White Paper

DriBank Labs

We are the new benchmark



DriBank Labs

Laboratory innovations for a more sustainable tomorrow

Driftless Region

Lanesboro, MN

Testimonials

"...for biomarkers [we] tested works better than -80°C...I like a lot of aspects of this unit

Dr. Lynne Bemis

Department Chair, Biomedical Sciences, University of Minnesota Medical School – Duluth Campus

"A practical and cost-effective shipping solution for biological samples; no dry ice required.

Dr. Kim Luke Laboratory Director, Intuitive Bioscience

"Accurate and meaningful next-gen sequencing data begin with optimal sample collection and preservation.

Dr. Aaron Ericsson Director, University of Missouri Metagenomics Center

Great product, works really well.

Dr. James Collins Professor, University of Minnesota Former Director Veterinary Diagnostic Laboratory "Surprised! ... replicate [RNA] samples dried and stored at room temperature in the Dri•Bank[®] container were identical to frozen controls after <u>two months.</u>

Dr. Daniel Purcell Molecular Biologist, University of New Mexico

"So far, we have been very pleased with the Dri•Bank[®] system...We plan to use Dri•Bank[®] for future nucleic acid projects to avoid the hassle of dry ice and potential spillage of samples during shipment.

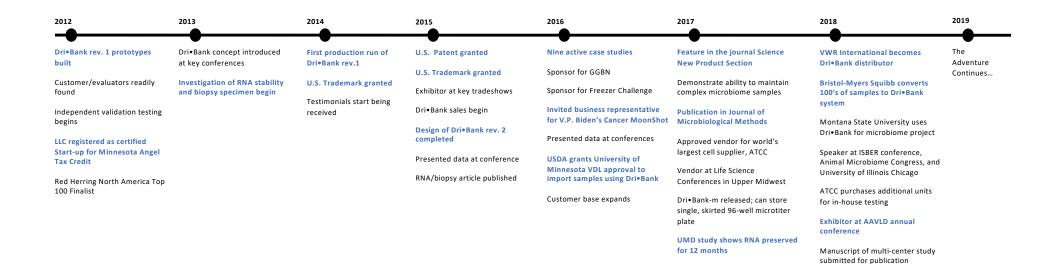
Dr. Stefan Green Director DNA Services, University of Illinois - Chicago

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DriBank Labs

Highlights



Additional publications being

Guest speaker at University of Minnesota Veterinary Diagnostic

Laboratory

written



Did you know that <u>one</u> typical ultra-low temperature freezer (ULTF), i.e. -80° C laboratory freezer, consumes 18,000 kWh of electricity and produces 35,000 pounds of CO₂ gas per year? ^{1,2,3}

This means for a typical R1 research university as classified by the Carnegie Institute for Higher Education, like the University of Wisconsin-Madison, can house approximately 1,000 ULTF on site, which can yield around 35 million pounds of greenhouse gas per year!



Background

Laboratories across the world procure, preserve and process a variety of biological specimens daily. These 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 punch biopsies to purified macromolecules in an aqueous solution. Common to all of these is the issue of how to preserve and store them best. Once collected, they need to be maintained in a manner that effectively preserves them from days to months later, or longer.

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 or decomposition*.

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

- 1.) preserved chemically and embedded in paraffin at room temperature; or,
- 2.) snap-frozen and kept refrigerated.

Both methods have their unique advantages and disadvantages, which are discussed later. For now, these approaches have allowed scientists to store biological samples to be used for a variety of analytical assays 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 freezer (ULTF) can house over \$200K of lab specimens—not including the cost of the freezer, utility or preventative maintenance fees; this only factors in the cost of the containers, samples and work-hours. Unfortunately, one has to look no further than the news from Harvard University⁴ 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[®] system based on research published in peer-reviewed scientific journals.^{5,6,7} Our U.S. patented product (#9,044,007 and other patents pending) can effectively preserve, store, and ship an assortment of biological specimens at room (ambient) temperature almost anywhere without the need for electric power, toxic chemicals or refrigerants. In fact, our innovation was highlighted in **Science Magazine's** new product section.⁸



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

-Dr. Purcell. Cancer Research Center, University of New Mexico

Problem

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. These are primarily stabilizing solutions, modified filter paper, or thermostable coatings, all which have known limitations (see **Table 1**).

