# DNA MICROARRAYS FOR MICROBIOLOGICAL DIAGNOSIS IN STEM CELL CULTURES

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## INTRODUCTION

In stem cell cultures there is the possibility of infectious disease transmission to the recipients. Any microbial contamination of the donor`s biological products or introduced during the manufacturing process can potentially present a serious hazard to the recipients. The majority of potential contaminants are mycoplasma and other bacterias, yeasts and fungi. Moreover, viral and prion contamination of cell cultures and "feeder" cells is also a common risk..

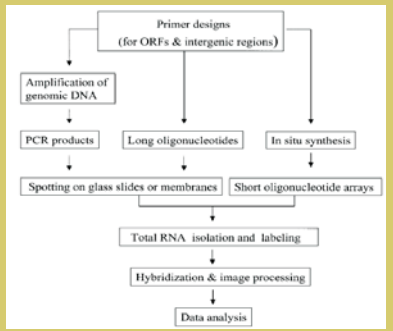
## NEED OF A MICROARRAY PLATFORM

To ensure the provision fo safe and reliable cells and tissues for these applications, stem cell banks should have a wide panel of tests to detect serious pathogens. Several of these methods are long standing techniques that need specialized facilities and prolonged time of detection. The new technologies based on the use of hybridization chips using microarrays of immobilized oligonucleotides or antigens/antibodies/genes for microorganisms can provide a rapid and useful methodology for the identification of contaminants.

## MICROARRAY TECHNOLOGY DESCRIPTION

DNA arrays consist of a series of nucleic acid targets immobilised on a solid substrate. Hybridization of fluorescently labelled probes made form nucleic acids in the test sample to these targets allows analysis of the relative concentrations of mRNA or DNA in the sample. Microbial diagnostic microarrays consist of nucleic acid probe sets, with each probe being specific for a given strain, subspecies, species, genus or higher taxon.

Figure. Construction and development of microarrays for stem cell cultures. (Obtained from Ye et al. 2001)




## DNA ARRAYS TYPES

PCR product-based DNA microarrays.

The first step is the design of primers to amplify specific regions of interest. Also an ideal approach could be the consideration of specific genes to each species it want to test.

Oligonucleotide-based DNA microarrays

Microarrays can also be constructed with short nucleotides. No reverse transcription or amplification steps are involved. A system using one probe per gene has also been developed. The main advantages of the oligonucleotide microarrays are that no amplification is required, there is less likelihood for contamination due to non specific amplification and mishandling, there is a reduction in cross-hybridization and high density oligonucleotide arrays enable high coverage of the genome.

Table. Microorganisms that should be included in a microarray platform for stem cell cultures.

Microorganisms of human origin:

Virus: HIV, HBV, HCV, HAV, HEV, CMV, VEB, HSV, etc

Bacterias: Mycoplasma spp, T. Pallidum, Staphylococcus spp, Bacillus spp, Corynebacterium spp.

Fungi: Aspergillus, Penicillium

Yeasts: Candida.

Microorganisms of animal origin:

Reovirus-3, Sendai virus, Hantaan virus, LDH virus, Ectromelia, CMV, Toolan, Kilham, rotavirus, etc.

## CONCLUSIONS

1- The safety of the products used in cell cultures could be obtained for application of an exhaustive program of microbiological screening.

2- The simplicity of the microarray protocols, together with their use of a large number of species-specific oligoprobes and their ability to analyze multiple samples in a short time offers clear advantages.