Introduction

Laboratories throughout the world procure, preserve and process a wide variety of biological specimens. These laboratory samples come in a variety of different formats. From thin sections of tissue affixed onto microscope slides to a pellet of cells in microcentrifuge tubes to purified macromolecules in an aqueous solution. Common to all of these is the issue of how to store them best. Once collected, they need to be maintained in a manner that effectively preserves them from days to months or longer. Hence, the worldwide market for preserving and stabilizing biological samples ranges between $34 – 500 billion (USD).¹,²

Once a biological sample is removed, either from the organism or the media it was grown in the process of decomposition begins. Specifically, as nutrients are exhausted normal cellular activity stops and powerful digestive enzymes once securely housed within intracellular compartments are released dissolving the cell. This is known as autolysis, or cellular degradation.

Autolysis, however, is abated by preservation. This process reduces or eliminates decomposition by holding, or “fixing” all the intracellular components and most biological molecules (bio-molecules) in place. For over 100 years there have been two predominant choices for maintaining biological specimens:

1.) preserved chemically and embedded in paraffin; or,

2.) snap-frozen and kept under ice-cold conditions.

Both methods have their unique advantages and disadvantages, which are discussed later. For now, these approaches have allowed scientist to store biological samples to be used for a variety of analytical techniques at a later point in time.

Once fixed and stored, these samples have acquired a tremendous cost, which its loss could be substantial. For example, a conventional upright -80°C ultra-low temperature (ULT) freezer can house over $200K of lab samples—not including the cost of the freezer, utility or preventative maintenance; this only factors in the cost of the containers, samples and man-hours. Unfortunately, one has to look no further than the recent news from Harvard University\(^3\) to illustrate the point of a freezer malfunction. Over 20 years of precious brain specimens were lost when both the freezer and its temperature alarm failed.

At DriBank Labs, we developed the Dri•Bank® container based on research published in peer-reviewed scientific journals.\(^4\)\(^5\)\(^6\)\(^7\) Our product effectively preserves and stores various biological specimens at room temperature anywhere without toxic chemicals or refrigeration.

**Problem Statement**

Laboratories performing work on biological samples, whether academic institutions, biotechnology companies, diagnostic businesses, government facilities, hospitals, medical device or pharmaceutical corporations have placed huge demands on acquiring and sustaining high-quality biological samples while keeping, or reducing their overhead costs. These organizations have recognized for years the hassle of shipping samples on dry ice, purchasing and maintaining expensive refrigeration systems or dealing with the complication of chemical preservatives. To date, there are only a handful of products available to store biological samples at room temperature for extended periods without the need for toxic chemicals, electricity or refrigeration. Most rely on a chemical coatings that must be dried over the sample of interest, which could take days\(^8\) and consequently allow for too much degradation to occur. The Dri•Bank® container however is the only one that can rapidly preserve and securely store biological samples at room temperature anywhere. No need for any coatings, chemicals or refrigeration. Samples such as unfixed tissue sections on glass slides, blood serum, biopsy specimens or purified nucleic acid aliquots have all been stored from weeks to months with no observable differences when compared to fresh counterparts. Unlike other room-temperature storage options, our product can also be used in conjunction with cold-chain methods having the following distinct advantages:

1.) attenuates carbonic acid formation from samples shipped with dry ice\(^9\) with the Atacama-C™ cartridge; and,

2.) prevents accumulation of frost build-up on uncovered samples or in the event of a refrigeration system failure, moisture condensing on exposed samples.

\(^3\) Clarke T. Hospital freezer fault destroys crucial brain data. Retrieved on Jun 14, 2014 from website: http://www.reuters.com/article/2012/06/11/health-brain-research-damage-idUSL1E8HB50V20120611


\(^5\) Sadler TR, Khodavirdi AC. High quality RNA extracted from biopsied samples dehydrated and stored dried at room temperature without chemical preservation for up to three months as evidenced by RT-PCR results. Appl Immunohistochem Mol Morphol. Accepted April 2014.


