



Solution Preserves Nucleic Acids in Body-Fluid Specimens

Specimens can be stored and transported at room temperature.

Lyndon B. Johnson Space Center, Houston, Texas

A solution has been formulated to preserve deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in specimens of blood, saliva, and other bodily fluids. Specimens of this type are collected for diagnostic molecular pathology, which is becoming the method of choice for diagnosis of many diseases. The solution makes it possible to store such specimens at room temperature, without risk of decomposition, for subsequent analysis in a laboratory that could be remote from the sampling location. Thus, the solution could be a means to bring the benefits of diagnostic molecular pathology to geographic regions where refrigeration equipment and diagnostic laboratories are not available.

The table lists the ingredients of the solution. The functions of the ingredients are the following:

- EDTA chelates divalent cations that are necessary cofactors for nuclease activity. In so doing, it functionally removes these cations and thereby retards the action of nucleases. EDTA also stabilizes the DNA helix.
- Tris serves as a buffering agent, which is needed because minor contaminants in an unbuffered solution can exert pronounced effects on pH and

Ingredient	Weight or Volume of Ingredient	Final Concentration of Ingredient in Solution
Sodium dodecyl sulfate (SDS)	1 g	1 percent
Ethylenediaminetetraacetic acid (EDTA)	0.037 g	1.0 mM
Tris(hydroxymethyl)aminomethane (also known as "Tris" and sold under the trade name Trizma™ Base)	0.12 g	10 mM
Water Free of Deoxyribonuclease and Ribonuclease	99 mL	
	Total Volume 100 mL	

The **Nucleic Acid Stability Solution (NASS)** contains ingredients that perform different roles essential to the preservation of DNA and RNA in specimens. In the preparation of this solution, the ingredients are mixed in the indicated quantities (or common multiples thereof), then the solution is sterilized by passing it through a 0.2- μ m filter.

thereby cause spontaneous degradation of DNA.

- SDS is an ionic detergent that inhibits ribonuclease activity. SDS has been used in some lysis buffers and as a storage buffer for RNA after purification.

The use of the solution is straightforward. For example, a sample of saliva is collected by placing a cotton roll around in the subject's mouth until it becomes saturated, then the cotton is placed in a collection tube. Next, 1.5 mL of the solution are injected directly

into the cotton and the tube is capped for storage at room temperature. The effectiveness of the solution has been demonstrated in tests on specimens of saliva containing herpes simplex virus. In the tests, the viral DNA, as amplified by polymerase chain reaction, was detected even after storage for 120 days.

This work was done by Duane L. Pierson of Johnson Space Center and Raymond P. Stowe. For further information, contact the Johnson Commercial Technology Office at (281) 483-3809. MSC-22891

Oligodeoxynucleotide Probes for Detecting Intact Cells

Cells can be detected, identified, and enumerated via chemiluminescence.

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A rapid, sensitive test using chemiluminescent oligodeoxynucleotide probes has been developed for detecting, identifying, and enumerating intact cells. The test is intended especially for use in detecting and enumerating bacteria and yeasts in potable water.

As in related tests that have been developed recently for similar purposes, the oligodeoxynucleotide probes used in this test are typically targeted at either single-copy deoxyribonucleic acid (DNA) genes (such as virulence genes) or the multiple

copies (10,000 to 50,000 copies per cell) of 16S ribosomal ribonucleic acids (rRNAs). Some of those tests involve radioisotope or fluorescent labeling of the probes for reporting hybridization of probes to target nucleic acids. Others of those tests involve labeling with enzymes plus the use of chemiluminescent or chromogenic substrates to report hybridization via color or the emission of light, respectively. The present test is of the last-mentioned type. The chemiluminescence in the present test can be detected easily with relatively sim-

ple instrumentation.

