### Culture Change and Stress in Western Samoan Youth: Methodological Issues in the Cross-Cultural Study of Stress and Immune Function<sup>†</sup>

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ABSTRACTThis study was designed to pursue three objectives: 1) investigate the impact of culture change on children and adolescents in Western Samoa; 2) introduce a cross-cultural perspective to studies of psychosocial stress and immune function; and 3) evaluate the utility of minimally invasive methods for assessing immune function. Seven hundred sixty individuals between the ages of 4 and 20 years were recruited from three distinct geographic regions within Western Samoa that differ in degree of westernization. Finger prick samples of whole blood were collected from each individual and analyzed for antibodies against the Epstein-Barr virus (EBV; an indirect marker of cell-mediated immune function) and C-reactive protein (a nonspecific marker of current infection). After controlling for age, sex, and current infection, EBV antibody levels were significantly elevated in urban Apia and rural Upolu, indicating lower levels of cell-mediated immune function. The results suggest a higher degree of psychosocial stress in these regions, possibly due to exposure to westernizing influences. Am. J. Hum. Biol. 12:792– 802, 2000. © 2000 Wiley-Liss, Inc.

EB virus: ubiquitous herpersvirus that infect 80-90% of adults by the age of 40 in industrialized countries, while 100% by the age of 5 in developing countries. Despite the virus is asymptomatic, adequate cell-mediated immune function is critical for maintaining the virus in a latent state.

Immunosuppression lead to the increase of EBV antibodies

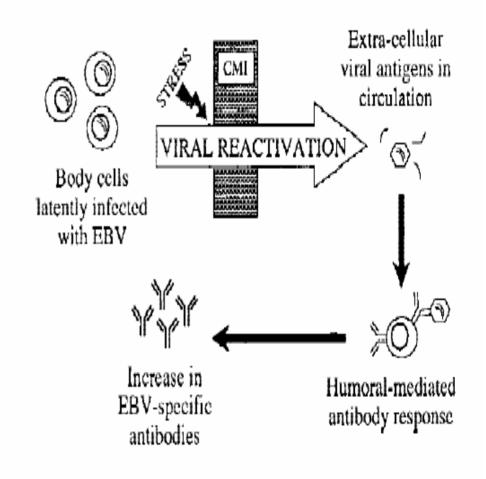


Fig. 1. Relationship between stress, cell-mediated immunity (CMI), and EBV antibody level.

TABLE 1. Age and sex distribution of the sample by region

Age (years)	Apia		Upolu			Savaii			Total	
	Female	Male	Total	Female	Male	Total	Female	Male	Total	by age
4-6	26	29	55	28	27	55	28	27	55	165
7-9	30	30	60	36	32	68	26	24	50	178
10-12	28	26	54	26	20	46	34	29	63	163
13-15	29	18	47	34	18	52	17	12	29	128
16-20	23	18	41	24	25	49	19	17	36	126
	136	121	257	148	122	270	124	109	233	
	Total fema	les: 413			Total m	ales: 357		Tot	tal sample	: 760

TABLE 2. Markers of lifestyle in Apia, Upolu, and Savaii

	Apia	Upolu	Savaii
Television			
Yes	76.6	57.1	$53.6^{\mathrm{a}}$
No	23.4	42.9	46.6
Car			
Yes	37.8	33.0	$21.6^{\mathrm{b}}$
No	62.2	67.0	78.4
Travel outside W. Samoa			
Yes	34.1	15.0	$7.8^{\mathrm{a}}$
No	65.9	85.0	92.2
Type of house			
European	57.6	40.9	$40.5^{ m b}$
Traditional	42.4	59.1	59.5
Father occupation			
Professional	26.7	6.3	$2.4^{\rm a}$
Unskilled labor	41.0	3.5	3.2
Planter	32.4	90.1	94.5
Mother occupation			
Professional	8.1	0.0	$0.9^{ m b}$
Unskilled labor	13.0	1.4	0.0
Housewife	78.9	98.6	99.2

 $<sup>^{\</sup>mathrm{a}}P < 0.01$  for  $\chi\text{-square statistic.}$   $^{\mathrm{b}}P < 0.05.$ 

