HLA Typing: Template Quality Control for SSP and SSO

Introduction

The Thermo Scientific NanoDrop™ 1000 Spectrophotometer has been integrated into the workflows of many HLA typing laboratories. The system requires only one microliter of undiluted sample to make a full UV-Vis absorbance measurement in 10 seconds. This feature has helped to increase the workflow efficiency by removing time-consuming traditional spectrophotometers.

The NanoDrop 1000 has been integrated into the HLA typing workflow in the following manner: DNA is extracted and purified from donor tissue using DNA extraction systems, then quantified on the NanoDrop 1000 to ensure adequate template prior to amplification. The NanoDrop 1000 employs a patented sample retention system that requires only 1ul of sample for DNA absorbance spectral analysis, providing a calculated DNA concentration and purity ratios. The DNA template is then amplified using sequence specific primers for sequence specific PCR (SSP) or amplified using universal primers for downstream sequence specific oligonucleotide (SSO) hybridization. The microsample capability of the NanoDrop 1000 provides HLA laboratories fast, accurate DNA quantitation with minimal consumption of sample, which is critical for HLA typing for samples of limited cell mass such as mononucleated bone marrow specimens.

Many HLA typing laboratory personnel are strong proponents of this novel microsample technology. This article describes some of the key reasons behind its growing acceptance by the HLA typing community.

How It Works

By using fiber optic technology and the inherent surface tension properties of liquid samples, NanoDrop Technologies' microvolume instrumentation can accurately quantitate a wide range of biomolecules in volumes as small as 1 microliter. The patented sample retention system enables absorbance measurements to be performed without traditional containment devices such as cuvettes or capillaries.

The system uses liquid surface tension to hold a droplet of sample in place between two optical surfaces during the measurement cycle. In order to make a measurement, 1ul of sample is pipetted directly onto the lower optical (measurement) surface (Figure 1a). An upper optical surface automatically engages the sample to form a liquid column of mechanically-controlled path length (Figure 1b). Once the measurement is complete, the user simply cleans both optical surfaces with a standard laboratory wipe to prepare for the next sample. The NanoDrop 1000 eliminates the need for traditional sample containment devices, enabling an automatic path length change from 1mm to 0.2mm during the same measurement cycle of a given sample. This allows for an unprecedented range of sample concentration to be assessed through absorbance spectroscopy (2 ng/ul to 3700 ng/ul for dsDNA), essentially eliminating the need to perform dilutions.

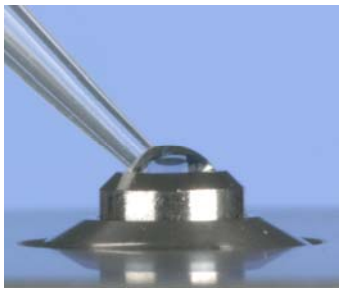

Figure 1a: Loading 1ul sample.

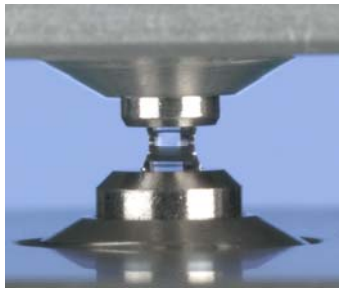

Figure 1b: Retention system.

Improved Efficiency and Time-To-Result

***Paula Wetzsteon**, C.H.S, Immunogenetics Laboratory Supervisor, Oregon Health & Science University, Portland, Oregon.*

The more significant the impact of a device on the workflow of an HLA laboratory, the more accepted it will be by the HLA testing community. The NanoDrop 1000 has proven to be a considerable labor-saving device by greatly reducing the time needed to perform spectrophotometric quantitation prior to DNA amplification for SSP and SSO typing.

Paula J. Wetzsteon supervises the Laboratory of Immunogenetics & Transplantation for the Oregon Health & Science University.

- "The time-limited nature of organ procurement and typing requires instruments that are efficient as well as reliable. The NanoDrop 1000 has proven to be an absolute labor-saving device. The difference between measuring one microliter of DNA in ten seconds versus making dilutions and taking measurements on a traditional cuvette spectrophotometer is significant, especially in higher-throughput situations. Reducing the amount of hands-on time without sacrificing reliability is the main reason the NanoDrop 1000 was integrated into our HLA typing program. Our robotic extraction system typically produces 300 ul of purified DNA ranging in concentration from 20 to 90 ng/ul. These concentrations would require time-consuming dilutions in order to be read on a traditional cuvette-based spectrophotometer. Dilutions must be performed accurately without pipetting errors. With a broad dynamic range of 2 to 3700 ng/ul for DNA, the NanoDrop 1000 removes the need to perform dilutions. The NanoDrop 1000 has improved our turn-around time. I endorse the NanoDrop 1000 for the pleasure of bringing it to the attention of my friends and colleagues in the field."

