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(54) Title: METHOD FOR STABILIZING BIOLOGICAL SAMPLES FOR NUCLEIC ACID ANALYSIS

(57) Abstract: Disclosed is a method for preparing nucleic acid containing biological samples for a nucleic acid integrity assay and/or multiple mutation analysis by incubating the biological sample with a stabilization solution that includes a buffer, a chelating agent and a salt.

# METHOD FOR STABILIZING BIOLOGICAL SAMPLES FOR NUCLEIC ACID ANALYSIS

## RELATED APPLICATIONS

5 This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 60/571,120, entitled “Method for Stabilizing a Biological Sample for a Nucleic Acid Integrity Assay” filed on May 14, 2004, the disclosure of which is herein incorporated by reference in its entirety.

## 10 FIELD OF THE INVENTION

The invention relates generally to assays to detect nucleic acid markers indicative of cancer and other diseases and more particularly to preparing nucleic acid-containing biological samples for use in these assays.

## 15 BACKGROUND OF THE INVENTION

Tissue and body fluid samples, including stools, contain shed cellular debris. In healthy patients, such debris is the result of apoptotic degradation as part of the normal cell cycle. Apoptosis reduces the integrity (intactness) of nucleic acids, proteins, and other cellular components in healthy individuals, so that only small fragments exist in the debris that results from the apoptotic process (e.g., exfoliated cellular debris).

20 In diseases in which cell cycle mechanisms are destroyed or impaired, cellular debris can include high-integrity cellular components, such as nucleic acids that have not been degraded by apoptosis. One class of disease in which cell cycle mechanisms are disrupted is cancer. An increased presence of high molecular weight nucleic acids in a biological sample therefore can reveal the presence of cancer in a patient from whom the biological sample was obtained. Disease detection assays known as nucleic acid integrity analysis assays have been developed that are based on the increased levels of non-degraded nucleic acid in a cancerous tissue or body fluid as compared to the level of non-degraded nucleic acid in a non-cancerous tissue or body fluid.

30 Nucleic acids in patient samples tend to degrade after they have been removed from the patient. This degradation can diminish the effectiveness of a nucleic acid integrity assay that scores a sample as diseased (e.g., cancerous) based on the presence of intact nucleic acids; if

the sample is excessively degraded, a sample that is actually positive may appear to be negative.

### SUMMARY OF THE INVENTION

5           The invention is based in part on the discovery of a method for stabilizing nucleic acids in tissue and body fluid samples so as to facilitate analysis of the samples in a nucleic acid integrity assay. It has been unexpectedly found that contacting a patient sample with a stabilization solution stabilizes the DNA so that intact nucleic acids indicative of diseased cells are more effectively detected in a nucleic acid integrity assay.

10           Accordingly, in one aspect, the invention provides a method for preparing a nucleic acid sample for a nucleic acid integrity analysis assay. In one aspect, the invention provides a method for preparing a nucleic acid sample for a nucleic acid integrity analysis assay. The method includes providing a patient sample that includes shed cells or cellular debris and a nucleic acid and contacting the patient sample with a stabilization solution under conditions  
15           sufficient to stabilize the nucleic acid for nucleic acid integrity analysis. In one embodiment, the stabilization solution includes a buffer, a salt, and a chelating agent.

          In some embodiments, the conditions are sufficient to detect at least a three-fold genomic equivalent (GE) increase in a nucleic acid integrity analysis of a patient sample having adenoma or cancer as compared to the GE detected in a nucleic acid integrity analysis  
20           of a sample from the patient that is not incubated with the stabilization solution. In one embodiment, the integrity analysis is performed by determining an amount of nucleic acid greater than about 200 bp in length using an assay that detects a nucleic acid (which can be a wild-type or mutant nucleic acid). The nucleic acid is present in a patient sample that includes shed cells or cellular debris. A patient is identified as having cancer or adenoma if the amount  
25           of nucleic acid is greater than an amount of nucleic acid expected to be present in a sample obtained from a patient who does not have cancer or adenoma.

          The patient sample can be obtained from a patient that is, e.g., a vertebrate, including a mammal, a reptile, or an amphibian. A mammal can be, e.g., a human, a non-human primate (such as a gorilla or monkey, including a chimpanzee), a rodent (such as a mouse, rat, guinea  
30           pig, or gerbil) dog, cat, horse, pig, goat, sheep, or cow. The patient sample can be any body tissue or fluid that is suspected of containing DNA from a diseased cell (such as a precancerous or cancerous cell). In one embodiment, the patient sample is a stool sample.

In some embodiments, the patient sample can be obtained as part of a screen for, e.g., a disease or disease-associated condition that impairs, or could lead to impairment of, the proper function of the gastrointestinal system. Gastrointestinal diseases can include, e.g., those associated with the stomach, small intestine, and/or colon. The disease or condition can include  
5 cancers or precancerous conditions such as an adenoma. Other conditions include inflammatory bowel syndrome, inflammatory bowel disease, Crohn's disease, and others in which a genomic instability is thought to play a role.