TABLE 3. Anthropometric dimensions by region and sex (mean (SD))\*

	Apia		Up	oolu	Savaii	
	Female	Male	Female	Male	Female	Male
HAZ	0.11 (0.99)	-0.21 (1.04)	0.10(1.03)	0.03 (1.13)	-0.07 (1.02)	-0.03 (1.23
WAZ	0.32(0.92)	0.08 (1.12)	0.36(0.99)	0.22(1.01)	0.19(0.99)	0.08(1.02)
WHZ	0.16(0.70)	0.26(0.93)	0.25(0.74)	0.30(0.69)	0.19(0.87)	0.16(0.71)
Triceps (mm)	12.4 (5.8)	9.2 (4.0)	12.6 (5.6)	8.7 (3.8)	12.1 (5.0)	8.2 (2.0)
Subscap (mm)	10.4 (4.6)	8.0 (3.7)	11.1 (5.9)	8.0 (4.4)	11.2 (5.4)	7.3(2.4)

<sup>\*</sup>Height-for-age (HAZ), weight-for-age (WAZ), and weight-for-height (WHZ) z-scores are based on NCHS reference values.

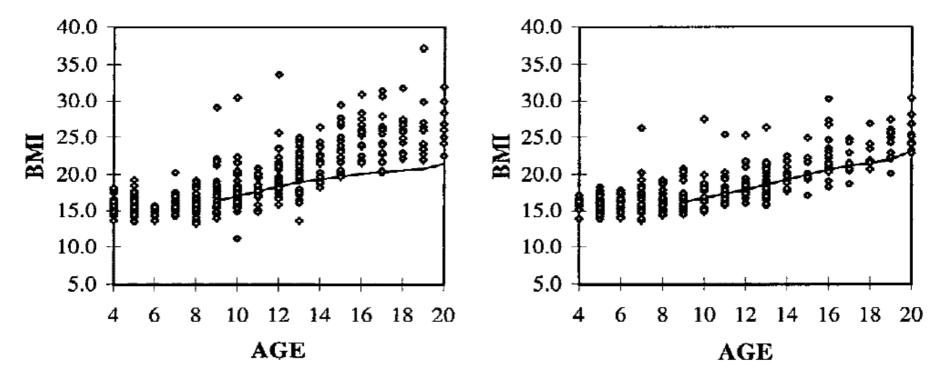


Fig. 2. Body mass index by age: girls. The NHANES reference median is indicated by the superimposed line.

Fig. 3. Body mass index by age: boys. The NHANES reference median is indicated by the superimposed line.

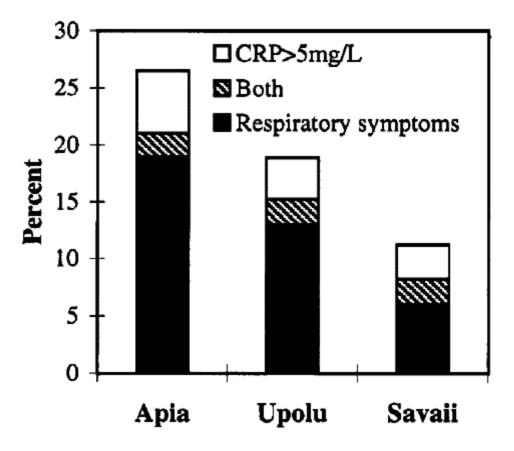


Fig. 4. Regional differences in the prevalence of current infection, as defined by the presence of respiratory symptoms and/or elevated CRP.

TABLE 4. Linear model results investigating the effects of age, sex, and region on log-transformed EBV antibody levels

	df	MS	$\mathbf{F}$	P
Model	4	0.559	6.32	0.0001
Sex	1	0.376	4.26	0.040
$_{ m Age}$	1	0.119	1.35	0.25
Region	2	0.804	9.09	0.0001

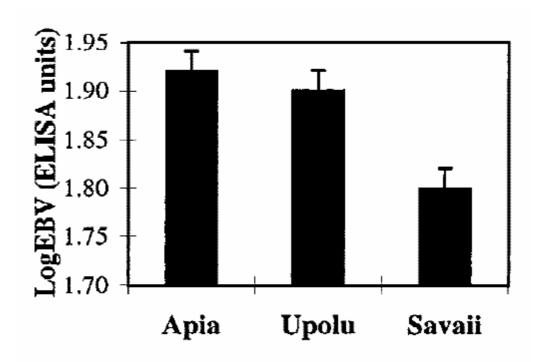


Fig. 5. EBV antibody level, by region (mean + SE).

