



Epstein-Barr Virus (EBV)

Blood Spot Measurement of Epstein-Barr Virus (EBV) Antibody Titers in Wave I of the National Social Life Health & Aging Project

Authors:

Andreea Mihai, University of Chicago, Department of Ob/Gyn

Thomas McDade, Northwestern University, Departments of Anthropology and Cells to Society (C2S)

Sharon Williams, Purdue University, Department of Anthropology

Stacy Tessler Lindau, MD, MAPP, University of Chicago, Departments of Ob/Gyn and Medicine – Geriatrics, Chicago Core on Biomeasures in Population-Based Health and Aging Research at NORC and the University of Chicago Center on Demography and Economics of Aging*

* Corresponding author.

E-mail address: slindau@uchicago.edu (S.T. Lindau).

Fax: +1 773 834 5664.

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Rationale

Epstein-Barr Virus (EBV) is a member of the herpes virus family, and it typically infects B-lymphocytes(Crawford 2001). It is usually latently expressed(Klein, Kis et al. 2007).

EBV infects about 90% of the world's population(Macsween and Crawford 2003). Infection is lifelong(Callan 2004). While most individuals remain asymptomatic, EBV has been known to contribute to several diseases, including infectious mononucleosis and Burkitt's lymphoma(Macsween and Crawford 2003).

Elevated levels of EBV antibodies are indicative of lower cell-mediated immune function(Glaser, Kiecoltglaser et al. 1985), which has been linked to psychosocial stress(Kiecoltglaser, Garner et al. 1984; Glaser, Kiecoltglaser et al. 1985). Lower cell-mediated immune function increases an individual's risk for B-cell lymphoproliferative disease(Crawford 2001). Additionally, those who test negative for EBV antibodies are at an increased risk for severe EBV-associated diseases if they become infected in later life(Crawford 2001). Compromised immunity due to any factor (including immunosenescence)(Srinivasan, Maestroni et al. 2005) also increases the risk for pathological EBV infection(Bennett 2007).

NSHAP's in-home biological data collection design was based on the principles of minimal invasiveness(Lindau and McDade 2007). Specimens for EBV antibody titer assays were collected in the form of dried blood spots; this is a minimally invasive process that can be safely carried out in the home by a trained lay interviewer(McDade, Williams et al. 2007). This method has been successfully implemented in a variety of studies in the US in addition to NSHAP (e.g. the Health and Retirement Study and the National Longitudinal Study of Adolescent Health) and internationally, including lowland Bolivia, Samoa, Kenya, Papua New Guinea, and Nepal(Worthman and Stallings 1997; McDade, Stallings et al. 2000b; Panter-Brick, Lunn et al. 2001; Shell-Duncan and McDade 2004; McDade, Leonard et al. 2005).

Measurement

The standard assay for clinical EBV detection involves indirect immunofluorescence using blood plasma(McDade, Stallings et al. 2000a). In NSHAP, blood was collected by finger stick using a disposable lancet and then captured on filter paper for transport and storage. (Details about collection equipment are described in the Availability section, below.) To determine EBV IgG antibody titers, a measure of EBV infection(McDade, Stallings et al. 2000a), the ELISA (enzyme-linked immunosorbent assay) assay was used on a 3.2 or 6.0 mm sample from the filter paper blood spot specimen. Previous research has established that the ELISA assay produces comparable results with indirect immunofluorescence, with a strong positive correlation between the two

(Pearson $R = 0.77$; $p < 0.01$)(McDade, Stallings et al. 2000a). The correlation between EBV antibody titers obtained from blood plasma and blood spots is also high ($R = 0.936$)(McDade, Stallings et al. 2000a).

The ELISA assay measures IgG antibodies against the p18 viral capsid antigen (VCA) complex. The dried blood spot protocol is an adaptation of a commercially available kit designed for use with serum or plasma (DiaSorin Corporation, Stillwater, MN, product no. P001606A)(McDade, Stallings et al. 2000a).

Population Norms

Early exposure to the virus is more prevalent in developing countries, where the rate of infection approaches 100% during the first 5 years of life(McDade, Stallings et al. 2000b). Related pathology is less likely to occur if a person is exposed to the virus early(Crawford 2001).

In industrialized nations, 25-50% of adolescents are infected with EBV by the age of 18. By the age of 40, 80-90% of adults are infected(Jones and Strauss 1987).

Each individual's viral load remains fairly constant over time (about 5-500 virus infected cells in every 10×10^6 circulating B cells)(Crawford 2001). Qualitative descriptions (low level of EBV antibodies, high level, etc) are matched with their quantitative measurements in Table 1. Because older adults are relatively immunosuppressed, their EBV antibody levels are expected to be in the mid-high to high range.

