Dried Blood Spot Specimens for Serological Testing of Rodents

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Abstract

The utility of dried blood spot (DBS) sampling technology for rodent colony health monitoring was investigated using a two-tiered approach. In the first approach, groups of ICR mice and SD rats were experimentally infected. Mice were inoculated with epizootic diarrhea of infant mice (EDIM) virus, mouse hepatitis virus (MHV), murine norovirus (MNV), mouse parvovirus (MPV) or Theiler's murine encephalomyelitis virus (TMEV). Rats were inoculated with Kilham's rat virus (KRV) or rat theilovirus (RTV). Paired DBS and serum specimens were collected at 2, 4, 6 and 12 weeks postinoculation. In the second approach, designed to evaluate real-world conditions, over 1,000 mouse and rat paired DBS and serum specimens were collected from collaborating North American institutions. DBS and serum specimens were evaluated by multiplex fluorescent immunoassay (MFI) against a comprehensive panel of infectious disease assays, and data was compared for magnitude of MFI signal, signal-to-noise ratio, correlation and stability. Results from these studies demonstrated that the magnitude of the mean positive MFI signal from the DBS specimens was equal to or higher than the corresponding serum specimens. Moreover, the assay signal-to-noise ratio from the DBS specimens was equal to or higher than the corresponding serum specimens. Evaluation of a large cohort of paired clinical DBS and serum specimens showed 100% diagnostic correlation and improved specificity. Finally, stability studies showed no degradation of MFI signal when DBS specimens were protected from humidity and stored at room temperature or 4°C for up to 14 days or when stored at –20°C for up to 3 months, compared to controls. A moderate decrease in MFI signal was encountered when specimens were stored at 37°C in 95% humidity for 24 hours; however, all specimens remained diagnostically positive.

Introduction

Over the past five decades, great strides have been made in the identification and eradication of infectious agents from laboratory rodents. As a result, most contemporary biomedical research rodent colonies are relatively free of the pathogenic viruses, parasites, bacteria and fungi that cause clinical disease. However, some microbes, especially those agents that cause subclinical disease, remain in an enzootic state in many research colonies. These agents, despite their insidious nature, have an impact on physiologic parameters of the host and thus on the results of animal experiments, independent of their pathogenic potential.

Timely and accurate diagnosis of infectious disease in rodent colonies is critical to the success of biomedical research. To this end, institutional veterinarians closely monitor the health of research animals through periodic systematic examination of specimen groups of research and sentinel animals against a predetermined list of infectious agents. Serological testing for the detection of antibodies to infectious agents is an important component of a comprehensive rodent health monitoring program. Serological evidence of naturally acquired infection is typically detectable within 2 weeks postinfection and a measurable antibody titer persists for many months after initial infection. Accordingly, serological health monitoring provides a means for detection of both recent and past infectious disease exposure. The ability to detect past infections by serology dramatically increases the window of detection for infectious agents that can be cleared by the host, such as viruses, which can therefore easily go undetected by PCR evaluation alone.

Serological testing is a critical component of a comprehensive rodent health monitoring program and has benefited significantly from recent technological advancements in serological testing platforms. One such advancement is the microsphere-based multiplex system that requires as little as 1 µL of undiluted serum for evaluation against a comprehensive panel of infectious agents. Despite the improvements in testing technology, there has been little change in blood specimen collection techniques. Conventional blood specimen collection from rodents is accomplished by cardiocentesis or superficial venipuncture and requires a relatively large volume of blood because of the logistical constraints associated with downstream processing, preparation and transport of serum; however, most of the serum specimen goes unused by the testing laboratory. Dried blood spot sampling technology offers a mechanism to harmonize blood specimen collection and specimen volume testing requirements.

Dried blood spot (DBS) sampling technology involves the collection of a single drop (~25 µL) of whole blood onto filter paper. The DBS specimen, once dry, is very stable and amenable to a variety of quantitative and qualitative analyses.[1] DBS sampling technology was first introduced in 1963 as a means to measure phenylalanine levels for the diagnosis of phenylketonuria in newborns.[2] Since that time, DBS sampling technology has been used as a sampling technique for newborn metabolic disease screening, molecular testing for infectious disease diagnosis and therapeutic drug monitoring.[3,4] Furthermore, pharmaceutical

companies have recently adopted DBS sampling methods for preclinical pharmacokinetic and toxicokinetic testing in rodents in order to improve animal welfare, drastically reduce animal numbers, improve study precision and reduce costs.

