The Value of Home-Based Collection of Biospecimens in Reproductive Epidemiology

John C. Rockett,[1] Germaine M. Buck,[2] Courtney D. Lynch,[2] and Sally D. Perreault[1]

[1]Reproductive Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [2]Epidemiology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Rockville, Maryland, USA

Detection, quantification, and prognosis of environmental exposures in humans has been vastly enhanced by the ability of epidemiologists to collect biospecimens for toxicologic or other laboratory evaluation. Ease of collection and level of invasiveness are commonly cited reasons why study participants fail to provide biospecimens for research purposes. The use of methodologies for the collection of biospecimens in the home offers promise for improving the validity of health effects linked to environmental exposures while maximizing the number and type of specimens capable of being collected in a timely and cost-effective manner. In this review we examine biospecimens (urine and blood) that have been successfully collected from the home environment. Related issues such as storage and transportation will also be examined as well as promising new approaches for collecting less frequently studied biospecimens (including hair follicles, breast milk, semen, and others). Such biospecimens are useful in the monitoring of reproductive development and function. *Key words:* **biospecimen, blood, breast milk, buccal cells, hair, home collection, nail, reproduction, saliva, semen, tissue collection, urine.** *Environ Health Perspect* **112:94–104 (2004). doi:10.1289/ehp.6264 available via** *http://dx.doi.org/* **[Online 24 September 2003]**

The design of studies examining the relation between environmental exposures and human health, including subtle markers of human reproduction, usually incorporates the collection of one or more biospecimens from study participants. Such specimens can both minimize misclassification bias associated with exposure or disease status and provide cellular and molecular data that can contribute to an understanding of the physiological processes being impacted. However, a major complicating factor for studies collecting biospecimens, especially large population-based studies such as the proposed National Children's Study (NCS 2003), is that sample collection can be encumbered by the logistics and expense of obtaining biospecimens from participants who live in a variety of locations and lead a variety of lifestyles.

One way to address this issue may be to incorporate home-based collection protocols that can be carried out by study participants with minimal to no oversight from study staff members. Such approaches have been widely if somewhat erratically used in previous epidemiologic studies. However, to our knowledge there has been neither discussion in the scientific literature on the utility or extent of the practice nor any assessment of its value in large longitudinal studies such as the NCS. We present a general overview of the utility of biospecimens and discuss those currently amenable to home collection, including how they can be collected and the type of research data they can yield.

Impact on Recruitment and Compliance Rates

One of the most useful aspects of home collection of biospecimens is the possible improvement of participant response. For example, a study by van Valkengoed et al. (2002) showed that in a screening program for asymptomatic *Chlamydia trachomatis* infections, mailing urine samples, as opposed to bringing them to the clinic, increased participation of male subjects by 18% (although no difference was noted among female participants). In another study, participation in a protocol to collect oral rinse samples was greater in the home-based group than in the clinic-based collection group (98% vs. 71%, respectively) (Harty et al. 2000b). There are several plausible reasons for this observed increase in participation by the home-based groups, primarily related to increased convenience for the participant. Special trips to the clinic are not required, and specimen acquisition and/or testing are performed in the privacy of the participant's home and at his/her convenience. In some cases the lack of interaction with the clinical environment may also add to a feeling of anonymity for the participant. For the aforementioned reasons, one could reasonably hypothesize that participation rates would be higher in studies using home-collection protocols than those requiring participants to attend clinics for specimen collection. However, at this time studies are insufficient to confirm this hypothesis, suggesting that such a study, encompassing different samples and different socioeconomic groups, would be well received and of great benefit to future epidemiologic research studies.

Feasibility Issues for Home-Based Collection of Biospecimens

When weighing the decision whether to use home-based biospecimen collection, investigators must consider five main issues:

- Specimen collection
- Specimen storage
- Transportation of the specimen to the clinic or analytical laboratory
- Stability of the specimen between collection and delivery
- Reception, storage, and analysis of the specimen in the laboratory

To address these issues, investigators must first determine the specifice use for these biospecimen(s). This in turn will determine how much sample will be needed, how it should be collected, when it should be collected, and how it should be stored and transported. Another consideration in feasibility assessment is the need for quality control of the samples to ensure their usefulness. Because many sources of error can be introduced in the collection and storage of biospecimens (Boone et al. 1995; Plebani and Carraro 1997), standard operating procedures must be developed and implemented regardless of whether specimens collected in the home are procured by research staff or by study volunteers themselves. Specific protocols must be prepared with

This article is part of the mini-monograph "Understanding the Determinants of Children's Health."