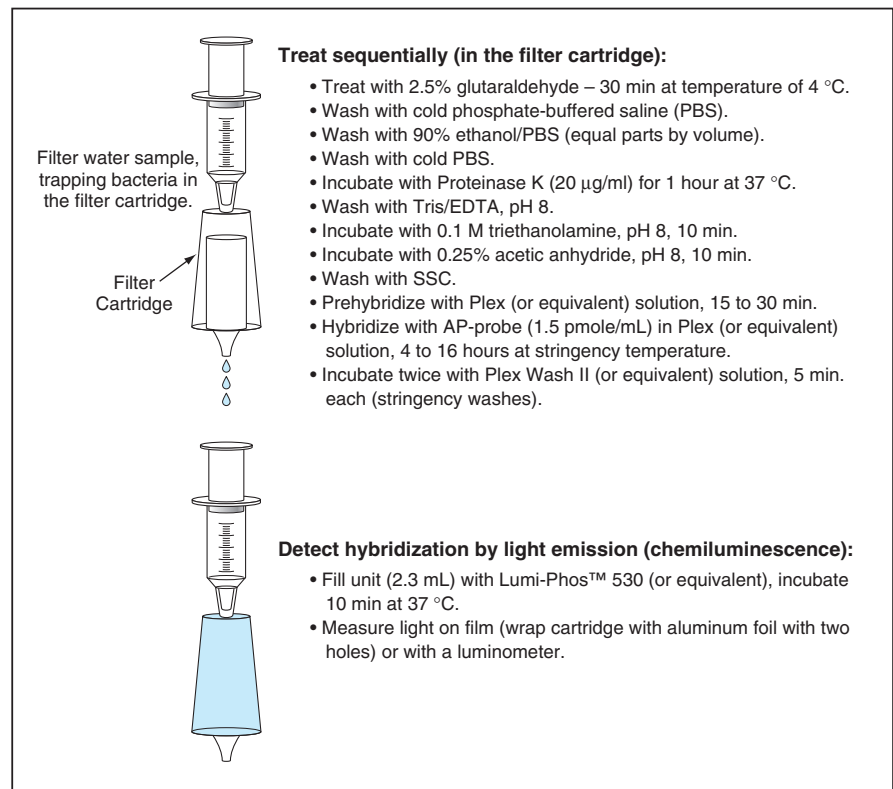
In developing the present test, the hybridization approach was chosen because hybridization techniques are very specific. Hybridization detects stable, inheritable genetic targets within microorganisms. These targets are not dependent on products of gene expression that can vary with growth conditions or physiological states of organisms in test samples. Therefore, unique probes can be designed to detect and identify specific genera or species of bacteria or

yeast (in terms of rRNA target sequences) or can be designed to detect and identify virulence genes (genomic target sequences). Because of the inherent specificity of this system, there are few problems of cross-reactivity.

Hybridization tests are rapid, but hybridization tests now available commercially lack sensitivity; typically, between 10^6 and 10^7 cells of the target organism are needed to ensure a reliable test. Consequently, the numbers of target bacteria in samples are usually amplified by overnight pre-enrichment growth. These tests are usually performed in laboratories by skilled technicians. The present test was designed to overcome the shortcomings of the commercial hybridization tests.

The figure summarizes the major steps of the test. It is important to emphasize that the hybridization process used in this test differs from that of other hybridization tests in that it does not extract target nucleic acids. This process is based on intact-cell hybridization (so-called "in situ hybridization"), wherein the intact cells act as immobilizing agents. The cells are identified and enumerated by measuring the chemiluminescence emitted from alkaline phosphatase-probe (AP-probe) hybridization; the chemiluminescence is detected or measured by use of photographic film or a luminometer, respectively.

This test provides rapid, simple, and sensitive detection of microorganisms in water. The test is very flexible: specific probes can be developed for almost any group, genus, and, in many cases, species of microorganisms. The



Treat sequentially (in the filter cartridge):

- Treat with 2.5% glutaraldehyde – 30 min at temperature of 4 °C.
- Wash with cold phosphate-buffered saline (PBS).
- Wash with 90% ethanol/PBS (equal parts by volume).
- Wash with cold PBS.
- Incubate with Proteinase K (20 µg/ml) for 1 hour at 37 °C.
- Wash with Tris/EDTA, pH 8.
- Incubate with 0.1 M triethanolamine, pH 8, 10 min.
- Incubate with 0.25% acetic anhydride, pH 8, 10 min.
- Wash with SSC.
- Prehybridize with Plex (or equivalent) solution, 15 to 30 min.
- Hybridize with AP-probe (1.5 pmole/mL) in Plex (or equivalent) solution, 4 to 16 hours at stringency temperature.
- Incubate twice with Plex Wash II (or equivalent) solution, 5 min. each (stringency washes).

Detect hybridization by light emission (chemiluminescence):

- Fill unit (2.3 mL) with Lumi-Phos™ 530 (or equivalent), incubate 10 min at 37 °C.
- Measure light on film (wrap cartridge with aluminum foil with two holes) or with a luminometer.

Intact-Cell Hybridization is performed in a filter cartridge using chemiluminescent oligodeoxynucleotide probes to detect target bacteria.

test can be performed in the field, or in a laboratory, using simple, battery-powered portable instrumentation. The test can be initiated and completed by non-technical persons. The test format can be automated easily. Probes for *E. coli* and the coliform group of bacteria have been developed for testing water. Results of a test can be obtained within 8 hours. Probes for *Vibrio cholerae*, *Burkholderia cepacia*, *Staphylococcus aureus*,

Staphylococcus epidermidis, and the *Salmonella* group have also been developed.

This work was done by Reinhardt A. Rosson, Julie Mawrina-Brunker, Kim Langley, and Christine M. Pynnönen of Bio-Technical Resources, L. P. for Johnson Space Center. For further information, contact the Johnson Commercial Technology Office at (281) 483-3809. MSC-22663