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Testing Protocols

Care of Biological Samples in a Rural Setting

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The purpose of this study is to evaluate collection, transport, and storage protocols of urine and air allergen samples from infants in a rural setting. Infant urine and aeroallergen samples ($n = 21$) are collected in the home setting in rural areas where time and distance to the central laboratory is a consideration. Urine samples are analyzed using ELISA tests after being transported using three different techniques: (a) ambient temperature, (b) dry ice, and (c) packaged on dry ice and shipped commercially. Following initial ELISA testing for levels of cytokines, urine samples are frozen at -70 degrees Celsius for 6 months. Samples are then reanalyzed for levels of cytokines. Dust samples are analyzed for levels of aeroallergens, stored at 4 degrees for 6 months, and reanalyzed. The integrity of samples varies by biomarker, shipment temperature, and storage time, creating a high degree of variability in results.

Keywords: *biomarkers; sample collection; rural; infant; respiratory*

Biomarkers are laboratory measurements that can be used to identify surrogate targets for therapy and clinical endpoints (Deykin, 2006). The use of peak expiratory flow rate (PEFR) and forced expiratory volume

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as biomarkers of lung function provide useful information about asthma control, but are not helpful in capturing the processes that influence the pathobiology of the disease. Recognition of biomarkers that can assist in managing asthma, or more importantly in identifying who might be at risk for inflammation associated with asthma, may assist in reducing the burden of asthma, particularly among children (Szefer, 2007).

Issues related to measuring biomarkers as an outcome of intervention studies among people living in rural settings have not been extensively studied, because of, in part, the unique difficulties encountered with this type of rural research. Those challenges include the separation of research team members by significant geographic distance and the distance of rural research participants from research institutions or laboratories where biological samples are analyzed. If interventions are to be studied in the rural setting, efforts must be made to maximize the effectiveness, reliability, and validity of data collection to ensure that the integrity of biological samples is preserved.

The central intent of this preliminary study was to develop and test protocols useful in collecting and transporting infant biomarkers and aeroallergens specimens in rural areas. Children without allergies or asthma were selected for this study as the primary purpose was to determine feasibility of transport of specimens. Though preliminary results of values of biomarkers measured are included here, the intent of the study was to learn about collection and transportation of specimens. Preliminary protocols for specimen collection, transport, and storage were developed. Establishing protocols that ensure both the stability of samples and complete data sets are vital to meet the unique challenges of collecting data in rural settings.

Asthma is the most prevalent serious chronic disease among children. Nearly eight million of the 214 million Americans who suffer from asthma are children (American Lung Association, 2002). Because the incidence of asthma is rising each year, prevention of this chronic illness is becoming a national emergency.

Problem

Use of Biological Markers in Research

Bearer (1998) defined biomarkers as observable properties of an organism that indicate a change in biochemical components, structure, or function. For measurements of biomarkers to be useful, they must be specific,

sensitive, robust, predictive, and noninvasive, and link preclinical and clinical systems (Paradigm Genetics, 2003). Bearer argued that, because biomarkers have not generally been measured in children, research validating biomarkers in children's health should be a priority.

This research team's interest in intervention research in childhood asthma led it to select biomarkers related to general inflammatory responses for measurement. These included C-reactive protein (CRP), interleukin 5 (IL-5), IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor alpha (TNF- α), and RANTES. These biomarkers, which have not been extensively studied in children, can be measured through noninvasive methods (via urine sample) (R&D Systems, Inc., 2004). Useful biomarkers for measuring inflammatory response in children include Eosinophil Cationic Protein [ECP] (Koeller, Wojnarowski, Herkner, Weinlander, Raderer, & Eichler, 1997; Oymar & Bjerknes, 1998; Reijonen & Korppi, 1998), eosinophil protein X [EPX] (Garofalo, Dorris, Ahlstedt, & Weliver, 1994; Martinez, Stern, Wright, Taussig, & Halojnen, 1998), and C-reactive protein [CRP] (Ford, 2003). Other relevant biomarkers include select cytokines, which play a critical role in the persistence of the inflammatory process associated with asthma. Measuring these inflammatory cytokines IL1, IL4, IL5, IL6, TNF- α , GM-CSF, RANTES, and EPX through non-invasive methods is beneficial.