In some embodiments, the patient sample is frozen and thawed prior to incubation with stabilization solution. In other embodiments, the patient sample is not frozen prior to  
10 incubation with the stabilization solution.

In general, the stabilization solution is added to the patient sample at a ratio of about 1 ml/gram of patient sample to about 20 ml/gram of patient sample. In some embodiments, the stabilization solution is provided at 1-15 ml/gram, 2-12 ml/gram, 3-11 ml/gram, or 4-7 ml/gram. However, higher or lower ratios may be used. When the patient sample is a stool  
15 sample, and the stabilization solution is 10 mM Tris-Cl, 1 mM EDTA, and 150 mM NaCl, a suitable ratio of stabilization solution to patient sample is 7 ml/gm.

In some embodiments, the patient sample and stabilization solution are incubated at about 4 to 28 degrees Centigrade. In some embodiments the temperature is 17 to 27 degrees Centigrade, e.g., about 20 to 25 degrees Centigrade. However, the sample and stabilization  
20 solution may be exposed to higher or lower temperatures (e.g., the sample and stabilization solution may be frozen). Also, a sample and buffer may be exposed to changing temperatures during transport and/or storage.

In various embodiments, the patient sample and stabilization solution are incubated at least 6 hours, e.g., at least 12 hours, at least 24 hours, or at least 36 hours.

In some embodiments, the buffer in the stabilization solution is 0.5 mM to 25 mM Tris, e.g., 5 mM to 15 mM Tris, 8 mM to 13 mM Tris or about 10 mM Tris. However, other buffers and/or concentrations may be used.

In some embodiments, the chelating agent in the stabilization solution is 0.01 to 2.5 mM EDTA, e.g., 0.75-1.25 mM EDTA, or 1mM EDTA. However, other chelating agents and/or concentrations may be used.  
30

In some embodiments, the salt in the stabilization solution is 75 mM to 225 mM NaCl, e.g., 100 mM to 175 mM NaCl, or 150 mM NaCl. However, other salts (e.g., KCl etc.) and/or

In some embodiments, the stabilization solution is provided at pH 7.0 to 9.0, e.g., pH 7.5 to 8.5, or pH 8.0. However, higher or lower pHs may be used.

In some embodiments, the method further includes determining in the incubated patient sample an amount of nucleic acid greater than about 200 bp in length using an assay that  
5 detects wild-type or mutant nucleic acid, and identifying the patient as having cancer or adenoma if the amount is greater than an amount of nucleic acid expected to be present in a sample obtained from a patient who does not have cancer or adenoma (e.g., more than about 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 50 fold, or more greater than an amount expected  
10 in a normal individual). In one aspect, a DNA integrity assay may include interrogating a sample for the presence of long DNA fragments (e.g., longer than 200 nucleotides, longer than 500 nucleotides, longer than 1,000 nucleotides, etc.) at two or more different loci. In one embodiment, a patient is identified as having cancer or adenoma if two or more loci (e.g., 3, 4, or more loci) are positive for the presence of abnormally high levels of long DNA.

In one embodiment, the invention provides a method for preparing a nucleic acid  
15 sample for a nucleic acid integrity and/or multiple mutation analysis assays for diagnosing a carcinoma or adenoma. The method may include providing a patient stool sample that includes shed cells or cellular debris and a nucleic acid, and incubating the patient sample with a stabilization solution under conditions sufficient to stabilize the nucleic acid for nucleic acid integrity and/or multiple mutation analysis. The stabilization solution may be about pH 7.5 to  
20 about pH 8.5 and may include 0.5 mM to 25 mM Tris, 0.01 to 2.5 mM EDTA and 100 mM to 200 mM NaCl. For example, the stabilization solution can be 10 mM Tris-Cl pH 8.0, 1 mM EDTA, and 150mM NaCl. In one embodiment, the conditions are sufficient to detect at least a three-fold genomic equivalent (GE) increase in a nucleic acid integrity analysis of a patient sample having adenoma or cancer as compared to the GE detected in a nucleic acid integrity  
25 analysis of a sample from the patient that is not incubated with the stabilization solution.

Aspects of the invention may be used for transporting or storing biological samples after they are obtained and before they are processed for analysis. For example, methods of the invention may be used to stabilize nucleic acid in biological samples (e.g., stool samples) for about 12 hours, about 24 hours, about 36 hours, or longer (e.g., 4 days, 5, days, 6 days, 1 week,  
30 or longer), even in the absence of refrigeration or freezing.

Aspects of the invention may be particularly useful for detecting indicia of adenomas, early stage cancers, and/or other diseases that may be characterized by very low frequencies of

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