Study Design

The utility of DBS sampling technology for rodent colony health monitoring was investigated using a two-tiered approach. To this end, 6 groups of 20 ICR mice each were inoculated by gavage or dirty bedding transfer with epizootic diarrhea of infant mice (EDIM) virus, mouse hepatitis virus (MHV), murine norovirus (MNV), mouse parvovirus (MPV), Theiler's murine encephalomyelitis virus (TMEV) or sham. In addition, 3 groups of 8 SD rats each were inoculated by gavage or dirty bedding transfer with Kilham's rat virus (KRV), rat theilovirus (RTV) or sham. Serial paired DBS and serum specimens were collected at 2, 4, 6 and 12 weeks postinoculation. Further, over 1,000 mouse and rat paired DBS and serum specimens were collected from collaborating North American institutions. Paired DBS and serum specimens were evaluated by multiplex fluorescent immunoassay (MFI) against a comprehensive panel of infectious disease assays, and data was compared for magnitude of MFI signal, signal-to-noise ratio, diagnostic correlation and stability.

Results

Experimental Infection of Mice (Fig. 1)—The results from this study demonstrated that seroconversion to EDIM, MHV, MNV, MPV and TMEV occurred by week 2 postinfection, and agent-specific antibody titers remained elevated throughout the duration of the study. Mice developed antibody exclusively to the specific infectious agent with which they were inoculated, and there was no antibody cross-reactivity to any of the other 27 assays. The magnitude of the mean positive MFI signal from the DBS specimens was equal to or higher than the corresponding serum specimens. Moreover, the assay signal-to-noise ratio from the DBS specimens was equal to or higher than the corresponding serum specimens.

Experimental Infection of Rats (Fig. 2)—The results from this study demonstrated that seroconversion to KRV and RTV occurred by week 2 postinfection, and agent-specific antibody titers remained elevated throughout the duration of the study. Rats developed antibody exclusively to the specific infectious agent with which they were inoculated, and there was no antibody cross-reactivity to any of the other 21 assays in the panel of tests. The magnitude of the mean positive MFI signal from the DBS specimens was equal to or higher than the corresponding serum specimens. Moreover, the assay signal-to-noise ratio from the DBS specimens was equal to or higher than the corresponding serum specimens.

Collaborative Studies (Fig. 3)—To evaluate the clinical utility of DBS sampling technology, over 1,000 mouse and rat paired DBS and serum specimens were collected from collaborating North American institutions. Paired specimens were evaluated by MFI against a comprehensive panel of infectious disease assays, and data was compared for magnitude of MFI signal and diagnostic correlation. Antibodies to MNV, MHV and MPV were detected by MFI in a subset of the paired serum and DBS specimens, and positive MHV and MPV results acquired by MFI were confirmed by immunofluorescence assay (IFA) of the DBS specimen. The magnitude of the MFI signal from all positive DBS specimens was equal to or higher than the corresponding serum specimen. Moreover, the paired clinical DBS and serum specimens showed 100% diagnostic correlation.

Stability Studies (Fig. 4)—The stability of DBS specimens was evaluated using whole blood collected from known antibody-positive mice. To simulate short- and long-term storage conditions, whole blood (25 μL) was deposited on multiple DBS strips, and specimens were stored in sealed plastic bags with desiccant at –20°C, 4°C or room temperature (~21°C). Further, to simulate the environmental extremes that specimens may encounter during shipment, DBS specimens were stored at 37°C in 0% or 95% humidity for 24 hours. Specimens were removed from storage at various time points and evaluated by MFI against a comprehensive panel of infectious disease assays, and data was compared for magnitude of MFI signal.

The stability studies demonstrated no degradation of MFI signal when DBS specimens were protected from humidity and stored at room temperature or 4°C for up to 14 days or when stored at –20°C for up to 3 months, compared to controls. DBS specimens stored at 37°C and 0% humidity for 24 hours showed a modest decrease in mean MFI signal compared to DBS specimens stored at room temperature (37°C, 0% humidity: 15,420.11 ± 5,055.55; room temperature: 20,016.83 ± 3,989.82). DBS specimens stored for 24 hours at 37°C and 95% humidity showed a more marked decrease in mean MFI signal compared to control specimens (9,574.78 ± 3,678.96); however, all specimens remained diagnostically positive.