Previous Options

For ease and convenience, biological lab samples are frequently placed in buffered formalin, which acts as a biological glue keeping cellular structures and macromolecules in place (i.e., fixing). This canonical method has been used for well over a century. However, this presents a number of problems. First, the fixation process is neither instantaneous nor rapid. That is, the chemical must penetrate all the cells that comprise the sample. Most accept the rate buffered formalin can penetrate tissue between 0.5mm - 1mm per hour, but fixation taking much longer, known as penetration-fixation paradox10 (see Appendix, Figure 1). To put this into perspective, a typical 5mm³ punch biopsy specimen may not be preserved for over two days. Regardless that the sample is placed in a refrigerator, those cells not preserved are decomposing. Second, these chemical fixatives are not compatible with most modern molecular assays. These tests include, but are not limited to proteomic investigations looking for specific disease markers or a polymerase chain reaction to amplify a gene of interest. Due to the chemical nature of these fixatives, they can only be removed by prolonged immersion into boiling caustic solutions. Typically this requires a trial-and-error approach to optimize the conditions for its elimination. Third, formalin and other similar preservatives are cancerous, toxic compounds that require special handling and disposal procedures. All of these points add significant time and cost to the analytical process.

Snap-frozen techniques on the other hand offer the advantage of almost instantaneous preserving the sample and greatly reducing the likelihood of degraded cells or macromolecules. These samples are considered the “gold standard” as they have not been adulterated by fixatives and are readily compatible with modern assays. Though it should be noted that while preservation at -50°C (or below) is intended for long-term storage, the activity of water, and therefore it's potent contribution to degradation of biomolecules still persists, albeit at a slower rate.11

“...storing RNA at room temperature seems ridiculous. However, after comparing the RNA samples on the bioanalyzer [Agilent 2100] after 10 days [dried and stored in the Dri•Bank® container] I was surprised to not see a reduction [in signal, i.e. no sample loss]…”

-Cancer Research Scientist

Once snap-frozen the sample must remain frozen, or cryopreserved until they are needed for analysis. Traditionally this requires several sample replicates to avoid the all-damaging freeze-thaw cycle. Not surprisingly, given the number of samples a laboratory may need to process requires several costly refrigeration systems with a dedicated utility, back-up power supply, temperature and system failure alarms. This is particularly true for academic institutions and commercial biobanks that utilize dozens to hundreds of freezers. Again, this too adds significantly to the analytical process.

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The Dri•Bank® Solution

Key to over 95% of all biological reactions, including autolysis, is water. Hence its partial removal from the specimen during the traditional preservation process, either chemically or cryogenically is critical. Whether a protease is breaking down proteins or a nuclease is dissolving nucleic acid polymers, water is incorporated in the enzymatic activity of these degradative molecules. Yet, recent publications have demonstrated that storage of biological samples under ultra-low, dry-air environment generated by powerful desiccants swiftly removes moisture from the samples of interest and reduces and/or eliminates any decomposition. In fact, with certain single-celled organisms, for instance Gram-negative bacteria, it activates natural self-preservation mechanisms that allow them to remain in stasis for years, a process known as anhydrobiosis, or “life without water.” The ability to rapidly eliminate as much water (intracellular or solvent) from a sample has the clear advantage of allowing the storage of biological samples for analysis to be left at room temperature for several months or longer. Due to the Dri•Bank® container’s unique design and functionality it is compatible with traditional cold-chain storage and/or shipping practices. Specifically with the added bonus of reducing carbonic acid formation with the Atacama-C™ cartridge if dry ice is employed or preventing condensation during warming up with the Atacama™ cartridge.

The Dri•Bank® container has undergone performance testing under different environmental conditions: normal laboratory surroundings (50% RH and 20˚C), simulated tropical setting if used in the field (95% RH and 30˚C) and cryogenic (-50˚C). When closed under laboratory conditions the interior has low moisture levels (<10% RH) within five minutes and no detectable humidity after 50 minutes when closed (see Appendix, Figure 2A).

When compared to a standard microscope slide box, our container maintained a dry-air environment when removed from cryogenic conditions. Specifically, to simulate either when a slide box is accidentally left out too long or if a refrigeration system fails, within 10 minutes the comparison is noticeable. After 7 minutes at -2˚C, the normal slide box has an internal humidity of 70% as moisture began to be released from its frozen state. Meanwhile the Dri•Bank® container rises to no more than 6%. If either container is left out until at room temperature (20˚C), the slide box is stable at 50% RH and the Dri•Bank® container has no detectable moisture (0% RH) as shown in Figure 2B.