Eosinophilia is characteristic of asthma and plays a role in the inflammatory response in the airways. Eosinophils excrete toxic granulocytic proteins on stimulation and are, therefore, specific markers for the presence of eosinophils. Urinary eosinophil protein X has been successfully used as a marker of airway inflammation in asthmatic children (Carlsen, 1997; Oymar & Bjerknes, 2001). With respect to CRP, Ford (2003) found that adults with asthma were more likely to have an elevated CRP than those who did not have asthma, or had asthma earlier during their lives; however, the relationship between CRP and asthma is not well defined in children. The use of urine samples to measure anti-inflammatory cytokines (IL1, IL4, IL5, IL6, TNF- α , GM-CSF, RANTES) has not been thoroughly studied in the young child. Although EPX has been used to monitor disease activity and level of disease control, there is conflicting information about the usefulness of this as a marker of inflammation in children (Carlsen, 1997; Oymar & Bjerknes, 2001). Whether the conflicting information is because of the differences in sampling protocol is unknown because the protocols for urine sample collection are not clearly elucidated.

Because the purpose of this study was to clearly identify best methods of collection and transport from a rural setting, this manuscript will focus on the

process and outcome related to this aim. If effective methods of transporting these biomarkers from remote geographic areas can be recommended, researchers in rural areas may be able to add to the growing body of literature that supports the importance of measuring biomarkers in children.

Transport of Biological Samples in the Rural Setting

The collection and transport of urine samples require particular attention at any time they are undertaken. According to Holland, Smith, Eskenazi, and Bastaki (2003, p. 217), when urine is collected, “careful handling and storage of this precious biological sample” is imperative because the stability of biomarkers in urine can be affected by “time of collection, containers used, transport means and length of transit time.” These samples become even more prized—and their stability more threatened—when they come from sparsely populated and geographically isolated rural areas. Samples become more prized because the “pool” of potential subjects from a sparsely populated geographic area is limited. The stability of the samples may be threatened because of the distance the samples must travel to arrive at a laboratory for analysis. It is possible that during transport, temperature may influence the stability of the biomarker.

Indoor Allergens

Common indoor allergens are generated by dust mites, cats, dogs, mice, and cockroaches, and by environmental tobacco smoke. Measurement of allergens can be conducted using the Enzyme-Linked Immunosorbent Assay (ELISA) procedure, which has been shown to be sensitive and reproducible in measuring indoor allergens (Indoor Biotechnologies, 2003). Establishing a collection and storage method for home sampling of aeroallergens, which may vary depending on the geographic location of the rural site, requires a specific set of skills and equipment.

Even though the ELISA measure is sensitive and valid (Gehring, Triche, van Strien, Belanger, Holford, Gold, et al., 2007), two major factors influence the measurement of these aeroallergens: the location of aeroallergen collection and the altitude of study sites. Basagana, Torrent, Atkinson, Puig, Barnes, Vail, et al. (2002) determined that samples taken from sofas in the living room area of homes had higher levels of dust mite allergens than samples taken from mattresses, whereas Boner, Pescolliderung, and Silverman (2002) found that dust mite allergens were higher in mattresses. Other environmental aeroallergens are more likely to be found and sampled

(cat, dog, mouse, and cockroach) on floors. In addition, elevation at the location of allergen collection is an important consideration. For instance, dust mites do not flourish in dry environments or higher than 3,000 feet above sea level (Aetna Intellihealth, 2004). Cockroaches are also rare at higher elevations.

Purpose of the Study

Establishing protocols that ensure both the short- and long-term stability of samples is vital to meeting the unique challenges of collecting data in rural settings. The objective of this study was to develop and test protocols for measurement of infant urine inflammatory biomarkers and environmental aeroallergens from the participants' homes. The group of infants aged 12-16 months who participated in this study had also participated in a previous study conducted by the researchers. Parents were also asked to evaluate the process used to collect the biomarkers.

Design

A descriptive design was used to develop and test protocols for the collection, transport, and storage of biomarkers and indoor aeroallergens. Long-term storage protocols were also explored to determine whether the integrity of the samples was maintained at a 6-month retest.