Address correspondence to J.C. Rockett, Reproductive Toxicology Division (MD-72), NHEERL, U.S. EPA, Research Triangle Park, NC 27711 USA. Telephone: (919) 541-2678. Fax: (919) 541-4017. E-mail: rockett.john@epa.gov

We thank D. Lobdel and C. Mamay (U.S. EPA) and members of the National Children's Study Advisory Committee's Fertility and Early Pregnancy Working Group for critically reviewing this manuscript prior to submission.

The information in this document has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

The authors declare they have no competing financial interests.

Received 6 February 2003; accepted 2 July 2003.

simple, clear instructions suitable for people at all levels of education and that (in some cases) can be conducted unsupervised within the limits and with the equipment found in a residential dwelling. This means, for example, that for unsupervised home collections, toxic substances should not be included in collection kits, and samples should be amenable to storage at room temperature 4°C or –20°C.

Biospecimens Amenable to Home Collection

An eclectic body of work on both the acquisition and analysis of home-collected samples has emerged over the past few years. The most common biospecimens collected by investigators to date are urine, blood, and semen. In most cases, these and a number of other samples can be collected in the home (Table 1). In the next section, we discuss the types of data these samples can provide and the issues related to their collection and transportation.

***Urine*. What can be measured in urine?** Urine is one of the biospecimens most amenable to home collection. Many currently used biomarkers of reproductive health such as steroid hormones or their metabolites can be measured in urine. Because hormones play such a vital role in the maintenance of reproductive health, knowledge of an imbalance in one or more hormones can help illuminate the cause of health problems. In particular, the levels of these hormones are excellent indicators for studying various aspects of the female reproductive cycle. For example, daily sampling of urine throughout the menstrual cycle can assist in the evaluation of the dynamic functions of the hypothalamo–hypophysial–ovarian axis (Kesner et al. 1999; Scialli et al. 1997). Relative levels of steroid hormones in urine can also be used to estimate the day of ovulation through measurement of luteinizing hormone (LH, the basis for most commercially available ovulation predictor kits) (Kesner et al. 1998), or

Table 1. Biospecimens amenable to collection in the home environment.

Biospecimen	Collection by	Storage	Transportation	Target analytes
Blood	Trained phlebotomists	Ambient temperature Ice N.B., assays can be sensitive to temperature, shaking and processing	Study staff collect and deliver specimens to laboratory Mail options for filter paper	Biochemical agents (e.g., hormones) Chemicals (e.g., solvents) Chemical metabolites (e.g., 3,5,6- trichloro-2-pyridinol) Drugs Drug metabolites (e.g., cotinine)
Breast milk	Female study participants	Freezer Refrigerator	Study staff collect and deliver specimens to laboratory Study participant transports sample to clinic Mailed on dry ice or with freezer pack	Chemicals (e.g., *p,p´*-DDT) Chemical metabolites (e.g., *p,p´*-DDE)
Buccal cells	Study participants Study staff members	If collected in mouthwash or cytobrush: Up to 1 week at 37°C	Study staff collect and deliver specimens to laboratory Study participant transports sample to clinic Mail options	DNA for genotyping Immunoassays Chemicals (e.g., solvents)
Hair/nail	Study participants Study staff members	Freezer Refrigerator Room temperature	Study staff collect and deliver specimens to laboratory Study participant transports sample to clinic Mail options	Heavy metals
Hair follicles	Study participants Study staff members	If collected in RNA later: Freezer—indefinitely Refrigerator—1 month Room temperature—1 week	Study staff collect and deliver specimens to laboratory Study participant transports sample to clinic Mail options	DNA for genotyping RNA for gene expression analysis
Saliva	Study participants Study staff members	Freezer Up to 1 week at room temperature	Study staff collect and deliver specimens to laboratory Study participant transports sample to clinic Mailed on dry ice (preferable) or with freezer pack (preferable) or at ambient temperature	Steroid hormones
Semen	Male study participants	Up to 1 day ambient or refrigerated for most measures Freezer for genomic integrity assays	Study staff collect and deliver specimens to laboratory Study participant transports sample to clinic Mail options	Biological agents (sperm parameters) Biochemical agents (e.g., hormones) Metals (e.g., lead, cadmium) Chemical metabolites (e.g., *p*-chlorophenyl ethane)
Urine	Study participants	Freezer Refrigerator	Study staff collect and deliver specimens to laboratory Study participant transports sample to clinic Mail options for filter paper or frozen specimens	Biological agents (microbes) Biochemical agents (e.g., hormones) Chemicals (e.g., solvents) Chemical metabolites (e.g., 3,5,6-trichloro-2-pyridinol) Drugs Drug metabolites (e.g., cotinine)
Vaginal swabs	Female study participants Study staff members	Freezer	Study staff collect and deliver specimens to laboratory Study participant transports sample to clinic Mail options	Biological agents (microbes)

