



Improved Whole-Blood-Staining Device

Additional applications have been identified.

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Dramatic improvements have been made in NASA's Whole Blood Staining Device (WBSD) since it was last described in "Whole-Blood-Staining Device," *NASA Tech Briefs*, Vol. 23, No. 10 (October 1999), page 64. The new system has a longer shelf life, a simpler and more effective operational procedure, improved interface with instrumentation, and shorter processing time. More specifically, the improvements have targeted bag and locking clip materials, sampling ports, and air pocket prevention.

The WBSD stains whole blood collected during spaceflight for subsequent flow cytometric analysis. In short, the main device stains white blood cells by use of monoclonal antibodies conjugated to various fluorochromes, followed by lysing and fixing of the cells by use of a commercial reagent that has been diluted according to NASA safety standards. This system is compact, robust, and does not require electric power, precise mixing, or precise incubation times.

Figure 1 depicts the present improved version for staining applications, which is a poly(tetrafluoroethylene) bag with a Luer-lock port and plastic locking clips. An InterLink® (or equivalent) intravenous-injection port screws into the Luer-lock port. The inflatable/collapsible nature of the bag facilitates loading and helps to minimize the amount of air trapped in the fully loaded bag.

Some additional uses have been identified for the device beyond whole blood

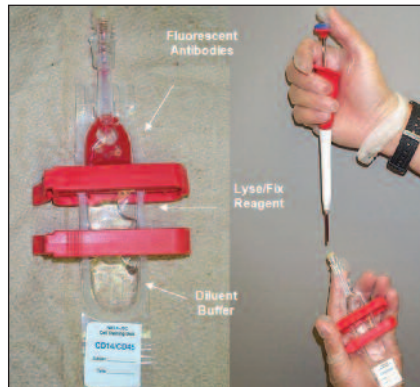


Figure 1. Photo of the Improved Version for staining applications.

staining. The WBSD has been configured for functional assays that require culture of live cells by housing sterile culture media, mitogens, and fixatives prior to use [Figure 2(a)]. Simple injection of whole blood allows cell-stimulation culture to be performed in reduced gravity conditions, and product stabilization prior to storage, while protecting astronauts from liquid biohazardous materials. Also, the improved WBSD has reconstituted powdered injectable antibiotics by mixing them with diluent liquids [Figure 2(b)]. Although such mixing can readily be performed on Earth by shaking in glass vials, it cannot readily be performed this way in outer space without entraining air bubbles. The present device can be preloaded with the powder and diluent(s) in separate

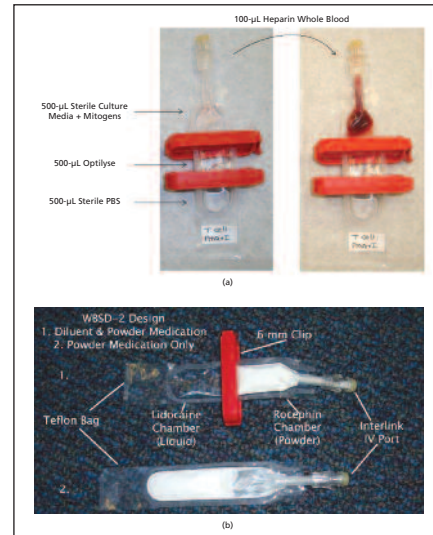


Figure 2. WBSD Configurations: (a) for functional assays, and (b) for powdered injectable antibiotics.

compartments. The powder and diluent(s) can be mixed, without introducing air bubbles, by removing the clip(s), then shaking. This use of the device could also be advantageous in terrestrial applications because it maintains the isolation of the constituents until the time of use.

This work was done by Clarence F. Sams of Johnson Space Center and Brian Crucian, Bonnie Paul, Shannon Melton, and Terry Guess of Wyle Laboratories. Further information is contained in a TSP (see page 1). MSC-24176-1/7-1/8-1

Monitoring Location and Angular Orientation of a Pill

System is part of targeted drug delivery.