Sample

A group of healthy infants ($n = 50$) whose mothers participated in a previous breastfeeding intervention study were invited to participate in this study. These potential participants resided in two rural communities in the western region of the United States. Of those invited, 21 mothers agreed to participate.

Methods

Once institutional review board approval was obtained from each institution, a date for data collection was identified. Mothers of the infants were contacted by telephone and invited to participate in the study. If the mother agreed, written consent was obtained during the first of three home visits

after the purpose, risks, and benefits of the study were explained. Before data collection began, all data collectors were trained during a 4-hr session to ensure that data would be collected in a consistent fashion.

Setting

Participants lived in two communities in the western plains in Wyoming and Nebraska. With a population of 494,423, Wyoming is the least populated state in the United States. Much of the state is designated as “frontier” because the population density is 5.04 people per square mile of land (United States Bureau of the Census, 2001). The western portion of Nebraska is also considered “rural.” Seven counties surrounding Scottsbluff County, where the study was conducted, have population densities that range from 0.7 to 11.3 persons per square mile, although Scottsbluff County is more populated with a density of 50 persons per square mile.

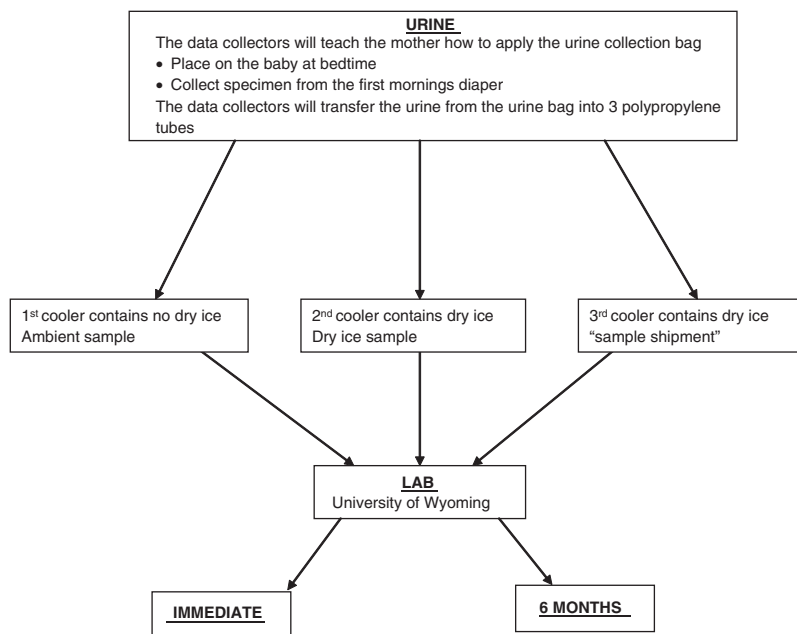
Sample Collection, Transport, and Storage

Data were collected by two research teams (one in each rural community). Each data collector was assigned to complete all home visits for four to six infants. Home visits were scheduled on the same day at both sites so that urine and aeroallergen samples could be collected at the same time. This was necessary for timely acquisition, packaging, and transport of the samples to the remote laboratory so that the initial biomarker assays could be completed within 36 hours after urine collection. The research team felt that working with the parents in the home would allow collection of dust samples for analysis, and would also provide an unhurried environment for education of the family with regard to the study and collection of the samples. All mothers were instructed in the same manner for application of the urine collection device (UCD) and handling the sample once it was collected. Written instructions (Hollister, 2004) about how to apply the urinary device were also provided to each parent (Figure 1). In addition to urine sample collection, aeroallergen samples were collected at the same time that the teaching regarding application/removal of the UCD was completed (Figure 2).