the relative concentrations of estrogen and progesterone metabolites in daily first-morning urine specimens (Baird et al. 1991, 1995). Pregnancy can of course be detected quickly and conveniently using kits that detect the sharp rise in urine of human chorionic gonadotropin (hCG). Because commercially available home pregnancy test kits are sensitive, specific (Ehrenkranz 2002), and easy to use, at least one study has used them in lieu of urine collection to ascertain early pregnancy losses (Buck et al. 2002). Wilcox and colleagues (2001) recently reported that home pregnancy kits may have a false positive rate of 10% when used the first day after expected menstruation (assuming ovulation is delayed). Corroboration of these findings will underscore the importance of estimating ovulation in protocols involved in day-specific exposures or outcomes.

Unfortunately, the relation between urinary hormone metabolites and abnormal reproductive function has not been well characterized across populations and particularly susceptible subgroups of the population or in individuals from disadvantaged or medically underserved backgrounds. As urinary hormone metabolites are not commonly reported during the evaluation of reproductive problems, Lasley and Overstreet (1998) called for clinical studies to compare concentrations in urine and blood as a first step in validating this approach. Nevertheless, numerous studies have been conducted on the actual measurement of reproductive hormones in urine, many of which are summarized in the Lasley and Overstreet review. Many of these assays have been refined over the past 10 years, making them cost effective, robust, and accurate. Furthermore, many are available commercially as kits for use at home by untrained personnel [e.g., for measuring estradiol and progesterone metabolites, LH, follicle-stimulating hormone (FSH), and hCG].

Steroid hormone data are not the only information that can be derived from urinary samples. An enormous range of biological, biochemical, and chemical substances can be detected in urine (e.g., CDC 2003), including microbes, pesticides, solvents, and drugs. It has long been known that microbial infection during gestation can adversely impact fetal development (e.g., *Rubella*) and in some cases may result in death (Embleton 2001). Such infections can sometimes be detected in the urine of parents through the use of enzyme-linked immunosorbent, ligase chain reaction, or polymerase chain reaction (PCR) assays. A variety of viruses (papillomavirus, hepatitis B virus, HIV, cytomegalovirus, polyomavirus, adenovirus), bacteria (*Neisseria gonorrhoeae* and *Chlamydia trachomatis*), and mycoplasma (*Mycoplasma genitalium*) can be detected in this way. Furthermore, because of the stability of DNA and the robustness of the PCR process, assays for infections such as cytomegalovirus can be performed on urine collected on filter paper (Yamamoto et al. 2001). This has the potential to facilitate shipping (it is easier to ship filter papers than specimen collection vials), reduce the biohazard potential of the sample during transportation (sample is effectively transported as a solid and therefore cannot leak), and make storage of the sample at the analytical facility more convenient.

Exposure to pesticides before or during pregnancy can adversely impact development, leading to impaired neurological, immunological, and reproductive function in the offspring (reviewed by Sever et al. 1997). For example, male and female greenhouse workers exposed to certain pesticides have an increased time to pregnancy compared with that of unexposed workers (Abell et al. 2000a, 2000b; Petrelli and Figa-Talamanca 2001). For children born to a cohort of male pesticide applicators, significantly more birth defects occurred in children conceived in the spring than in any other season (Garry et al. 2002). In the same study there was a modest but significant increase in risk (1.6- to 2-fold) for miscarriages and/or fetal loss occurring throughout the year, suggesting a potential association between pesticide exposure and reproductive outcome. In most cases it is unknown whether this connection is through a direct toxic effect on parental gametes or reproductive organs, an adverse impact on the paternal or maternal endocrine system, or direct toxicity to the developing embryo/fetus. However, it is clear that the ability to measure pesticide metabolites in urine may help explain delayed conceptions, aborted pregnancies, and developmental problems by determining if one or both partners have been exposed to pesticides. Many different pesticides have been measured in urine (reviewed by Aprea et al. 2002). They are generally measured using methods such as mass spectrometry, liquid chromatography–mass spectrometry, gas chromatography with electron capture, gas chromatography–mass spectrometry, or high performance liquid chromatography. For example, exposure to the organophosphorus pesticides chlorpyrifos and chlorpyrifos-methyl can be determined by measuring their specific metabolite, 3,5,6-trichloro-2-pyridinol, in urine samples (Koch et al. 2001).

