ZYMO RESEARCH

Sample Collection and Preservation in Public Health Studies

Public health research can be challenging and costly due to the lack of laboratory infrastructure at field collection sites, extensive sample collection periods, as well as need for specialized handling and transport of samples. These obstacles hinder the ability to effectively perform research in the environments where subjects reside. Zymo Research offers an innovative solution to overcome these challenges and enable accurate sample collection.

A Challenging Environment

Sampling for public health studies is frequently done in remote locations with little to no infrastructure, water or electricity, and can involve the collection of biohazardous specimens. Working in these conditions can be challenging. In addition, collection and transportation of samples for nucleic acid analysis adds another layer of complexity to these studies and impacts researchers around the globe. This is due to the sensitivity of nucleic acids to environmental factors such as heat, humidity, and nucleases. These conditions can result in modification or degradation of the genetic material during sample collection and transport. Furthermore, unless preserved at the time of collection, bacteria and fungi may continue to grow in the sample. This will lead to an inaccurate microbial profile that does not reflect the composition of the sample at the time of collection (Figure 1). This in turn leads to a misinterpretation of the data derived from a sample.

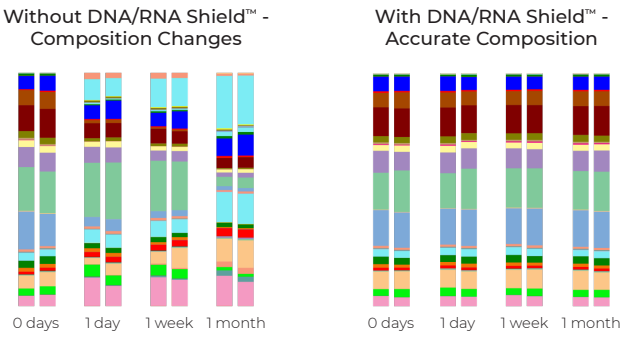


Figure 1: The microbial composition changes over time in the absence of preservative. Stool samples were stored at ambient temperature for one month. Total genomic DNA was extracted using the ZymoBIOMICS™ DNA miniprep kit and then subjected to microbial composition profiling using 16S rRNA gene-targeted sequencing. The resulting sequences (shown as genus-level taxa plots) demonstrate a shift in the microbial composition over time in the absence of preservation reagents (left panel) and no shift in the presence of DNA/RNA Shield™ (right panel).

Current Landscape

Currently accepted collection and transportation methods require the use of cold chain logistics (freezers, liquid nitrogen, cold packs) to slow down degradation and changes in sample composition. Transporting biological samples across international borders using a cold chain can be cost-prohibitive, laborious and hazardous.

Another option for nucleic acid preservation uses chemical solutions such as TRIzol®, which can inactivate biohazardous agents. However, reagents used for inactivation can pose their own set of health hazards during transit and processing in the laboratory.

An alternative is the use of chemical preservatives such as RNA*later*™. Although it has some effectiveness to preserve samples for storage, it has been shown to not effectively inactivate pathogens (e.g. HIV)[1], leaving samples potentially biohazardous. Additionally, the RNA*later*™ protocol requires removal of the reagent completely prior to nucleic acid extraction or it leads to ineffective or biased extraction of nucleic acids. RNA*later*™ preservative removal can bias the microbial composition of a sample by co-removing the nucleic acids of easy-to-lyse organisms that are disrupted when stored in the reagent[2]. This requirement makes the process more labor intensive, biased and does not lend itself to automated sample processing methods.

Solutions At Your Fingertips

Researchers across many institutions have found solutions to these issues by adopting DNA/RNA Shield™ collection devices offered by Zymo Research. DNA/RNA Shield™ is a reagent that preserves nucleic acid integrity at room temperature for > 2 years for DNA and 30 days for RNA, even for complex samples (Figure 1). Additionally, it protects nucleic acids against freeze/thaw damage (Figure 2). DNA/RNA Shield™ inactivates infectious agents making the samples safe for transport and handling in downstream applications (Table 1). Finally, it does not require reagent removal prior to nucleic acid extraction, eliminating bias issues observed for RNA*later*™ and enabling direct compatibility with automated workflows.