Conclusion

Dried blood spot sampling technology requires only a single drop (~25 μL) of whole blood, making antemortem sampling easily achievable and potentially reducing the number of mice used for sentinel monitoring. Unlike traditional serum, DBS specimens require no further processing before shipment to the testing laboratory, saving the investigator time and costs associated with specimen processing. DBS specimens are stable at room temperature for up to 14 days when protected from humidity; thus, allowing for shipment in a standard overnight envelope and avoiding additional shipping costs associated with refrigerated shipping boxes.

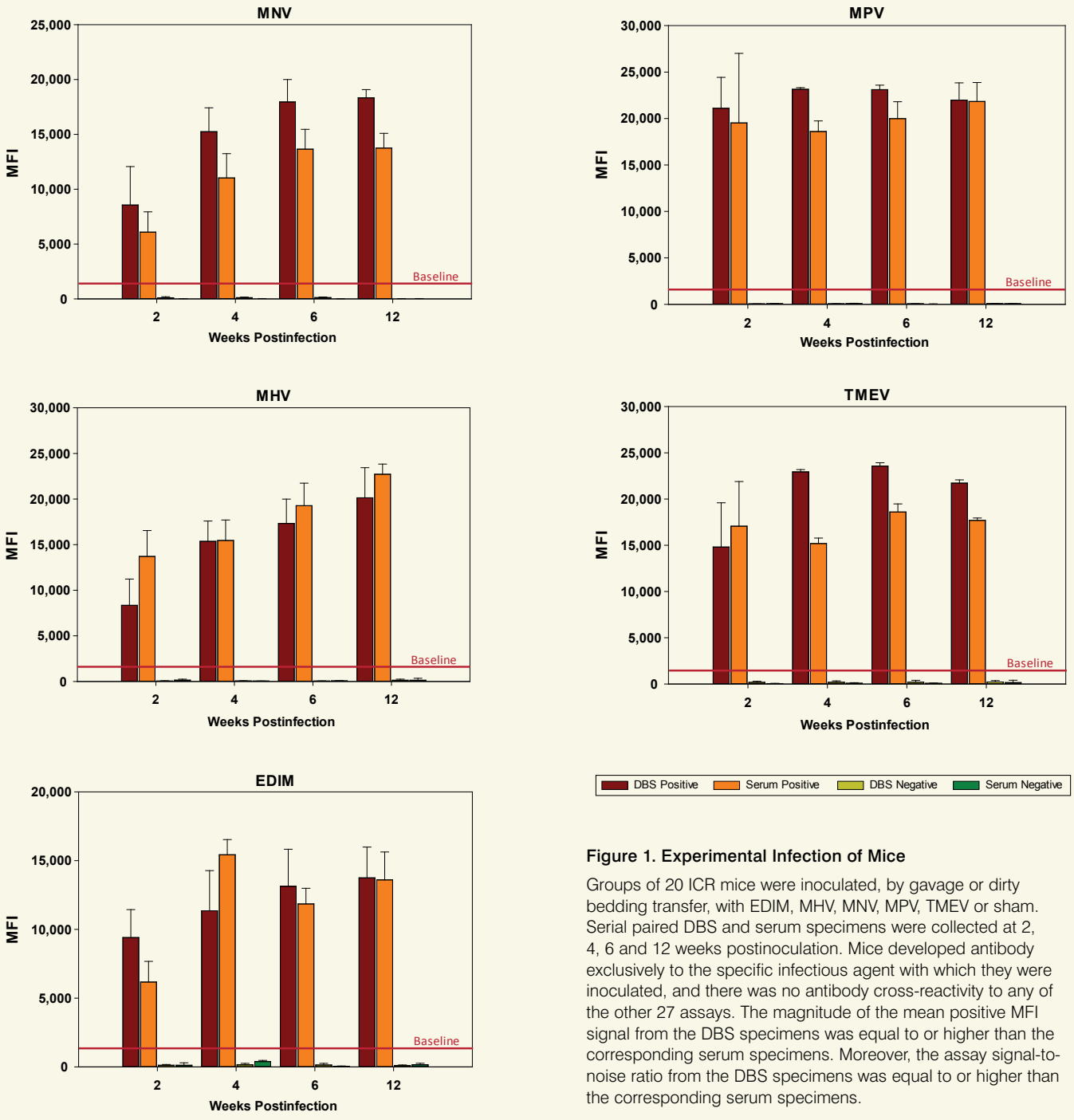

Figure 1. Experimental Infection of Mice

Groups of 20 ICR mice were inoculated, by gavage or dirty bedding transfer, with EDIM, MHV, MNV, MPV, TMEV or sham. Serial paired DBS and serum specimens were collected at 2, 4, 6 and 12 weeks postinoculation. Mice developed antibody exclusively to the specific infectious agent with which they were inoculated, and there was no antibody cross-reactivity to any of the other 27 assays. The magnitude of the mean positive MFI signal from the DBS specimens was equal to or higher than the corresponding serum specimens. Moreover, the assay signal-to-noise ratio from the DBS specimens was equal to or higher than the corresponding serum specimens.