As with pesticides, solvent exposure can lead to reduced fecundibility in both males (Cherry et al. 2001) and females (Sallmen et al. 1995) and lead to developmental problems when exposure occurs *in utero* (Scheeres and Chudley 2002; reviewed by Lindbohm 1995). Again, exposure to solvents can be detected in urine samples either directly or by measuring metabolites or biomarkers. For example, several investigators have measured toluene exposure in urine, using benzylmercapturic acid (Inoue et al. 2002), hippuric acid, *o*-cresol, and toluene itself (Kawai et al. 1996).

Analysis of cotinine (a metabolite of nicotine) in urine is used frequently as an indicator of exposure to cigarette smoke (active or passive), although blood or semen can be substituted for urine in this regard (Vine et al. 1993). Such exposures can have an adverse impact on fertilization and embryo development and are therefore of potential interest in fertility and pregnancy studies.

How can collection of urine be conducted in the home environment? Using simple collection protocols and storage procedures that are complicit with the facilities available in the home of an average study participant, investigators found that hormones [e.g., LH and FSH (Kesner et al. 1998, 1999)], solvents [e.g., benzene and toluene (Senzolo et al. 2001)], pesticides [e.g., 2,4-dichlorophenoxyacetic acid (Hu et al. 2000)], and/or metabolic products thereof [e.g., organophosphorous pesticides (Curl et al. 2003; Hu et al. 2000)] are stable in urine. Hence, home collection of urine samples by study volunteers is a feasible sampling approach (Macleod et al. 1999), and various protocols have been developed that create minimal inconvenience for study participants. When samples are collected at home, they are typically stored frozen. Freezing allows storage of a large sample volume, and multiple samples can be accrued and/or combined. Thus, the number of analytical measurements that can be made is essentially unlimited. In a study by Reutman et al. (2002), first morning urine samples were collected into vials containing glycerol at a final concentration of 7%. The glycerol prevents freeze-induced activity loss of LH and FSH (Kesner et al. 1995). The samples were stored in the participant's freezer (–20°C) until the end of the study, then shipped en masse in dry ice by express courier to the analytical laboratory. LH, FSH, estrone 3-glucuronide (a metabolite of estradiol), and pregnanediol 3-glucuronide (a metabolite of progesterone) were all successfully measured in these samples.

Although reproductive hormones are fairly stable in urine and home collection of urine for metabolite analysis has been carried out in collection vials such as those described above, a more convenient method for a large longitudinal cohort study might be to develop a system for home collection of urine samples on filter paper. Such samples would be even easier to store until collection or mailing. In most cases they could be mailed in an envelope at ambient temperature, speeding delivery and minimizing costs. Furthermore, storage of filter paper requires less space than vials, an important consideration for long-term studies where tens or hundreds of thousands of samples are accumulated. Such a convenient system would facilitate daily or more frequent

sampling with minimal inconvenience to study participants. The question remains as to what can be measured from such biospecimens. Shideler et al. (1995) used samples collected on filter paper for analyzing steroid hormone (estrogen and progesterone) metabolites in urine. Hormone metabolite analysis of the paper-stored samples was comparable to results obtained from analyses of the original liquid samples. Furthermore, storage of up to 1 year had no effect on hormone concentrations. This technology may also lend itself to the analysis of many other metabolites, including those derived from pesticides, drugs, and other toxicants. For example, McCann et al. (1995) quantified orotic acid (vitamin B13) from such samples. Despite these findings, it is clear that further pilot studies examining the optimal filter paper to use and the range of metabolites that can be detected need to be carried out before this method of storing and shipping home-collected specimens can be considered more seriously.

Collection of urine from infants, potentially one of the most highly sampled subgroups in a children's longitudinal study, offers a unique challenge. Several approaches have been used, including collection pads (placed inside diaper), U-bags, and clean catch into sterile bottles. A study by Liaw et al. (2000) found that all approaches were equally effective at excluding infection and avoiding contamination of samples. However, parents preferred collection pads because they were easier to use and most comfortable for the infant. Robertson and Fortmann (Unpublished data) are attempting to simplify the process even further by developing a system for extracting urine from disposable diapers and analyzing it for biomarkers of pesticide exposure and creatinine.

