Welcome to Mayo Medical Laboratories Profiles in Genetics.
These presentations provide short discussion of current genetics topics and may be helpful to you in your practice.
Our speaker for this program is April Studinski Jones, MS, CGC, a Genetic Counselor in the Biochemical Genetics Laboratory at Mayo Clinic, Rochester, Minnesota. In this presentation, Ms. Studinski Jones discusses the use of dried blood spot specimens for testing. Welcome April.
Disclosures

- Nothing to disclose

Thank you for the introduction. I have nothing to disclose.
The concept of applying biological fluids to filter paper was introduced about 100 years ago; however, the clinical application of dried blood spot testing wasn’t utilized until the 1960s. Dr. Robert Guthrie first used these specimens to measure phenylalanine for the detection of phenylketonuria, or PKU, in infants. This novel application for collecting blood led to the population screening of newborns for the detection of treatable, inherited metabolic diseases.

Today, hundreds of biological markers can be measured in dried blood spots. Such testing has been employed in a variety of settings including population screening, diagnostic testing, clinical trials, and research.
This slide and the next highlight just a few of the dried blood spot testing options available through Mayo Medical Laboratories, and is not intended to be all inclusive.

DBS testing first became available on a clinical basis through Mayo Medical Laboratories in the early 2000s when newborn screening via analyses of acylcarnitines and amino acids as well as second-tier newborn screening tests were introduced. These tests have been used to supplement the newborn care services offered by individual hospitals. In addition, state newborn screening programs utilize them to round out their test menu and, via the second-tier assays, both to reduce false-positive rates and to increase positive predictive values. More recently, work has been focused upon expanding the test menu to include newborn screening options for all conditions included in the Recommended Uniform Screening Panel as endorsed by the Advisory Committee on Heritable Disorders in Newborns and Children and subsequently approved by the Secretary of Health and Human Services.
That being said, dried blood spot testing is not limited to the newborn period. While some assays, such as the Supplemental Newborn Screen, are specifically designed for screening asymptomatic patients during the first week of life, other tests are intended to be employed for diagnostic purposes. The analysis of enzymes, biomarkers, DNA, and even postmortem screening can all be accomplished through DBS testing.

**DBS Testing Through MML**

- **Enzyme analysis**
  - PLSD / Lysosomal and Peroxisomal Storage Disorders Screen, Blood Spot
  - IDSBS / Alpha-L-Iduronidase, Blood Spot
- **Biomarker assays**
  - SUAC / Succinylacetone, Blood Spot
  - GPSY / Glucopsychosine, Blood Spot
- **Molecular genetic testing**
  - CFP / Cystic Fibrosis Mutation Analysis, 106-Mutation Panel
  - MITOP / Mitochondrial Full Genome Analysis by Next Generation Sequencing (NGS)
- **Postmortem screening**
  - PMSBB / Postmortem Screening, Bile and Blood Spots
Dried blood spot specimens are obtained by collecting and applying blood to specially manufactured absorbent specimen collection paper commonly referred to as filter paper. The Food and Drug Administration has classified such filter paper as a medical device. As such, filter paper manufactured for blood collection must meet certain criteria and quality standards with respect to sample absorption and lot-to-lot consistency. This was established, in part, to avoid variances that have the potential to impact performance metrics such as reproducibility. In addition, as a medical device, the filter paper collection cards must be labeled with an expiration date.
These photos highlight the demographic and specimen collection portions of the card along with the back cover, which contains the instructions for collection via heel or finger stick.

Specimen quality is impacted also by the collection process, which should occur as outlined on the back of the MML Blood Spot Collection Card. This involves obtaining a small amount of blood, typically via a heel or finger stick, and then applying the blood to the collection card. In fact, the amount of blood required for a single blood spot is approximately 75 to 80 microliters, so to fill a full specimen collection card with 5 spots requires less than a half milliliter of blood. Once acquired, the blood spots must dry for 3 to 4 hours in a horizontal position and at room temperature, away from sunlight and direct heat sources.
There are several reasons to choose dried blood spot testing over other specimen types. The ease of specimen collection, transport, and storage make dried blood spots an ideal specimen type. The small volume and avoidance of a venous blood draw can be particularly advantageous for individuals with complex medical needs including infants and others for whom it can be difficult to obtain a sufficient specimen volume via conventional collection methods. In fact, depending upon the testing needed, more invasive methods such as a skin punch biopsy, sometimes can be avoided or delayed until further evidence supports the need. For example, Niemann-Pick disease type C historically relied upon testing in cultured skin fibroblasts. While this testing is still available, the introduction of oxysterol analysis in blood spots allows for less invasive testing and at a fraction of the cost.

Furthermore, the savings of health care dollars can be realized through lower costs associated with the shipping and handling of dried blood spot specimens, which most commonly occurs at ambient temperature and require less packaging.