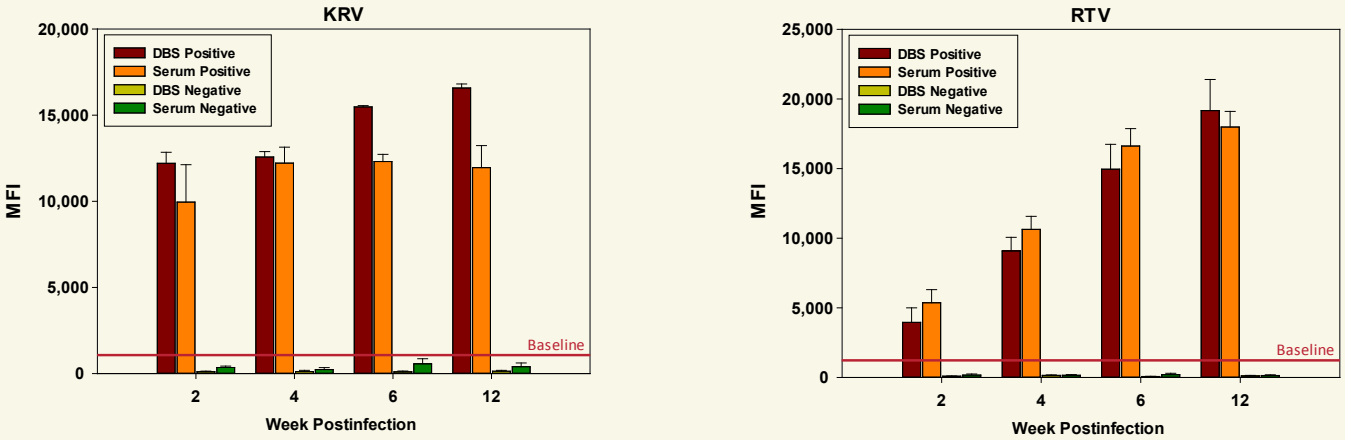

Figure 2. Experimental Infection of Rats

Groups of 8 SD rats were inoculated, by gavage or dirty bedding transfer, with KRV, RTV or sham. Serial paired DBS and serum specimens were collected at 2, 4, 6 and 12 weeks postinoculation. Paired specimens were evaluated by MFI against a comprehensive panel of infectious disease assays, and data was compared for magnitude of MFI signal and signal-to-noise ratio. The results from this study demonstrated that seroconversion to KRV and RTV occurred by week 2 postinfection, and agent-specific antibody titers remained elevated throughout the duration of the study. Rats developed antibody exclusively to the specific infectious agent with which they were inoculated, and there was no antibody cross-reactivity to any of the other 21 assays in the panel of tests. The magnitude of the mean positive MFI signal from the DBS specimens was equal to or higher than the corresponding serum specimens. Moreover, the assay signal-to-noise ratio from the DBS specimens was equal to or higher than the corresponding serum specimens.