TABLE 1. List of common non-destructive (e.g., formalin-free) preservation and storage methods, temperature range, and known limitations from publications. Laboratory refrigerator aside, all innovations are single-use only. * - refers to either fresh-frozen *ex vivo* specimens or aliquots in ultra-pure water. Filter paper refers to chemically-modified varieties used to preserve nucleic acid as well as other biomarkers such as dried blood spot cards

Method	Temperature Range	Limitations
Laboratory Refrigerator*	-80°C to 4°C	 specimens not cryopreserved (-130°C) biochemical reactions may continue, including decomposition high purchase, maintenance, and utility costs as well contributes to CO₂ emissions depending on sample, thawing times vary between 15 to 45 minutes on wet ice
Stabilizing Solution	-80°C to 25°C	 permeates all cells and tissues must be completely and thorough removed prior to downstream assays commercially sold is expensive, do-it-yourself takes time to prepare specimens are suitable at 25°C for 1 week, 4°C for 1 month, and -80°C "long-term" (i.e., undetermined/unknown)
Filter Paper	-80°C to 25°C	 drying time varies between 12 – 24 hours for liquid samples (50 – 100 μL) prone to cross or surface contamination during collection or processing eluting samples off of card matrix can take up to several hours final quantity biomarker eluted off the card can be low and sample quality varies
Thermostable Coating	25°C	 requires expensive vacuum centrifugation or lyophilization equipment without equipment, coating can require days at room temperature to completely dry room temperature drying process results in incomplete drying and preservation coating must be completely removed prior to analytical work-up

The Dri•Bank however is the only one that can preserve and securely store diverse biological samples at room temperature almost anywhere. No need for any coatings, chemicals or refrigeration. Specimens have included unfixed tissue sections on microscope slides, blood serum, fecal samples (microbiome), biopsy specimens, or purified nucleic acid aliquots, which have all been stored from weeks to months with no observable differences when compared to fresh-frozen counterparts.^{5,6,7}

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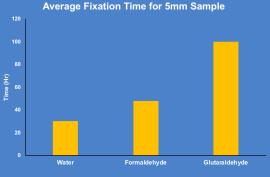


FIGURE 1. Comparison of chemical fixation times against dehydration for preserving a typical 5mm³ biopsy specimen. Note the differences in molecular weight affects rate, i.e., water equals 18g•mol⁻¹, formaldehyde 30g•mol⁻¹ and glutaraldehyde 100g•mol⁻¹.

Problem

CHEMICAL FIXATION

For ease and convenience, biological lab samples are frequently placed in buffered formaldehyde (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, formalin has several well-documented drawbacks, which include extremely slow fixation rate, destructive to critical bio-macromolecules, contributes to shrinkage of preserved tissue, and a toxic cancer-causing agent.¹⁰ Owing to its chemistry, when added to a buffered solution, formaldehyde is rapidly converted to methylene glycol in abundance (>99.9%). When specimens are submersed into formalin, it can take hours to days for most conventional laboratory samples to be preserved (Figure 1). Although formaldehyde is a small, polar molecule that can readily infiltrate most cells, this delayed fixation rate is known as the **Penetration-Fixation Paradox**.¹⁰ While next-generation methods such as cross-linked chromatin immunoprecipitation (X-ChIP) assays have exploited the fragmentation and binding properties of formaldehyde to generate data relating to DNA-protein interactions, this has lead to false-positive signals at highly expressed loci, promoting the development and use of formaldehyde-free ChIP (or native ChIP).^{11, 12, 13} Moreover, due to the chemical nature of these fixatives, they can only be removed by prolonged immersion into boiling caustic solutions (or Heat-Induced Epitope Retrieval, or HIER). Typically, this requires a sample-specific trial-and-error approach to optimize the conditions for the removal of the cross-linkages. Finally, 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, especially for those in remote, resource-limited areas.