# Lifestyle incongruity, social integration, and immune function in Samoan adolescents

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#### Abstract

The health consequences of rapid cultural and economic change have been explored for adults in a range of low-income countries, but comparable research in children and adolescents is currently lacking. Concurrently, the immunosuppressive effects of psychosocial stress have been documented in Western populations, but have yet to be considered in cross-cultural contexts. This study uses lifestyle incongruity (inconsistency between a household's material style of life and its socioeconomic status) as a model of culture change and stress, and considers its impact on immune function in a sample of 230 10–20 year-olds from (Western) Samoa. Anthropometric, lifestyle, and psychosocial data were collected, as well as finger prick blood spot samples for analysis of C-reactive protein (marker of infection) and antibodies against the Epstein–Barr virus (marker of cell-mediated immune function). Controlling for potential confounders, adolescents from households with a material style of life that exceeds its socioeconomic status have reduced cell-mediated immune function, indicating an increased burden of psychosocial stress. Social relationships moderate this effect: lifestyle incongruity stress is pronounced among adolescents with a high degree of social integration, and absent in adolescents with low social integration. This finding is counter to the buffering role of social support reported in previous applications of lifestyle incongruity to adults, and suggests that the moderating role of social integration may be unique to adolescents. The potential utility of the lifestyle incongruity model for future cross-cultural studies of child and adolescent stress is discussed. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Status inconsistency; Westernization; Psychoneuroimmunology; Adolescence; South Pacific

Table 1 Distribution of participants across region, as well as mean (standard deviation) values and ranges of selected variables

	Total sample	Apia	Rural Upolu	Savaii
N	230	77	104	49
Sex (% female)	57.4	61.0	58.7	49.0
Age (years)	13.8 (3.0) 10-20	13.9 (2.8) 10-20	14.0 (2.8) 10-20	13.4 (3.5) 10-20
BMI $(kg/m^2)$	20.8 (3.8) 11.2-33.6	21.0 (3.9) 15.5-31.8	21.0 (3.7) 14.8-33.6	20.0 (3.9) 11.2-28.4
SSF (mm)	23.0 (10.1) 11.0-68.7	23.5 (11.4) 12.6-68.7	23.0 (9.7) 11.0-64.0	22.2 (9.0) 11.7-51.5
Socioeconomic status	4.1 (1.4) 2.0-9.0	4.9 (1.4) 2.0-9.0	3.9 (1.1) 2.0-7.0	3.1 (1.0) 2.0-6.0
Material lifestyle	10.3 (3.9) 3.0-17.0	11.6 (4.0) 3.0-17.0	9.7 (3.5) 3.0-17.0	9.6 (4.3) 4.0-17.0
EBV antibody level (ELISA units)	101.0 (71.0) 20.5–301.6	116.2 (79.1) 21.2–301.6	102.6 (66.5) 21.9–266.1	73.7 (59.4) 20.5–289.4

Table 2 Material style of life scale<sup>a</sup>

Variable	Item-total correlation
Type of house (traditional = 1; European = 2)	0.61
Type of floor (soil, stone, coral=0;	0.58
cement = 1; linoleum/carpet = 2)	
Type of roof (leaves = $0$ ; tin/other = $1$ )	0.28
Method of cooking (Samoan oven, fire,	0.39
charcoal = 1; kerosene, gas, electric = 2)	
Does the household have: $(no = 0; yes = 1)$	
Indoor plumbing	0.43
Electricity	0.36
Toilet	0.27
Refrigerator	0.64
Stereo	0.33
VCR	0.57
Television	0.58
Chairs/sofa	0.51
Carpet	0.62
Car	0.46

<sup>&</sup>lt;sup>a</sup> Coefficient alpha (standardized) = 0.84.