APPLICATIONS

Asymptomatic Malaria

Researchers led by Dr. John Dame at the University of Florida are studying the underrepresented prevalence of malaria due to asymptomatic cases in remote and underdeveloped regions of Haiti. To solve the issue of blood sample transportation from remote collection sites in Haiti, the researchers collected small amounts of blood into vials containing DNA/RNA Shield™. The blood was rendered non-infectious and the nucleic acids were stabilized during transit to their laboratory, 5 to 15 days after collection[3]. Once in the lab, researchers stored the samples at -20°C until extraction, where removal of the reagent is not required, and the workflow is streamlined by using high-throughput processing. Upon isolation of the nucleic acids, the RNA was again stored in DNA/RNA Shield™ at -80°C to prevent

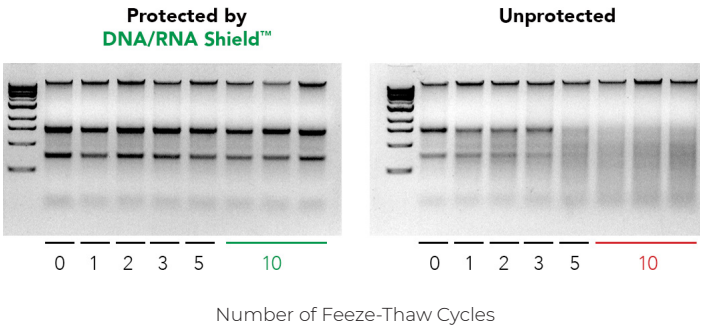


Figure 2: HeLa cells were freeze-thaw cycled either in 1xPBS or DNA/RNA Shield™ for the cycles indicated. DNA and RNA was co-purified using the *Quick*-DNA/RNA™ kit and resolved by agarose gel electrophoresis.

DNA/RNA Shield™ renders samples safe for handling and processing at different intervals while preventing freeze-thaw related degradation of nucleic acids.

freeze/thaw-derived degradation. They used a quick RNA cleanup (Zymo Research RNA Clean & Concentrator) to prepare the RNA for downstream applications, in this case RT-qPCR for malaria parasite detection. Dr. Dame's group developed a highly sensitive RT-qPCR test that was able to detect the malaria parasite in asymptomatic patients. With such a sensitive detection method, carriers of the parasite are readily identifiable, a critical point for preventing the spread and eradication of malaria within populations.

West Nile Virus Pathogenesis And Therapeutics

At the Department of Pediatrics, Tropical Medicine section at Baylor College of Medicine, a team led by Dr. Kristy Murray and researcher Dr. Shannon Ronca study the pathogenesis of various strains of West Nile Virus (WNV). They aim to identify therapeutics to combat the virus. West Nile virus is the lead cause of arboviral disease acquired in the USA[4]. Moreover, WNV infection in humans is currently untreatable and causes upwards of an 8% case fatality in patients affected by the neuroinvasive form of the disease. Since there is no current treatment other than supportive care available for WNV patients, research using live virus must be done carefully in a biosafety level 3 facility (BSL3) and samples must be free of live pathogens before exiting the facility. This added a level of complexity to Dr. Ronca's WNV research was accompanied by laborious measures to process the samples immediately to avoid freeze/thaw

Table 1: List of organisms that have been tested and inactivated by DNA/RNA Shield™.

Bacteria	Viruses	Yeast & Eukaryotes
B. subtilis	Parvovirus	*C. albicans*
E. faecalis	Chikungunya Virus	*C. neoformans*
E. coli	Dengue Virus	*S. cerevisiae*
L. fermentum	Ebolavirus	*P. malariae*
L. monocyto-genes	Herpes Simplex Virus-1	
M. tuberculosis	Herpes Simplex Virus-2	
P. aeruginosa	Influenza A	
S. enterica	Rhinovirus	
S. aureus	MERS-coronavirus	
S. pneumoniae	West Nile Virus	
X. fastidiosa		

cycling damage to the samples. To solve these problems Dr. Ronca incorporated DNA/RNA Shield™ into her protocols to stabilize the genetic material and neutralize the WNV in the samples. This treatment renders the samples safe for handling and ready to process at different intervals while preventing freeze-thaw related degradation of WNV nucleic acids. This improvement in workflow enabled a more consistent and sensitive detection of WNV and housekeeping marker controls (80 and 95% vs. 50 and 80% of samples positive for housekeeping controls in blood and urine, respectively) and increased the overall efficiency of the testing process.

