel plug assay in mice is a method of choice for the in vivo evaluation of pro- and anti-angiogenic molecules. However, quantification of the angiogenic response in the plug remains a problematic task. Here we report a simple, rapid, unbiased and reverse transcription-quantitative PCR (RT-qPCR) method to investigate the angiogenic process occurring in the Matrigel plug in response to fibroblast growth factor-2 (FGF2).

To this purpose, a fixed amount of human cells were added to harvested plugs at the end of the in vivo experimentation as an external cell tracer. Then, mRNA levels of the pan endothelial cell markers *murine* CD31 and *vascular endothelial-cadherin* were measured by species-specific RT-qPCR analysis of the total RNA and data were normalized for *human* GAPDH or β-*actin* mRNA levels. RT-qPCR was used also to measure the levels of expression in the plug of various angiogenesis/inflammation-related genes. The procedure allows the simultaneous, quantitative evaluation of the newly-formed endothelium and of non endothelial/inflammatory components of the cellular infiltrate in the Matrigel implant, as well as the expression of genes involved in the modulation of the angiogenesis process. Also, the method consents the quantitative assessment of the effect of local or systemic administration of anti-angiogenic compounds on the neovascular response triggered by FGF2.