In some cases, DBS specimen testing is considerably lower than other options and integration of these tests may result in additional savings. For example, Friedreich ataxia can now be diagnosed via analysis of frataxin in a dried blood spot specimen. As compared to the traditional trinucleotide repeat expansion testing for Friedreich ataxia, frataxin analysis is less expensive, has a shorter turnaround, and will detect the small percentage of cases that are not caused by the trinucleotide repeat.
Advantages of DBS Specimens

- Improved patient care
- Increased specimen stability
  - PLSD / Lysosomal and Peroxisomal Storage Disorders Screen, Blood Spot
  - CBGC / Galactocerebrosidase, Leukocytes
  - CBGT / Galactocerebrosidase, Fibroblasts
- Decreased turn around time
- Flexibility with adding tests to existing specimens
  - PSY / Psychosine, Blood Spot
  - GBAZ / Krabbe Disease, Full Gene Analysis and Large (30 kb) Deletion, PCR

Other reasons cited for the utilization of DBS specimens have direct impact on patient care including extended stability and shorter turnaround times. With leukocyte enzyme analyses, the specimens often need to be received within 72 hours, which can be a challenge for some clients. Similarly, viability of skin biopsies and fibroblast cultures can be impacted by transport conditions. These factors are less pronounced for DBS specimens. In addition, assays developed for application with dried blood spot specimens tend to be shorter, particularly if they replace a fibroblast assay that requires several weeks to grow the culture. Finally, patients benefit from the ability to perform multiple tests with a single blood spot collection card and testing can be approached in a tiered fashion by requesting test add-ons to existing specimens rather than requiring patients to return for a subsequent draw.

These factors are highlighted in the workup of an infant suspected to have Krabbe disease. The PLSD test performed on blood spots includes the enzyme for Krabbe disease, which is also available in leukocytes and fibroblasts. The leukocyte assay must arrive in the testing laboratory within 72 hours as compared to the blood spot assay, which has a stability of 7 days at ambient temperature and longer at refrigerated or frozen temperatures. In addition, patients with abnormal results can have the biomarker psychosine and DNA testing added to the same dried blood spot specimen.
Limitations

• Specimen collection concerns
  • Storage of collection cards; expiration date
  • Specimen quality
    • Sufficient drying requires adequate space and time
    • Finger/heel prick versus blood from collection tube

• Test availability
  • Sample volume
  • Molecular – validation not available for all tests
    • GBAZ / Gaucher Disease, Full Gene Analysis – validated
    • GAUP / Gaucher Disease, Mutation Analysis – not validated

Of course some of the same factors cited as advantages can be identified as limitations as well. As mentioned, the blood spot collection cards are classified as a medical device and with that comes an expiration date. While the collection cards do not require the same real estate for storage as venipuncture tubes, some attention still must be given to rotating stock and proper storage in a clean, dry location. Moreover, once a blood spot specimen is obtained, it must be handled appropriately. This includes fully drying the specimen and appropriately packaging it to comply with regulations.

Often, patients have several tests ordered at the same time and may require both a venous blood draw as well as a DBS specimen. It can be tempting to utilize blood collected into a vacutainer or other collection tube to spot onto the filter paper. However, caution should be taken to carefully review the specimen requirements or consult the laboratory performing the testing to ensure the appropriate sample is submitted for analysis. Some tests were validated on DBS specimens created with blood collected in tubes containing anticoagulants, but not all tests nor all anticoagulants were validated accordingly.

Unfortunately, not all tests are amenable to the DBS specimen type. In some cases, this is a direct result of the small specimen volume, which can be a limiting factor with respect to the development of certain tests. For example, DNA can be analyzed in a blood spot, but for large genes, DNA sequencing may not be possible. Whereas
other circumstances may have impacted the validation of the DBS specimen type for a particular test. In the current MML test menu, molecular genetic testing for Gaucher disease is available for the sequencing assay, but not the mutation panel.
Several recurring questions arise when people are considering dried blood spot testing for the first time.

Does the blood spot need to dry before packaging the specimen for shipment?

Yes. If the specimens are not fully dry, the quality may be compromised. The collection cards are designed to absorb a standard blood volume. Once dry, the blood spots should be visually inspected to ensure the circles are filled, completely saturated, and that the appearance is consistent on both sides of the card. Similarly, layering of blood, or multiple applications of blood to the same circle on the collection card impact the quality and are unacceptable. Accuracy of the test results is dependent upon the quality of the dried blood spot specimen.
Frequently Asked Questions

- Can dried blood spot testing be performed on adults?
  - Yes, for most tests
  - Some tests designed for patients of a specific age
  - Review specimen requirements and/or consult with MML

Can DBS testing be performed on adults?

With the majority of tests, age is not a factor and we would encourage people to use the dried blood spot assays. However, there are a few tests, such as the supplemental newborn screen specifically designed for asymptomatic newborns. This test should not be ordered on any individual who is clinically suspected of having a metabolic disorder included in this screen.
Frequently Asked Questions

- Can blood from a collection tube be spotted on the filter paper?
  - Test specific
  - Dependent upon validation studies for each individual tube type
  - Review specimen requirements and/or consult with MML
  - Note tube type, if applicable

Can blood from a collection tube be spotted on the filter paper?

Again, this is test dependent and is specifically related to whether validation studies were performed. As previously mentioned, it’s important to confirm either by consulting the specimen requirements or MML to ensure the appropriate sample is submitted for analysis. Similarly, if a DBS specimen has been created from blood in a collection tube, this should be noted so that appropriate determinations may be made regarding potential test add-on requests.
What collection cards can be used to submit dried blood spots?

MML supplies clients with the Blood Spot Collection Card, which can be obtained by ordering supply code T493. However, if a patient requires testing and there isn’t sufficient time to facilitate a request for the MML collection cards, there are alternatives that can be utilized. If these alternatives are not available either, your local newborn screening card may be used, but be aware that some newborn screening programs require that you pay for these collection cards in advance.
The clinical application of testing dried blood spot specimens will continue to expand. It’s important for clinicians and laboratorians to have a working understanding of the current testing options, their benefits and limitations, and how to best utilize available test menus in the care of their patients. While the benefits of DBS testing ultimately reduce costs and optimize patient care, one must be cognizant of how preanalytical factors such as proper specimen collection and handling impact both the quality and reliability of results.
Questions or requests…
Email to: MMLHotTopics@mayo.edu

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