EBV = 
$$a + b_1x_1 + b_2x_2 + b_3x_3 + b_4(x_4 + x_5)$$
  
+ $b_5(x_4 - x_5) + b_6x_6 + b_7x_7 + b_8x_8 + e$ ,

where  $x_1$ ,  $x_2$ , and  $x_3$  represent covariates such as age, sex, and region,  $x_4$  is standardized material style of life, and  $x_5$  is standardized SES. The quantity  $(x_4-x_5)$  models lifestyle incongruity, and  $(x_4+x_5)$  represents the aggregate lifestyle indicator that controls for the direct effects of the status variables. The remaining terms describe the interactions between lifestyle incongruity and covariates.

Table 3
Linear model results indicating the effects of lifestyle incongruity on log-transformed EBV antibody levels<sup>a</sup>

	df	MS	F	<i>p</i> -value
Model	8	0.423	4.69	0.0001
Age group	2	0.605	6.71	0.002
Sex	1	0.323	3.58	0.060
Region	2	0.512	5.68	0.004
BMI	1	0.046	0.51	0.48
Overall lifestyle	1	0.108	1.20	0.27
Lifestyle incongruity	1	0.646	7.17	0.008

<sup>&</sup>lt;sup>a</sup> Model  $R^2 = 0.15$ .

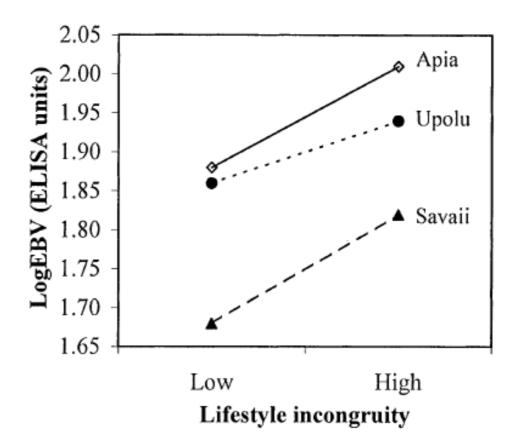


Fig. 1. Lifestyle incongruity is positively associated with EBV antibody levels, indicating higher levels of psychosocial stress.

Table 4
Linear model results indicating the effects of lifestyle incongruity and social integration on log-transformed EBV antibody levels<sup>a</sup>

	df	MS	$\boldsymbol{F}$	<i>p</i> -value
Model	10	0.386	4.36	0.0001
Age group	2	0.521	5.87	0.003
Sex	1	0.258	2.90	0.090
Region	2	0.383	4.31	0.015
BMI	1	0.063	0.71	0.40
Social integration	1	0.166	1.87	0.17
Overall lifestyle	1	0.130	1.46	0.23
Lifestyle incongruity	1	0.213	2.40	0.12
Lifestyle incongruity × social integration	1	0.459	5.18	0.024

<sup>&</sup>lt;sup>a</sup> Model  $R^2 = 0.17$ .

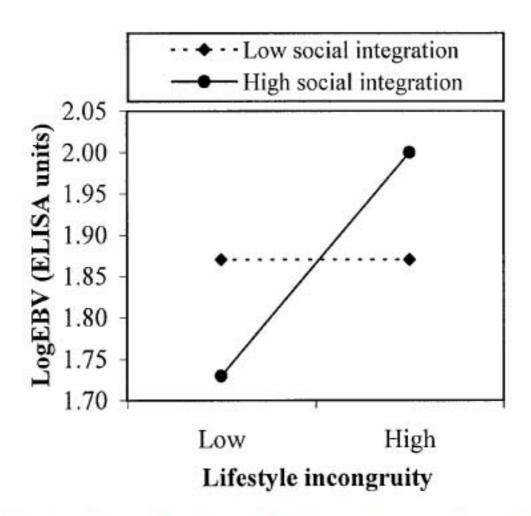
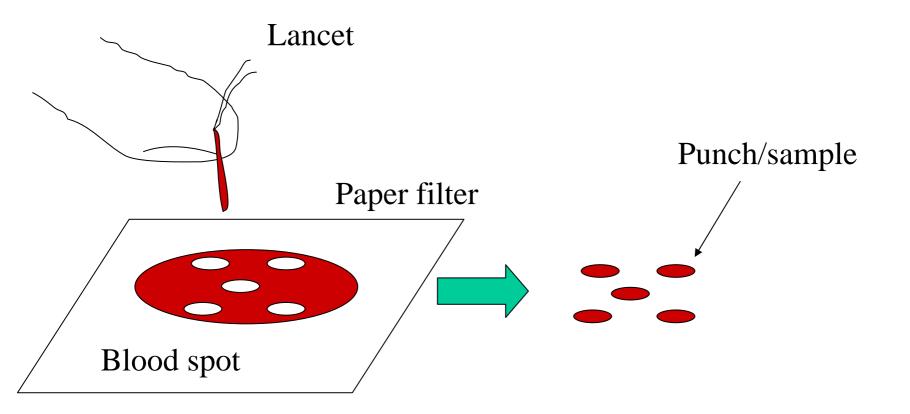