Storage and Transport of Specimens

Urine specimens. When the ELISA kits were obtained for this project, we ensured that the lot numbers for each specific kit were identical and that the expiration dates were after data were to be analyzed. It was in this

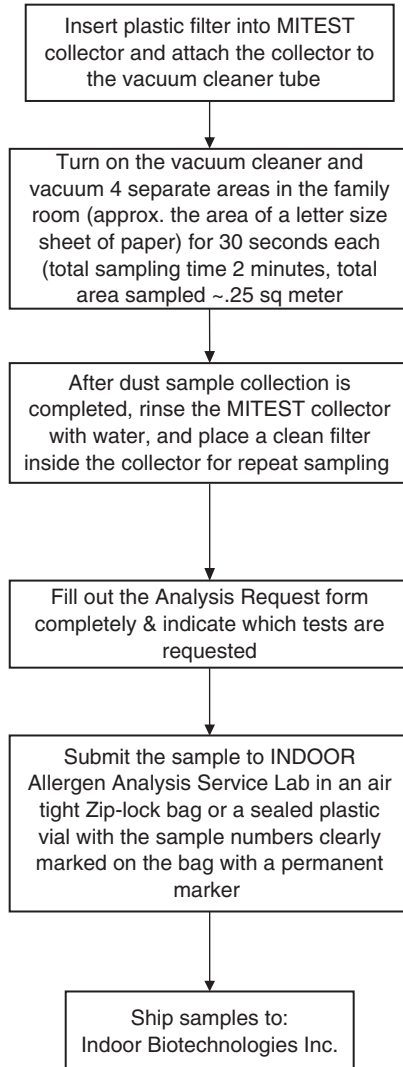
Figure 1
Urine Transport and Analysis



way that the consistency of the ELISA test results over time was taken into account.

The goal was to collect a minimum of 17 milliliters of urine from each infant to complete the six different ELISA tests (CRP, TNF-alpha, IL-5, IL-6, RANTES, and GM-CSF). Immediately after collection, urine samples were divided into five aliquots so that, after transportation, the same sample could be analyzed and stored in different ways. Because division of the samples took place immediately after collection, differences in sedimentation (within one sample) were unlikely. One aliquot was placed in a cooler at ambient temperature; one aliquot was placed in a cooler with dry ice; and three aliquots were placed in a cooler with dry ice, boxed for shipping, and handled as a FEDEX package. The ambient and cooler dry ice samples were transported by the research team via car to the laboratory, either 50 or 150 miles away, depending on the data collection site. The dry ice FEDEX samples were packaged as a single FEDEX package. All samples were

Figure 2
Dust Collection, Transport, and Analysis



scheduled to be collected on Friday before we realized that in these rural areas FEDEX does not deliver packages on weekends. Therefore, the researchers simulated the FEDEX shipment by driving the sample packed on dry ice in a cooler to the processing laboratory. The samples were neither unpacked nor temperatures of the samples recorded until the following afternoon (simulating the FEDEX schedule) when the FEDEX delivery would usually arrive. To determine whether urine samples would maintain integrity over time, the samples were analyzed at two time points: the first immediately after arrival at the laboratory and, the second, after they had been stored frozen at -700°C for six months.

Dust samples. Dust samples (sealed individually in a plastic bag) were packaged and sent to a laboratory via parcel post. The allergens were extracted in PBS (Phosphate buffered saline) with Tween 20 (0.05%). Aerosamples for dust mites [(Der f 1), cat (Fel d 1), dog (Can f 1), mouse (Mus m 1), and cockroach (Blag 1)] were analyzed and evaluated immediately after arrival at the laboratory, as well as after being stored at 40°C for six months by EMSL Analytical, Inc., in New Jersey.

Data Analysis

Urine Specimens

Urine concentrations of CRP, TNF-alpha, IL-5, IL-6, RANTES, and GM-CSF were determined in duplicate using commercially available, highly sensitive monoclonal antibody-based ELISA (ALPCO Diagnostics, Windham, NH, and R&D Systems, Minneapolis, MN). The sensitivity, intra-assay coefficients of variation and ELISA manufacturer are depicted in Table 1. Intra-assay coefficients of variation (CV) were determined for each kit at baseline and the 6-month time point using one internal standard control of known concentration and all detectable samples. Samples used for determination of intra-assay coefficient of variation were run in duplicate. The average intra-assay CV was determined for each specific ELISA from all detectable samples. Because the coefficients of variation were not statistically different between the baseline and 6-month time point, Table 1 reports the mean CV of both time points. Intra-assay CVs at baseline and 6 months were compared using the t-statistic with significance set *a priori* at 0.05.