FREEZING

Snap-frozen techniques on the other hand, provide the impression that samples are instantaneously preserved, which greatly reduces 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 here that according to the U.S. National Institute of Standards and Technology (NIST) cryopreservation does not occur until samples are at, or below the glassine state of water, or -130°C.¹⁴ Unless initially placed in liquid nitrogen, most other snap-frozen methods occur at or around -80°C, which still permits biochemical reactions, including degradation to occur, albeit at a slower rate.

Once frozen, the sample(s) must remain frozen, or cryopreserved until they are needed for analysis. Traditionally this requires several replicates to avoid potential damage from the freeze-thaw cycle. Not surprisingly, given the number of samples a laboratory may need to process requires several costly ULTF 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.

A practical and cost-effective shipping solution for biological samples; no dry ice required.

Dr. Kim Luke Laboratory Director, Intuitive Bioscience

Fact 🗸

Cold Facts About Cold-Chain Shipping^{1,16,17}

Fees for <u>one</u> shipment can run into the **thousands** of dollars due to:

- 1.) Time-sensitive nature (refrigerant loss over time)
- 2.) Final destination (domestic or international)
- 3.) Amount (weight) of refrigerant required & container size to accommodate up to 24-hour delay in transport
- 4.) Delays, including time spent in customs, if required
- 5.) Refrigerant restrictions based on destination country
- 6.) Hazardous Materials: dry ice and liquid nitrogen are known asphyxiants necessitating special handling and higher fees

Remedy

A re-useable system where one can easily preserve, store, and ship numerous, and diverse specimen types at room temperature



No cold-chain

No toxic chemicals

No high-costs

The Dri-Bank Innovation

Water is key to many of biological reactions, including autolysis. Hence its partial removal from a specimen during 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 into 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 effectively removes moisture from the samples of interest and reduces and/or eliminates any decomposition.^{5,6,7} 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 in challenging conditions such as those presented in resource-limited locations. 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 system has undergone performance testing under different environmental conditions: normal laboratory surroundings (30% RH and 20°C), simulated tropical setting if used in the field (>90% 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 had no detectable moisture (0% RH) after 20 minutes. During simulated tropical conditions, the internal environs are brought to less than 10% RH within 15 minutes and no detectable humidity after 50 minutes when closed.

When compared to a standard cryo-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 cryo-box has an internal humidity of 70% as moisture began to be released from its frozen state. Meanwhile the Dri•Bank rises to no more than 6%. If either container is left out until at room temperature (20°C), the cryo-box is stable at 50% RH and the Dri•Bank has no detectable moisture (0% RH).

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 these factors underscore an easier, more cost-effective means to preserve and store various biological samples at ambient conditions without costly refrigeration systems or necessitating the use and exposure to toxic, cancer causing chemicals, or troublesome filter paper methods.



The Dri•Bank® System

The Dri•Bank Innovation

Specimen preservation and storage at room temperature

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 physical interventions are not. These included amino acids, such as enzymes and most recently the "*unstable*" nucleic acid, RNA (see Figures on page 9).

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.^{2,3} To date, only aqueous DNA samples using filter paper-based technologies for example, have been verified to be reliably stored for several decades at room temperature. In fact, articles have demonstrated detection of soft tissue from dinosaur bones millions of years old.¹⁹ Nonetheless, the accepted dogma 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 have begun to demonstrate this inaccuracy, where RNA can be reliably stabilized under dry-air conditions at room temperature for several months without the aforementioned loss.^{2,4,5} 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 journals⁴ 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.^{6,7} 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.