Fig. 2. Moderating role of social integration on the relationship between lifestyle incongruity and EBV antibody level.

### Blood spot sample collection on filter paper



Each sample absorbed similar volume of serum?

## Use of Filter Paper for the Collection and Analysis of Human Whole Blood Specimens<sup>1</sup>

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ABSTRACT The Centers for Disease Control and Prevention and its partners have been operating the Newborn Screening Quality Assurance Program for >20 y. The program helps participating laboratories to evaluate and improve the quality of their newborn-screening testing efforts by providing quality control dried blood spot materials and proficiency-testing materials for the external evaluation of screening programs. The Newborn Screening Quality Assurance Program provides an independent evaluation of filter papers approved by the Food and Drug Administration for the collection of blood for clinical tests. These activities have created a mechanism for the validation of the filter paper blood collection device and the standardization of materials and methods for the analysis of dried blood spots. J. Nutr. 131: 1631S–1636S, 2001.

KEY WORDS: • newborn screening • dried blood spots • blood collection • quality assurance

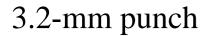
### Chromatographic effects

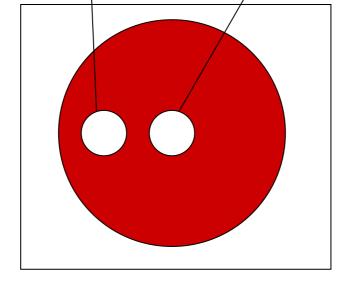
TABLE 1

Chromatographic effect on punch volume, where the center punch (3.2-mm, one-eighth-inch) was compared with peripheral punches taken from 100-µl volume dried blood spots of 55% hematocrit blood

Lot number	Peripheral punch average <sup>1</sup>	n	Center punch average	n	% difference2
Manufacturer 1 lot 2 Manufacturer 1 lot 3 Manufacturer 1 lot 4 Manufacturer 2 lot 1 Average % difference	1.445 ± 0.114 1.448 ± 0.104 1.459 ± 0.242 1.462 ± 0.066	500 700 300 700	1.474 ± 0.123 1.465 ± 0.104 1.487 ± 0.226 1.476 ± 0.073	125 175 75 175	1.97 1.16 1.88 0.95 1.49

<sup>1</sup> Serum volume (μL) in 3.2-mm punches representing north, south, east and west of the center punch.