Most research on EBV is based on populations with EBV-linked pathology. Normative data on EBV antibody titers have been described among younger adults under specialized conditions – ie, geographical location or infection of certain diseases(McDade, Stallings et al. 2000a; McDade, Stallings et al. 2000b; Shimoyama, Yamamoto et al. 2008). However, because both EBV-specific assays and data interpretations are not standardized, comparisons across studies should be interpreted cautiously(Hess 2004). Higher titers are associated with lower cell-mediated immune function; lower titers are associated with higher cell-mediated immune function (Glaser, Kiecoltglaser et al. 1985). Normative data on older adults without EBV-linked pathology, to the best of our knowledge, have not been previously generated.

Specimen Collection

The blood spot module was randomized to 5/6 of the total NSHAP sample (N=2494), of these, 84.5% (2,105) participated. There were no significant differences between those who agreed to provide blood spots and those who did not with respect to gender, race, ethnicity, age, education, income level, marital status, self-reported mental or physical health, or the reported number of doctor's visits. Due to collection difficulties, blood spots were not collected from an additional 57 individuals who originally agreed to participate. In total, dried blood spots were collected from 2,048 individuals. From these, 1981 EBV values were obtained.

Blood was obtained from free-flowing capillary blood via a single fingerstick using a retractable-tip, single-use disposable lancet. NSHAP acquired two lancets (model details in Product Availability section below). The Surgilance lancet was used routinely, contained a 2.3 mm blade and was slightly less penetrative. The BD lancet, which contains a slightly larger blade and is commonly used for newborn heel-sticks, was used for cases of thick calluses or where the fingerstick specimen was difficult to obtain with the Surgilance. The blood was allowed to pool on the respondents' finger and four drops of blood were dropped onto filter paper, the first drop being reserved for future genetic analysis. Overall, the procedure required approximately 8 minutes.

Interviewer instructions

- Angle Respondent's hand below their lap.
- Warm finger and stimulate circulation by gently kneading and squeezing the appropriate finger.
- Ask Respondent to gently shake their hand a few times.
- Wipe the index finger of the right hand with alcohol swab and wait a few seconds for the alcohol to dry (DO NOT blow on finger, wave hand, etc. to speed up drying).
- Squeeze the finger just below the area to be pricked.
- Firmly prick finger in the fleshy part of the pad, just off the center.
- IMMEDIATELY dispose of the lancet into the sharps container.
- Allow blood to well on tip of finger.
- If necessary, apply gentle pressure below the site of the prick
- Place first drop in discard circle of filter paper – marked D
- Place 3 (if possible) additional drops on filter paper.
- If unable to fill 3 spots (+ discard spot): Prick another finger. Place first drop in discard circle of filter paper (marked D) and place additional drop(s) in remaining circles.
- If necessary: Ask Respondent to hold cotton ball on finger and apply pressure until bleeding stops.
- Offer Respondent a bandage.
- Label filter paper with su_id.
- Fill out blood spot collection form.
- Place filter paper in plastic bag with desiccant and seal.
- Store at 4°C until shipment.

The filter paper was allowed to dry for the remainder of the interview before being placed in a plastic bag with desiccant for transportation.

Storage and Shipping

After the interview, the filter paper was placed in a Ziploc bag. Once the field investigator reached home, the filter paper was removed from the Ziploc bag and placed in a clear plastic container with the filter paper cover flipped up. A desiccant pack was placed in the container which was then sealed overnight and left at room temperature to ensure drying of the blood spots. In the morning, the filter paper was stored in a sealed Ziploc bag along with the desiccant pack and placed in a storage container. The storage container was kept in the refrigerator at 4°C until shipping day. Blood spots were shipped at room temperature to the designated location. Upon arrival at the laboratory,

the specimens were catalogued, analyzed for quality and quantity, and stored at -25°C until analysis.

Shipping Address	Thomas McDade, PhD Northwestern University Department of Anthropology Laboratory for Human Biology Research 1810 Hinman Ave. Evanston, IL 60208
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Assay

On day 1, samples were removed from the freezer and one 3.2 mm punch was removed from each sample and placed in buffer to elute overnight. On day 2, eluate was transferred to a 96 well plate for quantification of EBV antibodies using an ELISA protocol previously validated for use with dried blood spot samples (McDade et al. 2000). All analyses were conducted at the Laboratory for Human Biology Research at Northwestern University (Evanston, IL).