Low-Cost, High-Efficiency Alternative for Biological Sample Storage and/or Shipment

The more steps, or manipulations one has to perform on a specimen in order to obtain the information needed, the more time and money is consumed. The more samples that can be analyzed with minimal interventions, the more cost-effective and faster turn-around time is achieved. The Dri•Bank system offers a low-cost, high-quality, high-efficiency, greater flexibility and faster turn-around time for storing biological samples in various formats. Formalin-fixed, paraffin-embedded samples represent the highest average cost per sample at approximately \$2.⁵⁰, while room temperature storage offers a tremendous saving at approximately \$0.25 per sample (**Figure 2**).

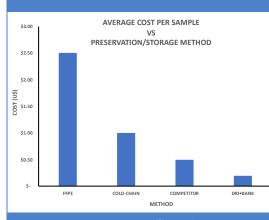


FIGURE 2. Cost comparison of sample preserved and stored using traditional and non-traditional methods. FFPE – formalin-fixed, paraffin-embedded. Cold-Chain = traditional cold storage and/or shipping with or without dry ice. Competitor = represents current ambient temperature preservation/storage products. 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.¹⁵ Did you know since the 1970's cryogenic storage in liquid nitrogen (LN2) has numerous faults?

Fact 🗸

This would include:

- 1.) Prone to external and internal contamination
- 2.) Asphyxiant hazard
- 3.) Burn hazard
- 4.) Refrigerant loss, and more!







DriBank Labs



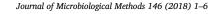
Journal of Microbiological Methods Utility of a portable desiccant system for preservation of fecal samples for ELSEVIEI

downstream 16S rRNA amplicon sequencing

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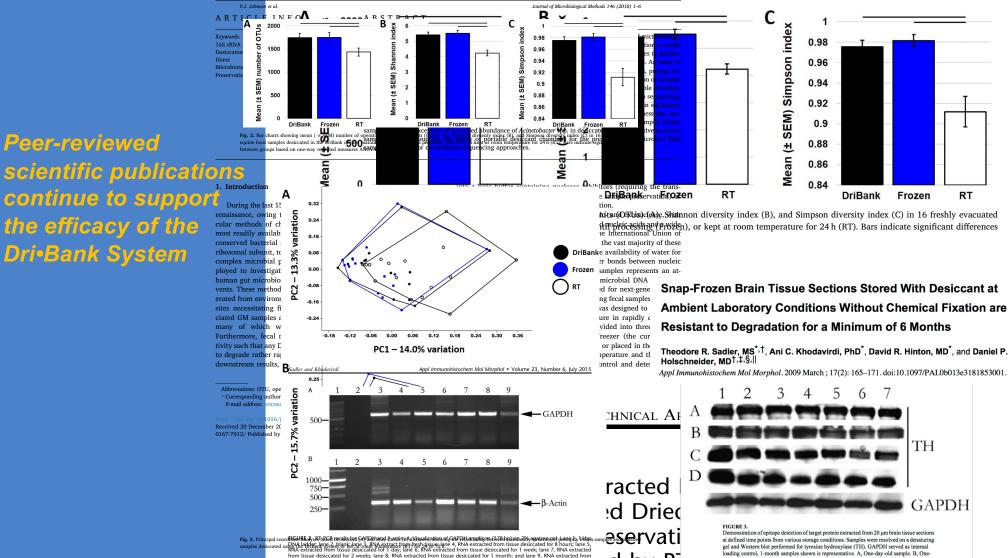
⁴ University of Missouri Metagenomics Center (MUMC), 4011 Discovery Dr., Columbia, MO 65201, USA ⁵ University of Missouri College of Veterbully Sulfacilie, 500 E. Campus Dr., Columbia, MO 65211, USA



week-old sample, C. One-month-old sample, D. Six-month-old sample, 1 = Fresh brain tissue section. 2 = Frozen brain tissue section (without heat). 3 = Ambient brain tissue section (without heat). 4 = Desiccated brain tissue section (without heat). 5 = Frozen brain tissue section (with

heat). 6 = Ambient brain tissue section (with heat). 7 = Desiccated brain tissue section (with