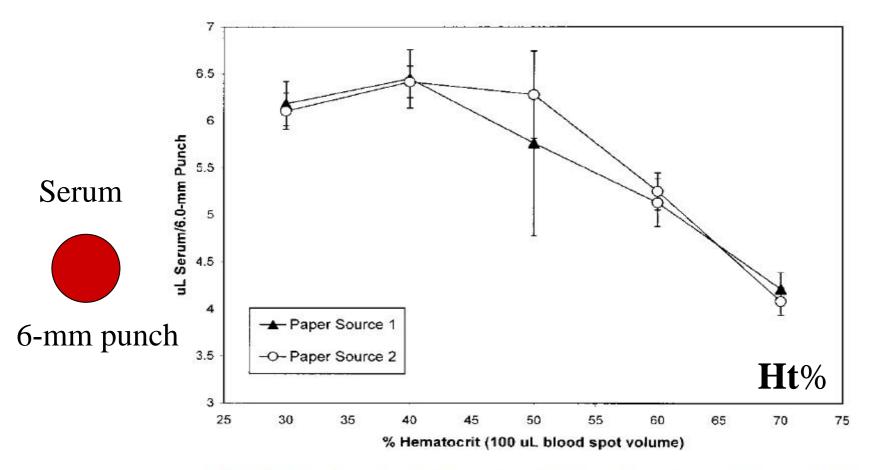




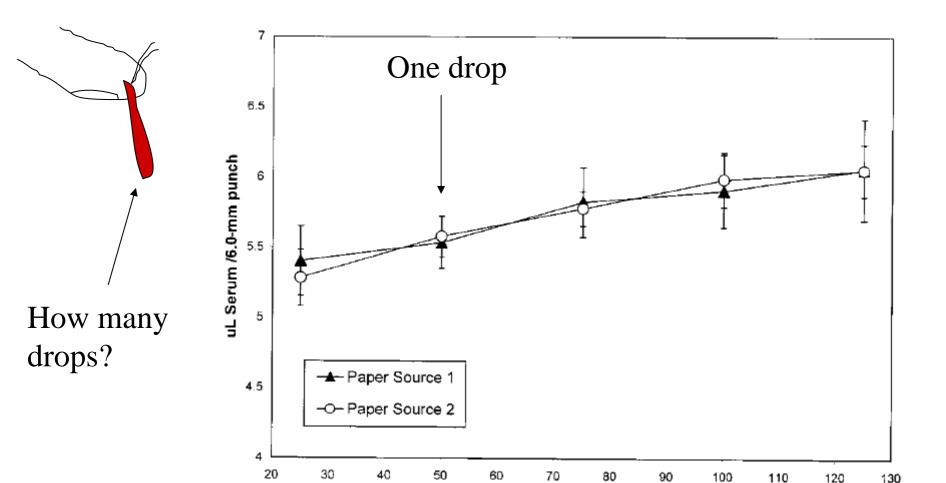
Chromatographic effects accounted for <2% of the overall variation

<sup>&</sup>lt;sup>2</sup> Percent difference calculated from the center punch to the peripheral punches.

#### Hematocrit effect



**FIGURE 1** The effect of hematocrit (%) on the volume of serum ( $\mu$ I) in 6.0-mm (one-fourth-inch) punches. As the hemacorit of the spotted blood increased, the amount of serum in the punch decreased. For each point, n=50.



**FIGURE 2** As the volume ( $\mu$ I) of whole blood applied to each spot increased, the amount of serum in a 6.0-mm center punch also increased. For each point, n=50.

Spot volume (uL, 55% hematocrit)

## Blood spot sample collection on filter paper

- Not applicable to the samples with large variation in Ht
- Number of blood drops should be controlled

#### Instructions for specimen collection

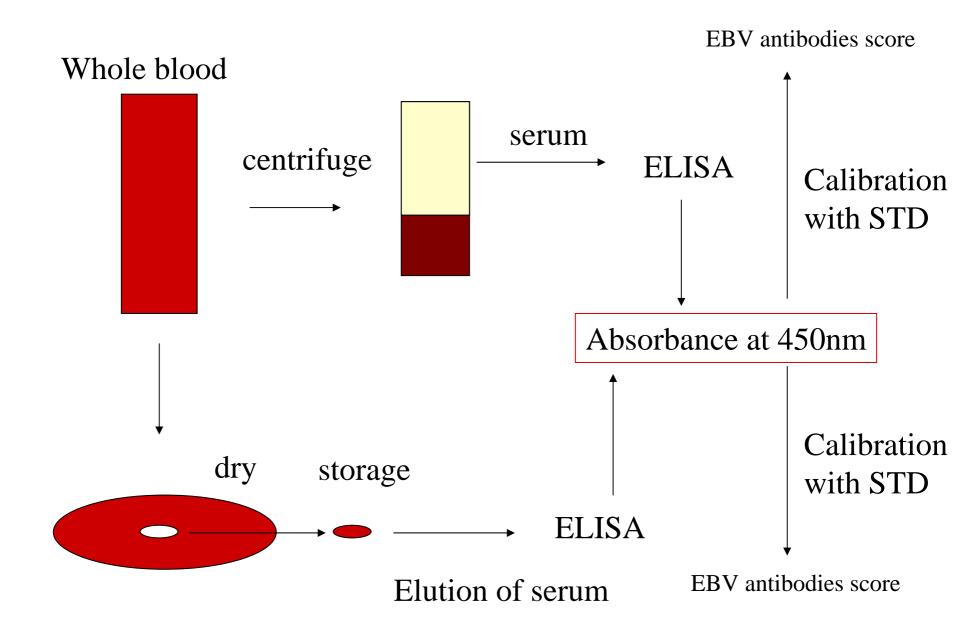
- Complete each item on the newborn screening collection form.
- Do not touch any part of the filter paper circle before or after collection.
- Select puncture site and cleanse with 70% of isopropanol.
- Use a sterile, disposable lancet with 2.0-mm point or less.
- Keep the infant's heel in a down position at or below the heart level. (Sampling after a feeding promotes better blood flow.)
- Wipe away the first blood of drop.
- Use the second large blood drop to apply to the surface of the filter paper circle.
- If not completely filled, add a second large drop immediately. Fill one circle at a time.
- Fill circles from only one side of the filter paper. Fill all required circles completely.
- Dry specimen at ambient temperature for 3–4 h in horizontal position.
- Forward specimen to the state newborn-screening laboratory within 24 h.
- Improperly collected samples will be rejected by the laboratory, require a second specimen and force a delay in the analysis, thus delaying treatment of affected newborns.