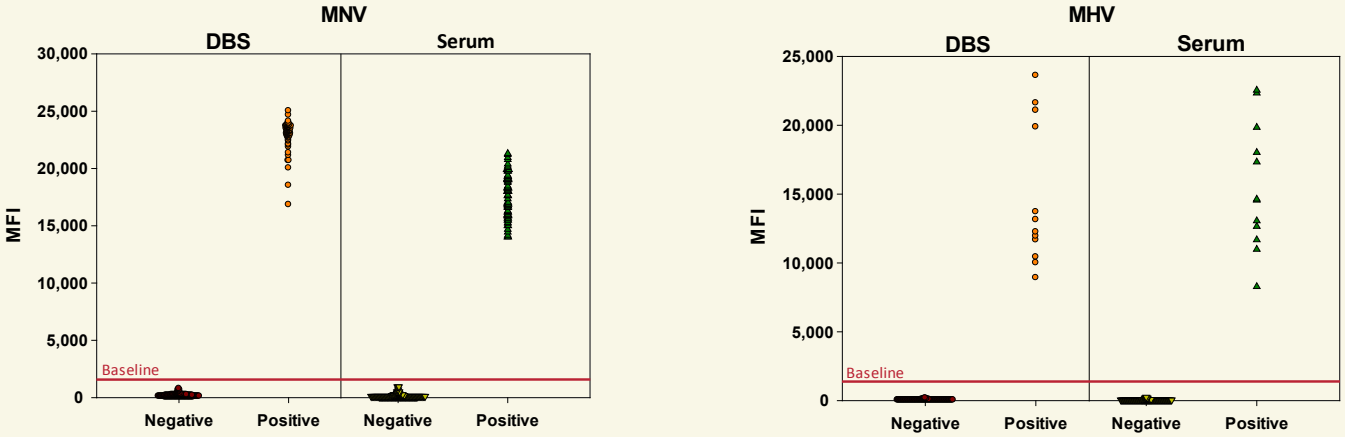

Figure 3. Collaborative Studies

To evaluate the clinical utility of DBS sampling technology, over 1,000 mouse and rat paired DBS and serum specimens were collected from collaborating North American institutions. Paired specimens were evaluated by MFI against a comprehensive panel of infectious disease assays and data were compared for magnitude of MFI signal and diagnostic correlation. Antibodies to MNV, MHV and MPV were detected by MFI in a subset of the paired serum and DBS specimens, and positive MHV and MPV were results acquired by MFI were confirmed by immunofluorescence assay (IFA) of the DBS specimen. Paired clinical DBS and serum specimens showed 100% diagnostic correlation.