heat), GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase



NVA extracted from ussue desiccated for 1 day; lane 6, KNA extracted from ussue desiccated for 1 veek; lane 7, KNA extracted form tissue desiccated for 2 veeks [lane 8, RNA extracted from ussue desiccated for 1 month; and lane 9, RNA extracted from tissue desiccated for 3 months. B, Visualization of β-actin amplicing (338 hp) on 2% agarose gel. Lane 1 = 10 kbp DNA ladder; lane 2, blank; lane 3, RNA extract from fresh tissue; lane 4, B hours RNA extract; lane 5, RNA extracted from tissue desiccated for 1 day; lane 6, RNA extracted from lissue desiccated for 1 veek; lane 7, RNA extracted from tissue desiccated for 1 day; extracted from tissue desiccated for 1 month; and lane 9, RNA extracted from tissue desiccated for 3 months. RT-PCR indicate everse transcription-polymerase chain reaction

d by R

Peer-reviewed

The Dri-Bank Innovation

Eco-Friendly

Given the Dri•Bank is reusable and does not require electricity or use of toxic chemicals, this greatly reduces the environmental impact for storing or shipping biological samples. A study conducted by Stanford University in 2009 investigated the cost and environmental impact of transitioning from cold-chain to room temperature storage.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.

Third-Party Evaluation

Several organizations representing large academic institutions (i.e., medical schools) and businesses have evaluated the Dri•Bank system. 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.

Evaluators were asked to provide their own impression after using our product. From the pool of evaluators, 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 for preparing samples for analysis by mass spectrometer. It was mentioned they typically must dry their samples overnight in an oven or at room temperature. However, using the Dri•Bank their samples were ready for analysis <u>after only 15 minutes!</u>

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. Lastly, a large diagnostic company examined primate blood serum dried and stored dried in the Dri•Bank over two weeks at room temperature. They compared the samples from the Dri•Bank to those normally kept at -80°C. Several markers were examined using Array Immuno-Multiplexing, all were detectable and equivalent to one another.

"Great product, works really well.

Dr. James Collins

Professor, University of Minnesota Former Director Veterinary Diagnostic Laboratory



Compared to other room temperature innovations, the reusable Dri•Bank® system preserves and stores biological samples with results equivalent to fresh or frozen samples at -80°C up to several months^{5,6,7} without the hassle of thawing, eluting, or washing!



Implementation

The Dri•Bank system 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 fixatives, elute samples off cards, or thaw specimens the samples preserved and stored in our product can be immediately used for analysis. For the majority of analytical specimens whose turn around time is from hours to a few months, the Dri•Bank is a powerfully efficient bench top innovation for any laboratory or field researcher. Plus for sample requiring longer-term storage and/or for more sensitive or untested biomolecules the Dri•Bank works with traditional cold-chain storage. Simply dry the sample with the Dri•Bank and then store as usual. The Dri•Bank will keep your samples preserved and last longer than normal due to its ability to effectively adsorb moisture.

For shipping samples, nothing could be easier than drying the sample within the Dri•Bank system, once confirmed dry, then package and ship to its destination without the need for Styrofoam boxes, dangerous refrigerants (dry ice or liquid nitrogen, or costly hazard surcharges.

Summary

The Dri•Bank system is the most convenient, easy to use, lowest maintenance and most cost-effective innovation on the market today for preserving and storing biological samples at room temperature. The Dri•Bank has demonstrated its compatibility to preserve and store tissue sections, biopsy specimen, blood serum, and purified nucleic acid, including RNA at room temperature, for several months. This is supported by both peer-reviewed scientific publications and successful third-party implementation. Where, customers have both liked the Dri•Bank 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.



Despite it's numerous drawbacks¹⁰, **formaldehyde** (formalin) continues to be one of the most frequently used preservatives across the globe

These include:

1. Incompatible with numerous modern molecular assays

2. Destroys nucleic acid biomarkers

3. Masks protein epitopes

4. Requires caustic, hightemperature solutions to remove

5. Toxic, cancer-causing compound



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We are the new benchmark

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U.S. Patent #9,044,007 and other patents pending.

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