## Epstein-Barr Virus Antibodies in Whole Blood Spots: A Minimally Invasive Method for Assessing an Aspect of Cell-Mediated Immunity

THOMAS W. McDade, PhD, Joy F. Stallings, PhD, MPH, Adrian Angold, MRCPsych, E. Jane Costello, PhD, Mary Burleson, PhD, John T. Cacioppo, PhD, Ronald Glaser, PhD, and Carol M. Worthman, PhD

Objective: Study 1: Introduce and validate a method for measuring EBV p18-VCA antibodies in whole blood spots to provide a minimally invasive marker of cell-mediated immune function. Study 2: Apply this method to a large community-based study of psychopathology in children and adolescents. Methods: The EBV antibody method was evaluated through analysis of precision, reliability, stability, and comparisons with plasma and indirect immuno-fluorescence methods. The effects of life events on p18-VCA antibody level were considered in a subsample of 9, 11, and 13 year-old children participating in the Great Smoky Mountains Study in North Carolina. The subsample was stratified by age, sex, and degree of overall life strain. Results: Dried blood spots provided a convenient, sensitive, precise, and reliable method for measuring EBV p18-VCA antibody titer. Life events were positively associated with p18-VCA antibodies in girls but not in boys. Conclusions: The validity of the blood spot EBV p18-VCA antibody assay, as well as the ease of sample collection, storage, and transportation, may provide an opportunity for psychoneuroimmunology to explore a wider range of stress models in larger, community-based studies. Key words: psychoneuroimmunology, cell-mediated immunity, stress, methods, life events, sex difference.

EBV antibodies = indirect measure of cell-mediated immune function



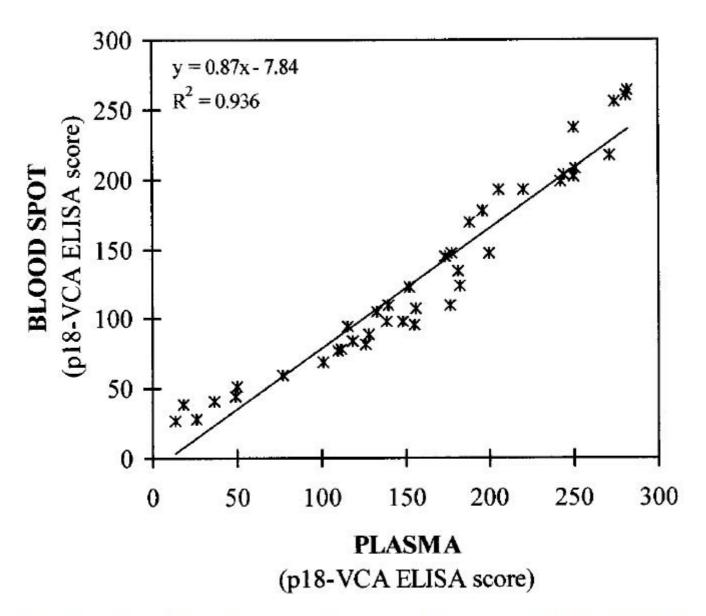


Fig. 1. Correlation between plasma and blood spot EBV p18-VCA antibody titer.