DNA/RNA Shield™

- Ensures genetic integrity and preserved expression profiles of samples (cells, tissues, blood, plasma, serum, saliva, urine, feces etc.) at ambient temperatures for > 1 month. In other words, there is no need for refrigeration or specialized equipment.
- Inactivates nucleases and infectious agents.
- DNA and RNA can be isolated directly without precipitation or reagent removal (compatible with most DNA and RNA purification kits).
- Available in various collection and storage devices (blood vacutainer tubes, swabs, lysis tubes, fecal, etc.)

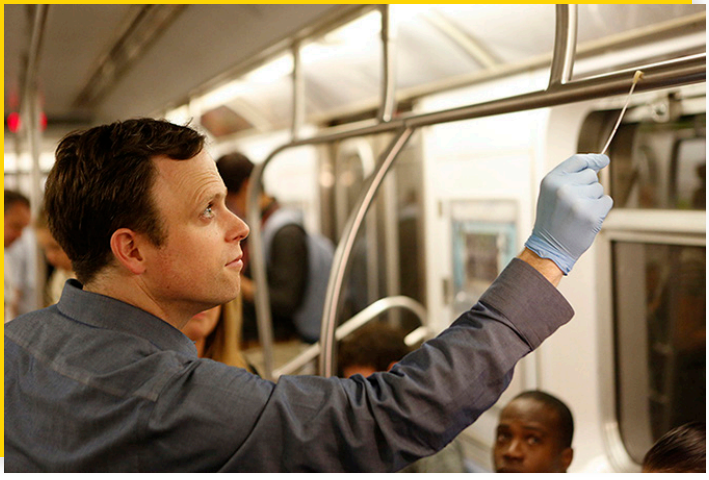
DETECTING COVID-19 IN NYC

In early March 2020, New York had just surpassed their 100th case of COVID-19. As the numbers continued to rise exponentially, a team of researchers led by Associate Professor Christopher Mason at Weill Cornell Medicine went onto the New York Metro system and began swabbing surfaces. In an effort to move science forward and gain insight on the spread of the novel virus, this heroic team took to one of the most crowded and most heavily used areas of the city to see if they could detect COVID-19 on surfaces, using DNA/RNA Shield™ for their sample collection and transportation medium. As the city shut down, they were able to swab other heavily used areas of the city like doorknobs and ATM machines.

DNA/RNA Shield™ enabled the team to safely transport samples that potentially contained COVID-19, without the fear of being infected. Without the need for cold-chain transportation, the researchers simply collected samples in DNA/RNA Shield and stored them in a backpack as they traveled around New York.

Through this study, the team hoped to understand the transmission dynamics of the virus in order to better inform city officials to establish policies that would keep the public safe. Additionally, as a more long-term goal, they were attempting to identify hotspots of infection within the city in order to predict which emergency rooms would see a spike upon lifting stay-at-home orders. The team also began to sequence the sewage in the city to pilot the idea of "sentinal sites" to track the virus.

Associate Professor Christopher Mason collecting samples on a subway (2016)



Hydraflock Swab

Swab

Screwcap Scoop

Stool

Ultra High-Density Beads

Tissue

Blood

Saliva

Urine

References

1. Uhlenhaut, Christine, and Maren Kracht. "Viral infectivity is maintained by an RNA protection buffer." Journal of virological methods 128.1-2 (2005): 189-191.
2. Bundgaard-Nielsen, Caspar, Søren Hagstrøm, and Suzette Sørensen. "Interpersonal Variations in Gut Microbiota Profiles Supersedes the Effects of Differing Fecal Storage Conditions." Scientific reports 8.1 (2018): 17367.
3. Maha A. Elbadry, Basima Al-Khedery, Massimiliano S. Tagliamonte, Charles A. Yowell, Christian P. Raccurt, Alexandre Existe, Jacques Boncy, Thomas A. Weppelmann, Valery E. M. Beau De Rochars, Jean F. Lemoine, Bernard A. Okech, and John B. Dame. (2015) High prevalence of asymptomatic malaria infections: a cross-sectional study in rural areas in six departments in Haiti. Malar J; 14:510.
4. Alexis Burakoff, Jennifer Lehman, Marc Fischer, J. Erin Staples, Nicole P. Lindsey. (2018) West Nile Virus and Other Nationally Notifiable Arboviral Diseases - United States, 2016. MMWR Morb Mortal Wkly Rep 2018; 67:13–17