Table 1
Sensitivity, Intra-Assay Coefficient of Variation, and
Manufacturer for ELISA Assays

Biomarker	Sensitivity*	Intra-assay CV** (%)	Manufacturer
CRP	0.12 ng/mL	3.8	ALPCO Diagnostics
TNF- α	0.12 pg/mL	9.6	R&D Systems
IL-5	< 3.0 pg/mL	11.8	R&D Systems
IL-6	< 0.7 pg/mL	8.9	R&D Systems
Rantes	2.00 pg/mL	2.9	R&D Systems
GM-CSF	< 0.26 pg/mL	8.0	R&D Systems

* Minimum detectable dose (MDD)

** Intra-assay coefficient of variation (CV) was conducted including all available samples and one known internal standard. Lot numbers for each test were identical (initially and at 6 months). CVs reported are the mean between baseline and the 6-month time point, because comparative statistics revealed no difference in CV between the time points.

Indoor Allergens

The dust samples were assayed using ELISA to detect levels of dust mites, cat, dog, mouse, and cockroach allergens. This antibody-based procedure captures the allergenic protein and generates a color for positive samples. The color was measured by a microplate reader, which reads the amount of light that passes through the solution. If positive, the intensity of the color is compared with the color of a series of allergenic protein standards. This concentration reveals the amount of allergenic protein in the original sample (EMSL, Analytical, Inc.).

Findings

Although the intent of this research is not to identify normal ranges for infant biomarkers discussed here, presenting the values of each biomarker measured according to type storage and transport is important to the overall goal of the study. Therefore, descriptive analysis of the results for both urinary biomarkers and aeroallergens are reported here to provide information about the best way to collect, transport, and store the urine for biomarker measurement, and for no other purpose.

Urinary Biomarkers

Infants ranged from 12 months to 23 months ($M = 17$ months; $SD = 3.9$). Required urine volume (17mL) to complete all six biomarker tests was collected from three infants, but collection volume was less than required for a majority of the infants. The urine collection device remained in place for only eight of the infants; nevertheless, some volume of urine was collected in instances of detachment of the urine collection device.

The temperature of the ambient samples was consistently 20.10 C. Dry Ice Samples ranged in temperature from 2-110 C, whereas the Shipment Samples were 00 C. The volume of urine was insufficient (<17 mL) in many cases because the urine collection devices did not remain in place overnight. Consequently, a full set of tests could not be completed on each infant urine sample. In three infants an abundant quantity (>30 mL) was available.

Not all infant urine samples contained detectable levels of inflammatory biomarkers. Detectable inflammatory biomarkers included CRP, TNF-alpha, IL-5, IL-6, RANTES, and GM-CSF. However, the number of samples detectable was highly variable between biomarkers assessed and between transport and storage protocols. Table 2 identifies the number of detectable samples and concentrations (mean + SD) of inflammatory biomarkers from the 21 infants and for all three shipment and storage protocols. Because there was insufficient urine volume in the aliquots shipped at ambient temperature, 6-month inflammatory biomarker retest of samples was not possible (Table 2). There was significant decline in the integrity of urine samples shipped on dry ice by the research team and shipped on dry ice by FEDEX for CRP, IL-5, IL-6, and RANTES at the 6-month follow-up analysis, evidenced by no detection capacity for CRP, IL-5, and IL-6 (Table 2). Although clinical norms are known only for CRP, all infant urine CRP analyses, regardless of transport and storage protocol, were within the clinical/expected normal range of less than 6.0 ng/mL ($M = 5.2 \pm 0.2$).