TABLE 1. EBV p18-VCA Antibody Assay Precision (Within-Assay CV) and Reliability (Between-Assay CV)<sup>a</sup>

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

<sup>&</sup>lt;sup>a</sup> Mean p18-VCA antibody titers are presented in standard ELISA scores.

### **Precision and reliability**

## **Storage condition**

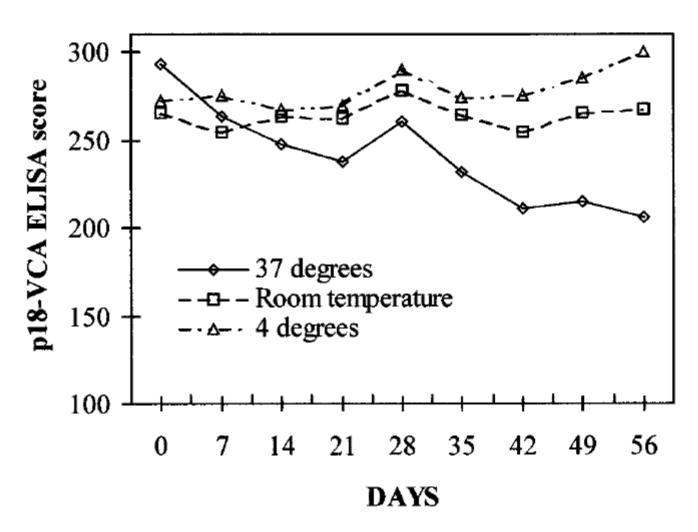


Fig. 3. Stability of EBV p18-VCA antibodies in whole blood spots over 8 weeks at 37°C, room temperature, and 4°C.

High-Sensitivity Enzyme Immunoassay for C-Reactive Protein in Dried Blood Spots, Thomas W. McDade,<sup>1\*</sup> James Burhop,<sup>2</sup> and James Dohnal<sup>3</sup> (<sup>1</sup> Laboratory for Human Biology Research, <sup>2</sup> Department of Neurobiology and Physiology, and <sup>3</sup> Evanston Northwestern Healthcare Research Institute and the Feinberg School of Medicine, Northwestern University, Evanston, IL; \* address correspondence to this author at: Laboratory for Human Biology Research, Northwestern University, 1810 Hinman Ave., Evanston, IL 60208; fax 847-467-1778, e-mail t-mcdade@northwestern. edu)

C-reactive protein: a central component of the acute phase response, a non-specific, systematic response to infection →index of current infection

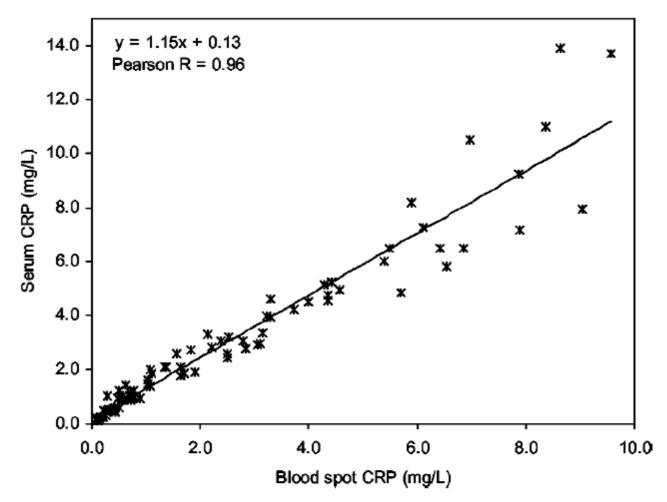


Fig. 1. Relationship between blood-spot and serum CRP concentrations in 84 paired samples.

The best-fit linear regression line is shown. Samples above the highest bloodspot calibrator (10.13 mg/L) are not included (n = 10).