Not surprisingly with these capabilities, biological samples can be preserved in much less time than would be required by traditional means and used in a wide variety of settings. Clearly, all of these factors underscore an easier, more cost-effective means to store or ship various biological samples at ambient conditions without costly refrigeration systems or necessitating the use and exposure to toxic, cancer causing chemicals.

Chemical- and refrigeration-free biological sample preservation and storage at room temperature for up to six months

Publications in peer-reviewed scientific journals have demonstrated bio-molecules previously thought to be highly susceptible to degradation at room temperature without chemical and/or cryo-preservation are not. These include amino acids, such as enzymes and most recently the "unstable" nucleic acid, RNA (Figure 3 and 4). Works published in recent years support the premise that chemical fixation of biological samples and/or refrigeration may not be necessary to effectively preserve samples of interest. 

To date, only aqueous DNA samples using FTA cards for example, have been verified to be reliably stored for several decades at room temperature. Point of note, numerous articles have demonstrated detection from biological samples thousands to millions of years in age. Nonetheless, general convention dictates that RNA is to be thought of as a highly sensitive and unstable macromolecule that necessitates precautionary measures to minimize its loss. Again, recent publications are beginning to prove this inaccurate, where RNA can be reliably stabilized under dry-air conditions at room temperature for several months without the aforementioned loss. Furthermore, proteins of different varieties, i.e. enzymatic, structural, signaling, etc. have been shown to be stable for six months dried and stored under dry-air conditions. Previous publications in scientific journals have demonstrated detection of enzymatic and structural proteins with no degradation observed. Finally at the cytological level, it was thought if specimen were dried and stored dried at room temperature that their cellular morphology would change over time. This, too, has been disproven through careful research published in peer-review journals. Where studies examined the morphology of unfixed, as compared to those chemically fixed, cells over the course of 1 month dried at room temperature with no noticeable change (Figure 5).
temperature storage.\textsuperscript{15} There, it was determined that a facility of its current storage capacity, around 2000 freezers across 350 laboratories could swap a quarter of its total number of biological samples (~13M) to room temperature storage. This would translate into a reduced carbon footprint by 18,000 metric tons and a cost savings of $16M over ten years. Currently the annual operational cost of all the freezers at Stanford run about $5.6M, producing an estimated 3,600 tons of carbon dioxide and consuming nearly 40,000 million BTU of energy. If this model were applied to all the larger academic institutions and biobanks across the US, 171 and 157 respectively the total annual carbon footprint reduction would be about 300,000 tons plus a cost saving of roughly half a billion US dollars.

\textit{Third-Party Evaluation}

Several organizations representing large academic institutions (i.e., medical schools) and businesses evaluated the Dri•Bank\textregistered container over a one-year period. These evaluators were not financially compensated for their time or supplies used, and performed tests when their schedule permitted. A variety of biological samples were used during the trial period: from animal tissue to yeast to human cell lines to primate serum to purified nucleic acids.

Upon receipt of the Dri•Bank\textregistered container, the evaluators were asked nine (9) questions regarding their initial impression of our product. The questions were ranked on a scale from zero (low or “no”) to five (high or “yes”). The average score for all nine questions was 4.4 with a standard deviation of 0.9. Table 1 summarizes the questions and the values obtained.

Evaluators were further asked to provide their own impression after using our product. From the group, four provided comments. One, a large international biotech company stored fresh-frozen tissue sections in our container at room temperature for one week. These samples were compared to samples kept at -80°C, which were subsequently stained using immunohistochemistry methods. After staining they reported their results were equivalent to those stored cryogenically for the same period. A different evaluator from the same biotech company used the Dri•Bank\textregistered container for preparing samples for analysis by mass spectrometer. It was mentioned they typically have to dry their samples overnight in an oven or at room temperature. However, using the Dri•Bank\textregistered container their samples were ready for analysis after only 15 minutes. A research scientist from a cancer research center was completely surprised that aliquots of purified RNA could be dried, stored dried at room temperature and then reconstituted days later without any signs of decomposition. This investigator analyzed the dried aliquots using qRT-PCR on a Agilent 2100 Bioanalyzer. Summary results found in Figure 7A. Lastly, a large diagnostic company examined primate blood serum dried and stored dried in the Dri•Bank\textregistered container over two weeks at room temperature. They compared the samples from the Dri•Bank\textregistered container to those normally kept at -80°C. Several markers were examined using Array Immuno-Multiplexing, all were detectable and equivalent to one another (Figure 7B).

