

Hydrofocusing Bioreactor for Three-Dimensional Cell Culture

Hydrofocusing reduces shear while “herding” cells and air bubbles.

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The hydrodynamic focusing bioreactor (HFB) is a bioreactor system designed for three-dimensional cell culture and tissue-engineering investigations on orbiting spacecraft and in laboratories on Earth. The HFB offers a unique hydrofocusing capability that enables the creation of a low-shear culture environment simultaneously with the “herding” of suspended cells, tissue assemblies, and air bubbles. Under development for use in the Biotechnology Facility on the International Space Station, the HFB has successfully grown large three-dimensional, tissue-like assemblies from anchorage-dependent cells and grown suspension hybridoma cells to high densities.

Conventional bioreactors rely on agitation to suspend cells and attachment materials and to facilitate the mass transfer required for the growth of cells and tissue assemblies. However, the shear force generated by agitation, can affect cell-cell interactions and degrade three-dimensional tissue development. Johnson Space Center has developed rotating-wall perfused-system (RWPS) bioreactors that create low-shear culture environments and support three-dimensional tissue development. However, their ability to control the locations of cells and tissue aggregates within vessels is limited. Moreover, air bubbles that form within the culture media in the vessels cannot be removed, although such removal is critical for operation in orbit around the Earth.

The HFB, based on the principle of hydrodynamic focusing, provides the capability to control the movement of air bubbles and removes them from the bioreactor without degrading the low-shear culture environment or the suspended three-dimensional tissue assemblies. The HFB also provides unparalleled control over the locations of cells and tissues within its bioreactor vessel during operation and sampling.

The HFB includes a rotating, dome-shaped cell-culture vessel with a centrally located sampling port and an internal rotating viscous spinner attached to a rotating base (see Figure 1). The vessel and viscous spinner can rotate at the same speed or different speeds and directions to create the desired levels of hydrodynamic force within the vessel. Both the low-shear suspension of cells and control of the locations of cells and air bubbles are effected by means of the hydrodynamic force created by the flow within the vessel and fluid drag along the surface of the viscous spinner. A gas-per-

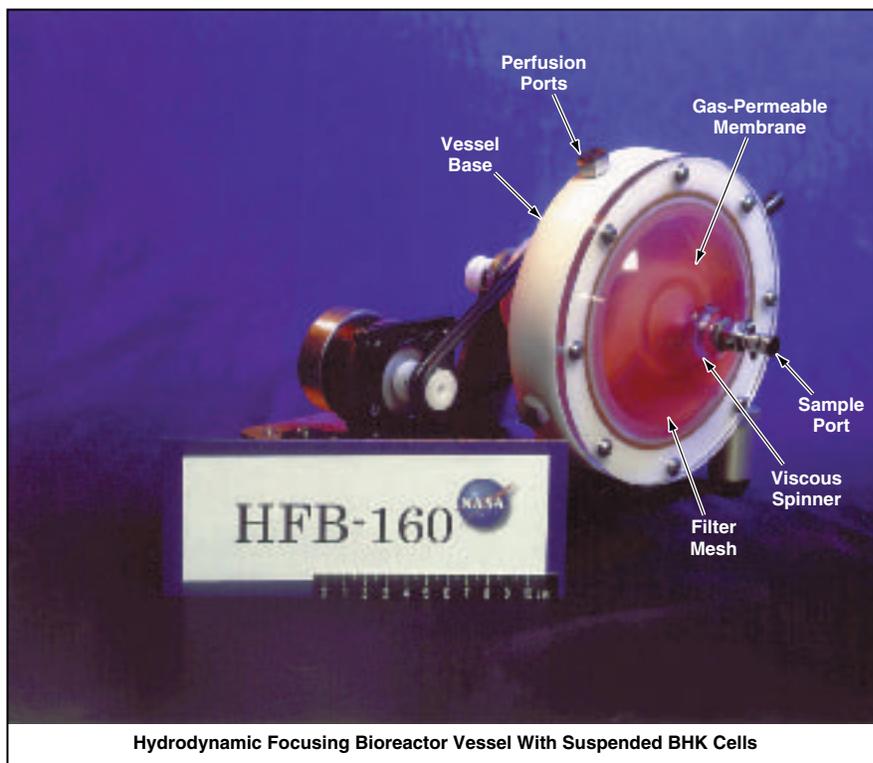


Figure 1. The **Hydrodynamic Focusing Bioreactor** utilizes rotation and viscosity to generate hydrodynamic forces for controlling locations of cells and bubbles while maintaining low shear.