Collection of samples from toilet-trained children (approximately 2–5 years of age) is less challenging, though special measures may still be needed. For example, in a study of organophosphorous pesticide exposure by Curl et al. (2003), parents were provided with a commode specimen collection pan and polypropylene bottles to store the samples. Children urinated either into the commode inserts, the contents of which were then poured into a polypropylene bottle, or directly into the bottles. Urine collection bottles were stored inside the plastic container in the families' refrigerators overnight until researchers retrieved them the following day.

***Blood*. What can be measured in blood?** Blood is a relatively accessible and informative tissue, although its collection is somewhat invasive and many study participants refuse to donate blood specimens for research purposes. Numerous naturally occurring biochemical molecules can be found in blood, including hormones, various other proteins, and chemical metabolites. Environmental pollutants such as organochlorine compounds (Mussalo-Rauhamaa 1991), dioxins (Smith et al. 1992), polychlorinated biphenyls (PCBs) (Schuhmacher et al. 2002), hexachlorobenzene, and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p*,*p*′-DDE) (Becker et al. 2002) can also be measured in blood. Metals such as beryllium, cadmium, manganese, mercury, and lead (Becker et al. 2002; Schuhmacher et al. 2002), and drugs such as nicotine (or its metabolite cotinine), cocaine, and caffeine (Dempsey et al. 1998) can be measured in blood as well. Nucleated cells (primarily leukocytes) can be obtained from blood. These are useful in that they can be cultured *ex vivo* to examine how they respond to various cytokine and chemical exposures, which may give some idea how the individual would respond if exposed to the same. Leukocytes can also provide RNA for gene expression analysis (Rockett et al. 2002) and DNA, which can be used for polymorphism and sequencing analysis and to detect chromosomal aberrations (Sorokine-Durm et al. 1997) and DNA adduct formation (Poirier 1997).

How can collection of blood be conducted in the home environment? Proper extraction, handling, and transportation procedures are most important in blood collection to prevent injury to the donor, protect the collector from accidental exposure to infectious microbes, and maintain specimen integrity. With few exceptions, the home collection of blood requires the presence of a trained phlebotomist. Even so, if a blood draw is prepared and transported under improper conditions, laboratory test results can be altered. A recent review by Becan-McBride (2002) examines in detail how samples collected in nonclinical environments such as the home should be transported to avoid specimen transportation errors. Depending on the analyte(s) to be measured, the blood may need to be kept within a certain temperature range. It is also necessary to use appropriate collection tubes. A variety of tubes are available from several suppliers. Different additives are included in the tubes depending on what the sample will be used for. For example, sodium heparin and sodium–EDTA tubes are generally used where trace element, toxicology, and nutritional analyses will be conducted. Potassium oxalate/sodium fluoride tubes are used for glucose determinations. Tubes containing clot activators and gel are used for preparing serum for hormone and other analyses. For further details see the BD Vacutainer tube guide (Becton, Dickinson and Company 2003). Other tubes are available (PaxGene blood collection tubes; Qiagen Inc., Valencia, CA; http://www1.qiagen.com/default.aspx) whose contents "freeze" cell transcription and preserve RNA for gene expression studies, though these are currently approved for research use only.

In most cases, unnecessary shaking must be avoided to prevent hemolysis, and samples must sometimes be returned to the analysis laboratory within a strict time frame to permit certain assays to be performed.

Blood is transported in a collection tube. However, as with urine, studies determining the value of collection and transportation of blood specimens on filter paper have been conducted. The idea of collecting blood samples on filter paper has been around for more than 30 years (Hill and Palmer 1969). Parkes et al. (1999) recently reported an at-home test that combined a filter paper technique for spotting capillary blood with an immunoturbidometric assay for measuring hemoglobin A1c. Others have successfully used a similar approach to measure glucose (Ward et al. 1996) and HIV infection (Spielberg et al. 2000), and these discoveries have prompted the commercial development of home collection kits. At least two companies currently market products for consumers to check their HIV status. Both kits require the individual to collect a small blood sample from her/his fingertip and mail it to a designated medical laboratory for analysis. Study data seem to support the accuracy and reliability of HIV testing through at-home collection, demonstrating 99.9% accuracy.