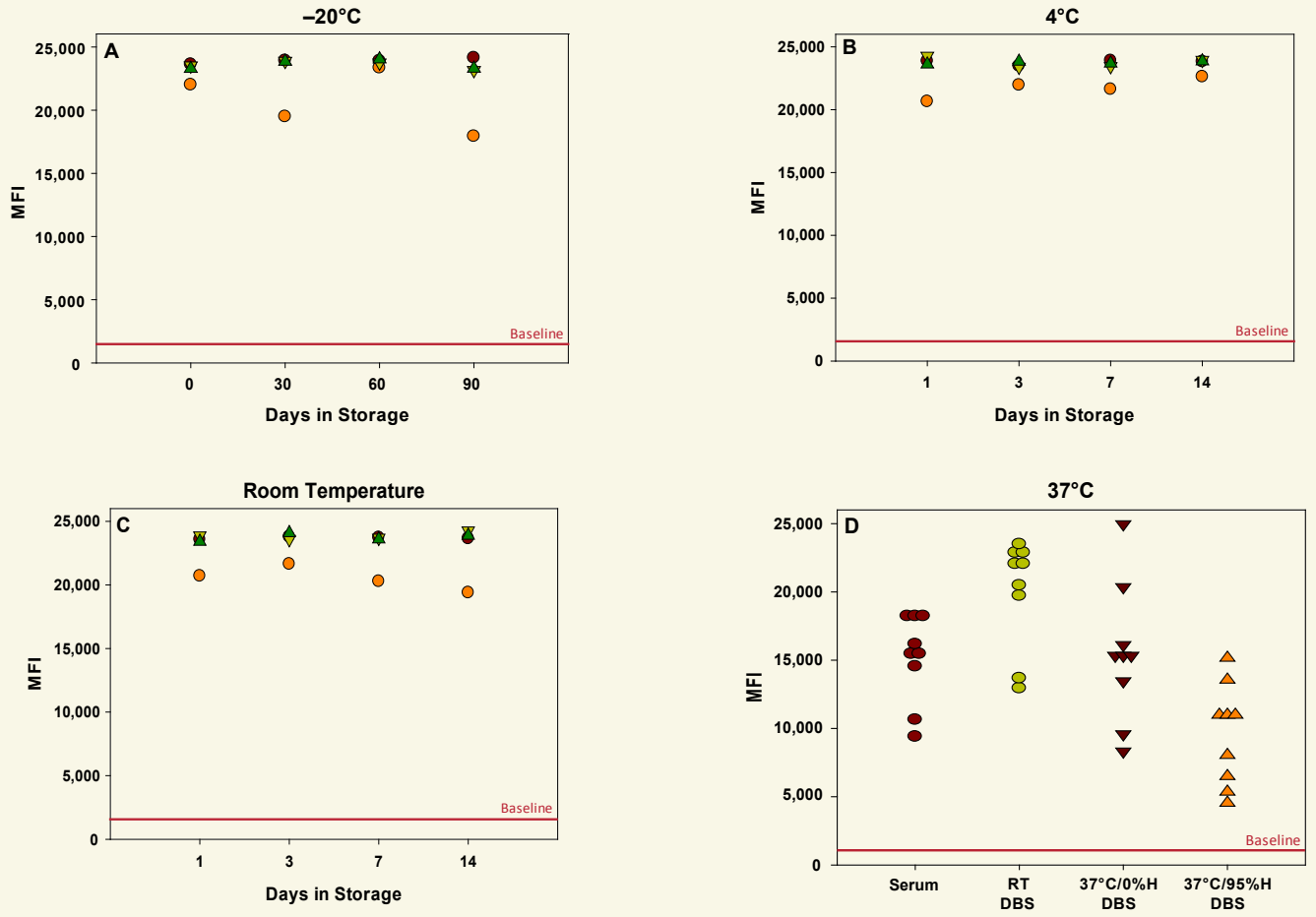

Figure 4. Stability Studies

The stability of DBS specimens was evaluated using whole blood collected from known antibody positive mice. To simulate short- and long-term storage conditions, whole blood (25 µL) was deposited on multiple DBS strips, and specimens were stored in sealed plastic bags with desiccant at (A) –20°C, (B) 4°C or (C) room temperature (~21°C). Further, to simulate the environmental extremes that specimens may encounter during shipment, DBS specimens were stored at (D) 37°C in 0% or 95% humidity for 24 hours. Specimens were removed from storage at various time points and evaluated by MFI against a comprehensive panel of infectious disease assays, and data was compared for magnitude of MFI signal. The stability studies demonstrated no degradation of MFI signal when DBS specimens were protected from humidity and stored at room temperature or 4°C for up to 14 days or when stored at –20°C for up to 3 months, compared to controls. DBS specimens stored at 37°C and 0% humidity for 24 hours showed a modest decrease in mean MFI signal compared to DBS specimens stored at room temperature (37°C, 0% humidity: 15,420.11 ± 5,055.55; room temperature: 20,016.83 ± 3,989.82). DBS specimens stored for 24 hours at 37°C and 95% humidity showed a more marked decrease in mean MFI signal compared to control specimens (9,574.78 ± 3,678.96); however, all specimens remained diagnostically positive.

References

1. Adam BW, Hall EM, Sternberg M, et al. The stability of markers in dried-blood spots for recommended newborn screening disorders in the United States. *Clin Biochem.* 2011;44(17–18):1445–1450.
2. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics.* 1963;32(3):338–343.
3. Dowd JB, Aiello AE, Chyu L, Huang YY, McDade TW. Cytomegalovirus antibodies in dried blood spots: a minimally invasive method for assessing stress, immune function, and aging. *Immun Ageing.* 2011;8:3. www.immunityageing.com /content/8/1/3. Published January 13, 2011. Accessed December 6, 2013.
4. Corran PH, Cook J, Lynch C, et al. Dried blood spots as a source of anti-malarial antibodies for epidemiological studies. *Malar J.* 2008;7:195. www.malariajournal .com/content/7/1/195. Published September 30, 2008. Accessed December 6, 2013.

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