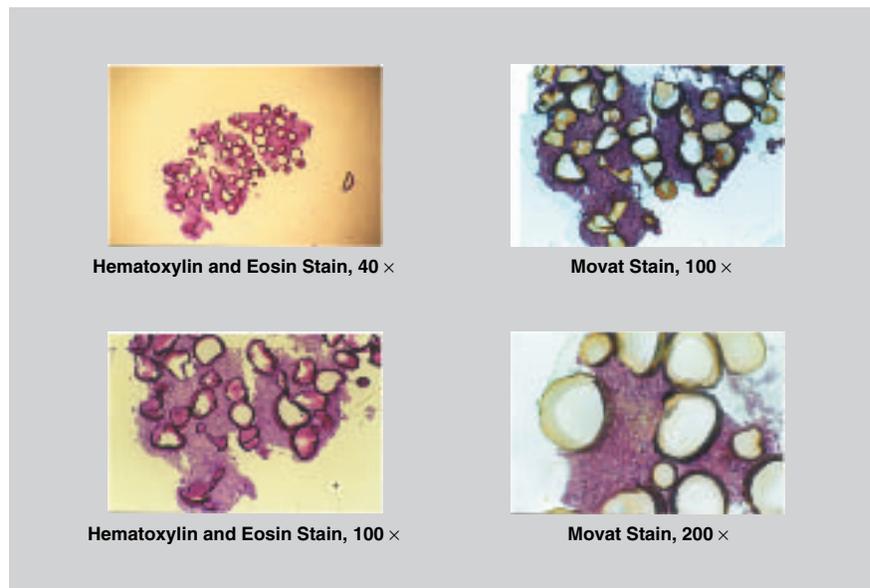


Figure 2. **Aggregates of Baby-Hamster Kidney Cells** grown in the HFB during ten-day intervals were photographed at two different magnifications after histological staining.

meable membrane connected to the base of the vessel enables the exchange of gas between the medium in the vessel and an incubator environment in which the vessel is

placed. Average shear values of 0.001 dynes per square centimeter were estimated for a rotation rate of 10 rpm — a rate at which efforts to suspend large, three-

dimensional, tissue-like assemblies have been successful.

Anchorage-dependent cells, including primary cells, transformed cells, genetically engineered cells and cell lines, and suspension hybridoma cells were successfully cultured either as mono- or co-cultures in the HFB for times as long as 2 weeks. Cultures were initiated by inoculating cells, variously with or without attachment materials, into the vessel through the sampling port, filling the vessel completely with a culture medium, and setting rotation rates to maintain suspension. Large three-dimensional, tis-

suelike assemblies were obtained from HFB cultures of anchorage-dependent cells (see Figure 2). In addition, critical stages in three-dimensional, tissue-like growth (cell attachment, three-dimensional aggregation, and formation of an extracellular matrix) were observed with all anchorage-dependent cells cultured in the HFB.

This work was done by Steve R. Gonda and Glenn F. Spaulding of **Johnson Space Center**, Yow-Min D. Tsao, Scott Flechsig and Leslie Jones of **Wyle Laboratories**, and Holly Soehnge of **Universities Space Research Associates**.

Title to this invention, covered by U.S. Patent No. 6,001,642 has been waived under the provisions of the National Aeronautics and Space Act {42 U.S.C. 2457 (f)}. Inquiries concerning licenses for its commercial development should be addressed to