\textit{Implementation}

The Dri•Bank\textregistered container was developed as an efficient way to store numerous samples so that they are “load and go”. With no need to remove chemical coatings or removed chemical fixative the samples preserved and stored in our product can be immediately used for analysis. For the majority of samples whose turn around time is from hours to a few months, the Dri•Bank\textregistered container is a powerfully efficient bench top preservation and storage solution. Plus for sample requiring longer-term storage and/or for more sensitive or untested biomolecules the Dri•Bank\textregistered container works with traditional cold-chain storage. Simply dry the sample as usual.

place into the Dri•Bank® container and then store as usual. The Dri•Bank® container will keep your samples preserved and last longer than normal due to its ability to scrub out any moisture.

For shipping samples, nothing could be easier than drying the sample as usual, place into the Dri•Bank® container, then package and ship to its destination without the need for dry ice.

**Summary**

The Dri•Bank® container is the most convenient, easy to use, lowest maintenance and most cost-effective solution on the market today for preserving and storing biological samples at room temperature. The Dri•Bank® container has demonstrated its compatibility to preserve and store tissue sections, blood serum, and purified nucleic acid, including RNA at room temperature, for several months. This is supported by both peer-reviewed scientific journals and successful third-party evaluations. Where, evaluators both liked the Dri•Bank® container and were happily surprised by its effectiveness to both preserve and store biological samples at room temperature. Finally, this effectiveness of preserving and securely storing biological samples at room temperature translates into tremendous cost savings.
Appendix

Figure 1........................................................................Comparison of chemical fixation times against dehydration for preserving a typical 5mm³ biopsy specimen.

Figure 2........................................................................Hygroscopic performance of the Dri•Bank® container under different environmental conditions.

Figure 3........................................................................Molecular biology data from samples dried and stored dried at room temperature for up to six months.

Figure 4........................................................................Generation of cDNA via RT-PCR from RNA extracted from 5mm³ liver biopsy specimen dried and stored at room temperature. Storage time was three months.

Figure 5........................................................................Photomicrographs of buccal mucosa cells fixed with conventional chemical fixatives compared to cells stored desiccated at room temperature. Storage period was 1 month.

Figure 6........................................................................Cost comparison of sample preserved and stored using traditional and non-traditional methods.

Figure 7........................................................................Data from third-party evaluators.

Table 1........................................................................Questions and response values from evaluators initial impression of the Dri•Bank® container.

Table 2........................................................................Summary of the results of tests performed using the Dri•Bank® container or known to be compatible with the Dri•Bank® container from peer-review scientific literature.
Fixation Time for 5mm$^3$ Sample

From a processing perspective, preservation via desiccation is significantly faster than chemical fixation and avoids penetration-fixation paradox.

**Figure 1.** Comparison of chemical fixation times against dehydration for preserving a typical 5mm$^3$ biopsy specimen. Note the differences in molecular weight affects rate, i.e., water equals 18g•mol$^{-1}$, formaldehyde 30g•mol$^{-1}$ and glutaraldehyde 100g•mol$^{-1}$. 
Figure 2. Hygroscopic performance of the Dri•Bank® container under different environmental conditions. (A) Atacama cartridge rapidly eliminates moisture from internal space when closed, shown at time zero. Ambient = room temperature (~20˚C). (B) Hygroscopic performance of the Dri•Bank® containers internal space during transition from -20˚C to +20˚C over 45 minutes as compared to a Conventional slide box.
Figure 3. Molecular biology data from samples dried and stored dried at room temperature for up to six months. (A) Photomicrograph of basic staining as well as stains for a couple of markers performed on 10um thick tissue sections. There was no difference between fresh tissue and sections that were desiccated and stored at room temperature (i.e., ambient) for six months. GFAP = glial fibrillary acidic protein; H&E = hematoxylin and eosin; IP-TH = immunoperoxidase-tyrosine hydroxylase. (B) Western blot comparing signal of enzyme tyrosine hydroxylase (TH) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from 20ug of total protein extracted from 20um thick tissue section. Storage period was six months.
Figure 4. Generation of cDNA via RT-PCR from RNA extracted from 5mm$^3$ liver biopsy specimen dried and stored at room temperature. Storage time was three months.
**Figure 5.** Photomicrographs of buccal mucosa cells fixed with conventional chemical fixatives compared to cells stored desiccated at room temperature. Storage period was 1 month.
Figure 6. Cost comparison of sample preserved and stored using traditional and non-traditional methods. FFPE = formalin-fixed, paraffin-embedded. Cold-Chain = traditional cold storage and/or shipping with or without dry ice. Competitor = represents current ambient temperature preservation/storage products. NOTE: clinical samples as reported by Jensen et al. 2009 are not included, but it is acknowledge these can run between $1,000 to $10,000 USD each.⁶
Figure 7. Data from Third Party Evaluators. (A) qRT-PCR data from 3rd Party Evaluator demonstrating the Dri•Bank® containers ability to dry and store DNA and RNA aliquots at room temperature without chemical. Storage time was 10 days. (B) Summary result of several markers analyzed by Arrayed Immuno-Multiplexing after primate blood serum was dried and stored dried at room temperature in the Dri•Bank® container.
Table 1. Questions and response values from evaluators initial impression of the Dri•Bank® container. Number of evaluators equals six, one abstained from answering, total number of response equals five. Questions were scored from 0 (low, negative impression) to 5 (high, positive impression).