Performance Characteristics

A. Precision

Table 1. EBV p18-VCA Antibody Assay Precision (Within-Assay Coefficient of Variation) and Reliability (Between-Assay Coefficient of Variation)^a

Control Level	Within-Assay (N=10 Determinations)		Between-Assay (N=12 Runs)	
	Mean ± SD	% CV	Mean ± SD	% CV
Low	29.4 ± 2.7	9.2	30.6 ± 4.3	14.1
Mid-low	111.4 ± 3.7	3.3	100.2 ± 5.0	5.0
Mid-high	212.1 ± 11.1	5.3	206.3 ± 8.8	4.3
High	271.7 ± 12.1	4.5	278.3 ± 19.1	6.9

^aMean p18-VCA antibody titres are presented in standard ELISA scores.

Reproduced with permission from McDade, Stallings et al. 2000a

B. Sensitivity

Values below 20 ELISA units were assumed to be seronegative. For a discussion of the sensitivity of the blood spot based EBV assay, please see McDade, Stallings et al. 2000b.

C. Correlation with Plasma

The correlation between blood spots and plasma was pre-determined by assaying 40 matched samples. A drop from each whole blood sample (EDTA-anticoagulated) was sampled onto filter paper while the other portion was centrifuged in order to withdraw the plasma. Previous literature determined the correlation to be high with an r value of 0.936(McDade, Stallings et al. 2000a).

The correlation between the standard indirect immunofluorescence technique for EBV analysis and ELISA technique used in NSHAP was likewise high (r=0.77, p<0.01)(McDade, Stallings et al. 2000a). One hundred eleven assays were matched in the validation study.

Quality Control

Blood spots were obtained on a card containing five pre-printed circles of standard size (1/2 inch/12.7mm diameter). Excellent samples filled the entire circle. Good samples were large enough to provide sample for a large (6.0mm) hole punch. Poor samples were ones that did not provide enough for a single (3.2mm) hole punch. Cards were adequate if they allowed for one large and three small punches and excellent if they allowed for more. Throughout the study, the number, quality and condition of the blood spots was recorded by personnel, and if consistent problems were observed from a single field interviewer, they were contacted to discuss problems and techniques to improve the quality of blood spot collection(Williams and McDade 2007).

Four blood spot control samples were included with each assay to monitor between-assay variation.

Table 2. Between-Assay Variation in Blood Spot Control Samples

	1	2	3	4
average	25.53	118.60	237.30	286.70
SD	3.42	10.42	12.19	17.01
CV	0.134	0.088	0.051	0.059

Product Availability

Product Name	Epstein-Barr virus VCA IgG ELISA
Manufacturer	DiaSorin, Inc.
Location of Manufacturer	1951 Northwestern Avenue - P.O. Box 285 MN 55082-0285 Stillwater Tel: +1.651.439.9710 +1.800.328.1482 Fax: +1.651.351.5669 (USA & Canada only)
Catalogue No.	P001606A
Product Name	903™ Multiple-part Neonatal Card

Manufacturer	Schleicher & Schuell BioScience
Location of Manufacturer	Keene, NH 03431 Contact: Judy Peter 800- 437-7003
Catalogue No.	10 537 279
Product Name	SUD-CHEMIE Performance Packaging; SORB-IT Harmless Absorbant
Manufacturer	Süd-Chemie Performance Packaging
Location of Manufacturer	926 S. 8th St. PO Box 610 Colton, CA 92324 Tel: +1 909 825 1793 +1 800 966 1793 Fax: +1 909 825 6271
Catalogue No.	n/a
Product Name	SurgiLance Safety Lancet, 2.3 mm (blue)
Manufacturer	SurgiLance, Inc.
Location of Manufacturer	Norcross, GA Tel: +1 (770) 448 9493 (US) Fax: + 1 (877) 804 5240 (Toll Free) (US)
Catalogue No.	SLB250
Product Name	BD Quikheel™ Premie Lancet (pink)
Manufacturer	Becton, Dickinson and Company
Location of Manufacturer	Franklin Lakes, NJ
Catalogue No.	368100

Sources

Procedure developed December 2004 from recommendations by Stacy T. Lindau, University of Chicago, Thomas McDade, Northwestern University and Sharon Williams, Northwestern University. Assay developed by Thomas McDade at Northwestern University Laboratory for Human Biology Research (currently unpublished).

Scoring & Usage

Values are reported in enzyme-linked immunosorbent assay (ELISA) units. Here, they are a measure of the amount of p18-VCA in the blood spot (McDade, Stallings et al. 2000a).

If the sample has less than 20 ELISA units, the subject is seronegative. If the sample has more than 20 ELISA units, the subject is seropositive. The recode category is positive/negative (McDade, Stallings et al. 2000a).

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