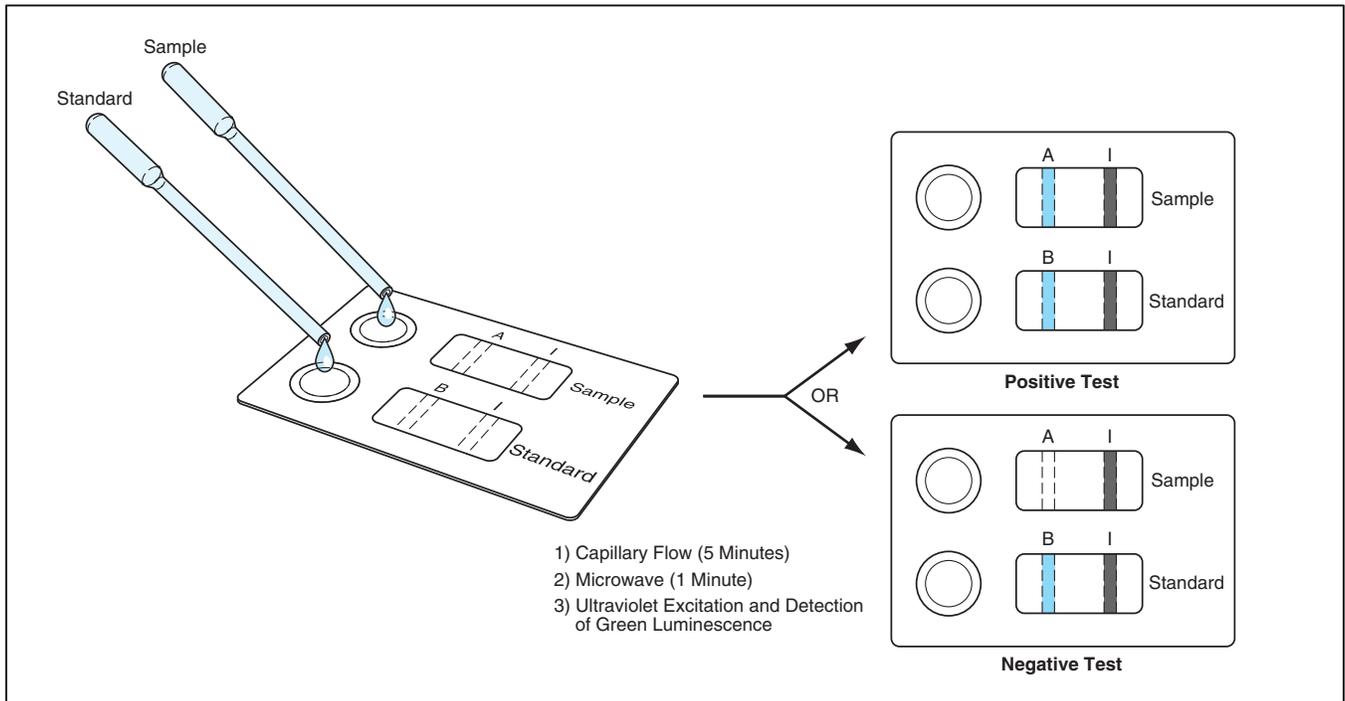
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Refer to MSC-22538, volume and number of this NASA Tech Briefs issue, and the page number.

Species Specific Bacterial Spore Detection Using Lateral-Flow Immunoassay With DPA-Triggered Tb Luminescence

A highly-sensitive assay can be performed in minutes.

NASA's Jet Propulsion Laboratory,
Pasadena, California



An Assay Is Performed on a sample suspected of containing bacterial spores of a species of interest, in parallel with an assay of a standard containing a known concentration of *Bacillus subtilis*. The intensity of luminescence in region A under ultraviolet illumination is proportional to the number density of the spores.

A method of detecting bacterial spores incorporates

- A method of lateral-flow immunoassay in combination with
- A method based on the luminescence of Tb³⁺ ions to which molecules of dipicolinic acid (DPA) released from the spores have become bound.

The second-mentioned component method, denoted the method of DPA-triggered Tb luminescence, was described

in somewhat more detail as a precursor of a related development reported in "Improved Technique for Detecting Endospores via Luminescence" (NPO-21240) *NASA Tech Briefs*, Vol. 26, No. 7 (July 2002), page 56.

The present combination of lateral-flow immunoassay and DPA-triggered Tb luminescence was developed as a superior alternative to a prior lateral-flow immunoassay method in which detection involves the

visual observation and/or measurement of red light scattered from colloidal gold nanoparticles. The advantage of the present combination method is that it affords both

- High selectivity for spores of the species of bacteria that one seeks to detect (a characteristic of lateral-flow immunoassay in general) and
- Detection sensitivity much greater (by virtue of the use of DPA-triggered Tb luminescence instead of gold nanoparticles) than