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<tr>
<th>Question</th>
<th>Average Response Value</th>
<th>Standard Deviation</th>
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<tr>
<td>Do you like the appearance of the Dri•Bank® container?</td>
<td>4.5</td>
<td>1.0</td>
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<tr>
<td>Were the instructions easy to read?</td>
<td>4.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Was it clear what/how the Dri•Bank® container can be used?</td>
<td>3.8</td>
<td>1.5</td>
</tr>
<tr>
<td>How was the construction of the Dri•Bank® container and Atacama™ cartridge?</td>
<td>4.5</td>
<td>0.6</td>
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<tr>
<td>Was it easy to insert the Atacama™ cartridge into the Dri•Bank® container?</td>
<td>4.8</td>
<td>0.5</td>
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<tr>
<td>Was the Dri•Bank® container easy to open and close?</td>
<td>5.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Do you feel the Dri•Bank® container is a sturdy, well-constructed product?</td>
<td>4.8</td>
<td>0.5</td>
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<tr>
<td>With your initial impression, do you like the Dri•Bank® container?</td>
<td>4.3</td>
<td>1.0</td>
</tr>
<tr>
<td>With your initial impression, would you recommend this product to a colleague?</td>
<td>4.0</td>
<td>1.4</td>
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<tr>
<td>Overall Response</td>
<td>4.4</td>
<td>0.9</td>
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Table 2. Summary of the results of tests performed using the Dri•Bank® container or known to be compatible with the Dri•Bank® container from peer-review scientific literature.

<table>
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<tr>
<th>Time Span</th>
<th>DNA (a)</th>
<th>Biological Molecule (Biomolecules) Investigated for Dry Room Temperature Storage</th>
<th>RNA</th>
<th>Proteins</th>
<th>Enzymatic (e)</th>
<th>Signaling</th>
<th>Structural (e)</th>
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<td>1 hour</td>
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<td>tRNA</td>
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<td>8 hour</td>
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<td>mRNA</td>
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<td>24 hours</td>
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<td></td>
<td>rRNA</td>
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<td>1 week</td>
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<td>3 months</td>
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<td>6 months</td>
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<td>12 months</td>
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<td>Over one year</td>
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</table>

Eukaryotic cells: a – human reproductive tissue, b – human tonsil tissue (16 hours), c – human colon tissue (16 hours), d – gallus liver tissue (3 months), e – rat brain tissue

Prokaryotic cells: gram-negative bacteria

References:
3 – Sadler T and Khodavirdi A. “High-quality RNA extracted from biopsied samples dehydrated and stored dried at room temperature without chemical preservation for up to 3 months as evidenced by RT-PCR results.” Appl Immunohistochem Mol Morphol. 2014 July;22(6);
4 – Sadler T, Khodavirdi A, Hinton D, et al. “Snap-freezing brain tissue sections stored with desiccant at ambient laboratory conditions without chemical fixation are resistant to degradation for a minimum of 6 months” Appl Immunohistochem Mol Morphol. 2009;17:165 – 171
5 – Garcia A. “Anhydrobiosis in bacteria: From physiology to applications.” 2011;36:1 – 12