Indoor Allergens

Samples revealed evidence of some of the indoor air allergens (cat, dog, and mouse). However, there was no evidence of cockroach or dust mites in the dust samples. This is unusual for one of the sites in particular where dust mites are known to be a contributing factor for allergy and asthma symptoms. When levels were compared across time, the correlation between the two analyses was high for all detectable aeroallergens (cat, $r_{(20)} = .99$; dog, $r_{(20)} = .88$; mouse, $r_{(20)} = .93$), but the mean decreased by half for dog

Table 2
Number of Detectable Inflammatory Biomarkers
and Concentrations (Mean+SD) From Infant Urine

	Baseline Samples Detectable (<i>N</i>)	Baseline Mean Concentrations	6-month Samples Detectable (<i>N</i>)	6-month Mean Concentrations
CRP				
Ambient	3	5.3 + 0.3 ng/mL	x	x
Dry Ice	3	5.2 + 0.1 ng/mL	0	ND
FEDEX	4	5.1 + 0.1 ng/mL	0	ND
TNF				
Ambient	11	0.7 + 0.6 pg/mL	x	x
Dry Ice	10	0.9 + 0.9 pg/mL	9	0.9 + 0.9 pg/mL
FEDEX	9	1.2 + 1.5 pg/mL	9	1.0 + 1.4 pg/mL
IL5				
Ambient	3	11.9 + 5.7 pg/mL	x	x
Dry Ice	2	9.1 + 0.2 pg/mL	0	ND
FEDEX	1	8.7 + 0.2 pg/mL	0	ND
IL6				
Ambient	0	ND	x	x
Dry Ice	8	1.3 + 1.0 pg/mL	0	ND
FEDEX	6	0.5 + 0.3 pg/mL	0	ND
GMCSF				
Ambient	0	ND	x	x
Dry Ice	9	1.6 + 0.9 pg/mL	6	2.9 + 1.3 pg/mL
FEDEX	9	1.4 + 1.0 pg/mL	5	2.9 + 1.3 pg/mL
Rantes				
Ambient	14	17.5 + 11.7 pg/mL	x	x
Dry Ice	13	16.6 + 11.9 pg/mL	1	28.8 pg/mL
FEDEX	7	15.4 + 12.0 pg/mL	2	24.3 + 3.3 pg/mL

ND = non-detectable; x = insufficient urine volume for measurement

(from 327 μ /g to 174 μ /g) and mouse (from .03 μ /g to .013 μ /g). The difference was significant for levels of the mouse allergen ($t(20) = 2.48$; $p = .02$).

Protocol Evaluation for Urine Sample Collection

Parents were provided written instruction and verbal explanation regarding the correct procedure for securing the urine sample collection devices. However, some urine collection devices did not remain in place.

In reviewing the instructions given to parents for application of the urine bag, we note that we had suggested that parents place the collection device after the evening bath—just prior to placing the infants in bed for the night. We did not ask parents to refrain from putting lotion on, at, or near the placement site of the urine collection device; something that, in retrospect, should have been done and may have improved collection volume.

Protocol Evaluation for Dust Sample Collection

All participants found that “having someone collect dust samples” was not at all disruptive. The mean amount of time for dust sample collection was almost 5 min.

Discussion

Limitations

Limitations of the study were related to urine collection problems, sample size, number of specimen freeze–thaw cycles, and the good health of the infants. Urine collection devices remained in place for only 8 of the 21 infants. In addition, the amount of urine collected was not sufficient to complete all of the ELISA testing. Consequently, the ability to complete analyses was disappointing. Another limitation was that the sample was of insufficient size to justify conducting inferential statistical tests, so only descriptive results are reported. However, results do provide valuable information for those transporting specimens for research in rural settings. Additionally, samples shipped on dry ice via FEDEX froze during shipment, had to be thawed for the initial (baseline) testing, and then re-frozen for the 6-month retest. Similarly, the samples shipped on dry ice and transported by the investigative team were nearly frozen (2–11° C). It is generally recommended that biological specimens such as this do not undergo more than one freeze–thaw cycle. The fact that the samples shipped on dry ice froze or were nearly frozen and had to be re-frozen could have contributed to the observed decline in concentration at the 6-month retest for most of the samples. Although the containers used to transport the samples were identical, the amount of dry ice varied. In the future, the amount of dry ice in each cooler should be the same so the temperature of the samples is more closely controlled. In addition, there were no efforts to ensure that sediment in the urine was equally distributed in the specimens. This could be addressed by mixing the specimens before dividing the samples into aliquots.