Instrument Validation and Acceptance

***Paul Warner**, PhD, D (ABHI), Co-Director of the Immunogenetics Laboratory, Puget Sound Blood Center, Seattle, Washington.*

All HLA typing laboratories must meet the standards set by ASHI to be accredited for clinical histocompatibility testing. Every instrument that is to be integrated into the workflow of an HLA typing laboratory must first go through a rigorous validation process. Not only has the NanoDrop 1000 passed the validation process for dozens of HLA typing laboratories across the country, the system is often recommended by certified laboratory inspectors.

Paul Warner co-directs the HLA typing laboratory at the Puget Sound Blood Center and is also an ASHI-certified inspector of HLA laboratories.

- "The standards and regulations that govern the testing in diagnostic facilities is much more strict than those for basic research labs. Before an instrument is accepted as part of an HLA typing laboratory, it is put through rigorous validation testing. Each lab needs to verify the performance of an instrument before they put it in routine use. The NanoDrop 1000 was thoroughly tested to ensure linearity and sensitivity as compared to other methods. When I inspect a lab, I check to see how a facility evaluates the concentration and purity of DNA templates prior to PCR-based amplification. To ensure robust amplification, accurate quantitation and purity assessment of template DNA is essential. If a laboratory does not have a NanoDrop spectrophotometer, I suggest evaluating the NanoDrop 1000 because it is much faster and easier to use than standard cuvette-based specs without compromising accuracy. The fact that the system requires only 1ul of undiluted sample becomes significant in situations that produce limited material. For example, solid organ labs usually produce decent yields of DNA, but bone marrow transplantation labs can have difficulty acquiring enough mononuclear cells to get good DNA yields. The NanoDrop 1000 is ideal for such situations because it minimizes the amount of material necessary to assess the quantity and purity of the DNA prior to amplification.

HLA Typing laboratories are always looking for ways to improve the speed and accuracy of their work. Solid-organ deceased donor workups require rapid turn-around times in order to speed the allocation process and minimize cold ischemic time. Any instrument that can reduce the time necessary for the work-up process is a definite advantage. The NanoDrop 1000 has met the following criteria which are essential for an instrument to be introduced into an HLA typing workflow: First, the instrument should be as foolproof as possible without requiring a lot of troubleshooting or preparation time. Second, it must meet the strict standards for accuracy and precision without compromising quick turn-around time. Speed and accuracy cannot be overemphasized. As HLA typing labs continually increase the volume and variety of testing being performed, not only are speed and accuracy of great importance, but so are the instrument's footprint and how the instrument fits into the workflow. The NanoDrop 1000 is ideal in that its footprint is quite small (about the size of a tissue box), yet still maintains a high level of speed and accuracy.

Training time is also important. It can take a year, or more, to adequately train a new HLA technologist, and any instrument that is user-friendly and easy to operate really helps to streamline the training process. In fact, I think if I were to take the NanoDrop1000 away from the lab and go back to our old cuvette spec, the techs in the lab would stage a revolt! That's how much they like the ease of use of the instrument. I appreciate the fact that it's easy to maintain, it's fast, and most of all, it's accurate. In short, the NANODROP 1000 fulfills all the requirements necessary for an instrument to be placed into routine use in our lab."

Bridging Key Steps in the HLA Workflow

***David Boyer**, Product Manager, GTI Diagnostics, Waukesha, Wisconsin.*

The NanoDrop 1000 has proven to be an attractive quality control device situated between the DNA extraction and HLA assay steps of the HLA typing workflow.

David Boyer is the product manager at GTI Diagnostics, an instrumentation company that provides automated human DNA extraction as well as genotyping assays.

- "High-throughput and high-complexity labs that perform nucleic acid testing and genotyping share some common needs. One of these needs is for instrumentation that bridges between two platforms and does not interfere with the work flow or increase labor demands. A bridge device should be able to accommodate the pace and quantity of the upstream process and accurately test at the sensitivity required by the downstream device or assay. The NanoDrop Spectrophotometer in my estimation fills these requirements exceptionally. In our application GTI's automated instrument, QuatroProbe, can isolate human genomic DNA from 48 samples in less than 1 hour. Most of the DNA is then HLA and HPA (platelet) typed by our genotyping assays, ThromboType® and HLA EZ Type®. To efficiently process this number of samples the genotyping assays need to be started immediately after DNA isolation.

The ease and speed of the NanoDrop is remarkable and has removed the slow and wasteful process of measurement by our old spectrophotometer. In the past it would require an enormous amount of time to process this number of samples. We would also lose a significant amount of the DNA in the process, especially for samples with low yield. NanoDrop has efficiently sped up the process significantly and has been greatly appreciated by the technicians.

For GTI Diagnostics, a supplier of both automated DNA isolation instrumentation and FDA cleared genotyping assays to clinical laboratories, NanoDrop has been the ideal bridge between these two processes. In our use the NanoDrop has largely replaced our old cuvette-based spectrophotometer. And as an *in vitro* diagnostics manufacturer that is extremely sensitive to the demands of our clinical lab partners we do not hesitate to recommend the NanoDrop to all of our customers interested in DNA isolation and genotyping."