Ames Research Center, Moffett Field, California

A mobile pill transmitter system moves through, or adjacent to, one or more organs in an animal or human body, while transmitting signals from its present location and/or present angular orientation. The system also provides signals from which the present roll angle of the pill,

about a selected axis, can be determined. When the location coordinates angular orientation and the roll angle of the pill are within selected ranges, an aperture on the pill container releases a selected chemical into, or onto, the body. Optionally, the pill, as it moves, provides a se-

quence of visually perceptible images. The times for image formation may correspond to times at which the pill transmitter system location or image satisfies one of at least four criteria.

This invention provides and supplies an algorithm for exact determination of

location coordinates and angular orientation coordinates for a mobile pill transmitter (PT), or other similar device that is introduced into, and moves within, a GI tract of a human or animal body. A set of as many as eight nonlinear equations has been developed and applied, relating propagation of a wireless signal between either two, three, or more transmitting antennas located on the PT, to four or more non-coplanar re-

ceiving antennas located on a signal receiver appliance worn by the user.

The equations are solved exactly, without approximations or iterations, and are applied in several environments: (1) association of a visual image, transmitted by the PT at each of a second sequence of times, with a PT location and PT angular orientation at that time; (2) determination of a position within the body at which a drug or

chemical substance or other treatment is to be delivered to a selected portion of the body; (3) monitoring, after delivery, of the effect(s) of administration of the treatment; and (4) determination of one or more positions within the body where provision and examination of a finer-scale image is warranted.

This work was done by John F. Schipper for Ames Research Center. Further information is contained in a TSP (see page 1). ARC-15810-1

Molecular Technique to Reduce PCR Bias for Deeper Understanding of Microbial Diversity

This technique has applications in medical manufacturing, food processing, and municipal water treatment.

NASA's Jet Propulsion Laboratory, Pasadena, California

Current planetary protection policies require that spacecraft targeted to sensitive solar system bodies be assembled and readied for launch in controlled cleanroom environments. A better understanding of the distribution and frequency at which high-risk contaminant microbes are encountered on spacecraft surfaces would significantly aid in assessing the threat of forward contamination. However, despite a growing understanding of the diverse microbial populations present in cleanrooms, less abundant microbial populations are probably not adequately taken into account due to technological limitations. This novel approach encompasses a wide spectrum of microbial species and will represent the true picture of spacecraft cleanroom-associated microbial diversity.

All of the current microbial diversity assessment techniques are based on an initial PCR amplification step. However, a number of factors are known to bias PCR amplification and jeopardize the true representation of bacterial diversity. PCR amplification of a minor template

appears to be suppressed by the amplification of a more abundant template. It is widely acknowledged among environmental molecular microbiologists that genetic biosignatures identified from an environment only represent the most dominant populations. The technological bottleneck overlooks the presence of the less abundant minority population and may underestimate their role in the ecosystem maintenance.

DNA intercalating agents such as propidium monoazide (PMA) covalently bind with DNA molecules upon photolysis using visible light, and make it unavailable for DNA polymerase enzyme during polymerase chain reaction (PCR). Environmental DNA samples will be treated with suboptimum PMA concentration, enough to intercalate with 90–99% of the total DNA. The probability of PMA binding with DNA from abundant bacterial species will be much higher than binding with DNA from less abundant species. This will increase the relative DNA concentration of previously “shadowed” less abundant species

available for PCR amplification. These PCR products obtained with and without PMA treatment will then be subjected to downstream diversity analyses such as sequencing and DNA microarray. It is expected that PMA-coupled PCR will amplify the “minority population” and help in understanding microbial diversity spectrum of an environmental sample at a much deeper level.

This new protocol aims to overcome the major potential biases faced when analyzing microbial 16S rRNA gene diversity. This study will lead to a technological advancement and a commercial product that will aid microbial ecologists in understanding microbial diversity from various environmental niches. Implementation of this technique may lead to discoveries of novel microbes and their functions in sustenance of the ecosystem.

This work was done by Parag A. Vaishampayan and Kasthuri J. Venkateswaran of Caltech for NASA's Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1). NPO-48200