Another limitation of the present study was that the infants were relatively healthy; therefore, the lack of inflammatory biomarkers in some of their samples could have been because of an absence of these biomarkers in their urine. Moreover, if inflammatory biomarkers were present, the concentration might have been below the detectable limit of the assay. The problem of non-detectable or minimally detectable levels of urinary biomarkers compounds the design of this study for evaluating the different storage and shipment protocols. The investigative team is conscious of this problem, which precluded any formal statistical evaluation. However, we believe that our findings may be of use to others investigating protocols for collection, storage, and shipment of urinary biomarkers when proximity to the processing laboratory is a limitation.

Implications

The descriptive and anecdotal evidence from this study suggests that the collection and analysis of biomarkers in household aeroallergens in remote rural regions is feasible, whereas the analysis of infant urinary biomarkers collected in this context is very limited. To our knowledge, this is the first study to evaluate protocols for measuring biomarkers from the urine of infants. Significantly, our results indicate a high degree of variability in detecting inflammatory biomarkers in infants; and the integrity of samples, both short and longer term, varies by biomarker of interest and shipment temperature. There are multiple explanations for the variability reported in this study, the foremost of which is likely to be the unstable pH of urine and the likelihood that this pH environment may have denatured or altered the molecular structure of the inflammatory biomarkers. Possible solutions to this could include collection of the urine into a stabilizing and preserving medium.

The use of infants' urine provides a noninvasive way to measure inflammatory markers. For overnight urine collection to be successful, devices must be applied after the evening bath so that all oils and skin lubricants are removed. In addition, the time and accessibility of nurses or data collectors experienced in such procedures must be figured into data collection time. A potentially viable option to overcome the issue of inadequate urine volume collection may be to use a mannequin to assist in showing parents how to apply the urine collection device. The time necessary to include this strategy in the instructions would need to be considered.

Additionally, transportation of urine samples on dry ice was neither necessary nor desirable. Results from this study indicate that CRP, IL5, IL6, and RANTES values are detectable only in urine samples that have not

been frozen. Levels of TNF-alpha and GM-CSF appeared to maintain some stability over time. However, because of the limitations of the present study, these results should be interpreted with caution.

The method of dust sample collection was appropriate for some air allergens but may not be appropriate for all aeroallergens investigated in this study. Determination of the best site for aeroallergen collection samples is an area of future study. Based on the reports of Basagana et al. (2002) and Boner et al. (2002), it would be prudent to determine the location of the highest concentration of mite allergens. Moreover, allergen exposure is a product of concentration and time, so evaluating regions of the home such as the infant's bedroom, where a significant amount of time is spent, may be appropriate. Finally, determining which tests are appropriate based on the geographic location of the community is another consideration.

Finally, this intense data collection time frame had the advantage of requiring participation from mothers and babies for only a limited but concentrated amount of time. The disadvantage for the researcher was the time commitment of two days. Because several of the biomarkers included in this study need to be analyzed at room temperature shortly after collection to obtain valid results, this type of data collection protocol may have to be used in similar studies. Therefore, if rural research requiring analysis of samples is being conducted, it is crucial that those in a rural setting determine (a) whether the proximity to the analysis laboratory is acceptable, (b) what constitutes the most efficient and efficacious specimen storage and transport protocols, and (c) whether the analysis needs to be done on arrival at the laboratory or if the samples are viable after being stored.

Conclusions

Results from this preliminary study suggest that infant urine samples collected from a rural setting, transported at ambient temperatures, and stored at -70°C for 6 months may maintain some integrity for analysis of GM-CSF and TNF- α . Samples analyzed for levels of CRP, IL-5, and RANTES can be transported at ambient temperatures but should be analyzed immediately after collection (within 36 hours). CRP, IL-5, and IL-6 appear to be minimally detectable in infant urine. Moreover, IL-6 requires shipment at a cooled temperature ($0-11^{\circ}\text{C}$) and must be analyzed immediately. Dust samples to be tested for dog and mouse aeroallergens should also be analyzed immediately after shipment.

This study is the first step in establishing collection, shipment, and storage protocols that ensure the integrity of urine and dust samples collected from infants in rural areas where samples are difficult to collect and analysis may be delayed. Identifying efficacious rural research protocols is imperative in light of the regional incidence of asthma, and the need for effective nursing interventions in often underserved rural geographic regions.

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