Other companies (e.g., FlexSite Diagnostic Inc., Palm City, FL; http://www.flexsite.com/pgs/3.html) specialize in the development of products and services that allow complex diagnostic tests to be conducted in the home and other nonlaboratory settings. A FlexSite product near release is a cholesterol profile kit that uses a patented device to collect a dried blood sample for measuring total cholesterol, high-density lipoprotein cholesterol, and triglycerides, along with a computed low-density lipoprotein cholesterol. The device (SerSite) separates serum from blood cells as it absorbs the blood. The blood sample is then mailed in a dry state to the FlexSite clinical laboratory for analysis. Although such kits exist or are under development, they are marketed primarily to medical workers who take patient samples outside the clinical environment and thus are not designed for use by the general public.

Home collection of blood is a limited option for large studies for the following reasons:

- Most important, obtaining reasonable quantities of blood (a few milliliters) via a venous puncture is a somewhat invasive procedure with possible adverse side effects if carried out incorrectly. As such, it requires the supervision or assistance of a trained phlebotomist.
- Although small amounts of blood can be obtained by finger prick, many people are reluctant about lancing their fingers or are hesitant about the sight of their own blood.

- Blood is often harder to obtain from children and parents are sometimes reluctant to approve the procedure.
- Many potentially useful analytes in blood are labile and samples require either immediate processing or a controlled storage of the type not normally found in the home.

Thus, although blood is one of the most useful of biospecimens on which a variety and types of assays can be conducted, home collection may not be feasible for a large epidemiologic study that relies on study participants alone for the collection of blood. Perhaps the only exceptions to this are certain groups of individuals (e.g., diabetics, nurses) who are trained and/or are familiar with taking small samples of their own blood. Such people may provide good cohorts for blood-based subsampling studies.

Semen. **What can be measured in semen?** Of late, semen collection is receiving increasing attention with respect to home collection. Semen can be analyzed to evaluate sperm characteristics and to measure hundreds of biological and chemical components of seminal fluid including steroid hormones, sugars, vitamins, enzymes, proteins, and metals. In addition, environmental exposures can introduce xenobiotic compounds such as pesticides and heavy metals into seminal fluid (Kumar et al. 2000). This has two possible ramifications. First, many kinds of exposures and chemicals affect human sperm quantity and quality. Second, the vagina absorbs a number of components of semen that can be detected in the female bloodstream within a few hours of sexual intercourse (Benziger and Edelson 1983; Sandberg et al. 1968). This has possible implications for the exposure of embryos and fetuses to components of seminal fluid by both intracanicular and bloodborne routes and may even serve as a route of exposure for women.

Many xenobiotics, metals, and naturally occurring biochemical components of semen have been measured to determine how their presence relates to fertility (Lay et al. 2001; Younglai et al. 2002). In addition to measuring biochemical components of the semen, one can conduct routine sperm analysis (concentration, motility, morphology) (Davis and Katz 1989; WHO 1999), evaluate more specific markers of sperm function such as genetic and chromosome integrity (Evenson et al. 2002; Perreault et al. 2000), and conduct gene expression profiling experiments (Ostermeier et al. 2002).

How can collection of semen be conducted in the home environment? Various investigators have developed prototype collection and transportation kits, such as the TRANSMEM100 (Royster et al. 2000). These kits can be distributed to study participants, and then collected by the study personnel, delivered by the participant, or shipped directly to a central laboratory for analysis once the sample has been collected. The TRANSMEM100 collection system was intentionally made simple, requiring only that the subject collect the semen sample in a toxicology-tested specimen jar, place the jar in a biohazard bag and secondary container, close the package, and call the overnight courier for pickup. Illustrated instructions are included with the kit. The initial pilot study on the utility of the TRANSMEM100 indicated that 65–80% of the samples were received in the laboratory the day after they had been collected and were of sufficiently good quality to carry out a number of standard measurements such as semen volume, and sperm number, concentration, and morphology.

Recently, the TRANSMEM100 was tested for sample stability with regard to newer, more specific tests of sperm nuclear integrity. For example, the sperm chromatin structure assay (SCSA; SCSA Diagnostics, Inc., Brookings, SD; http://www.scsadiagnostics.com/) detects increased susceptibility to acid-induced DNA damage in sperm and is a measure of sperm genomic integrity (Evenson et al. 2002). SCSA results were comparable when semen samples were frozen right after collection or after 24-hr storage at 4°C, but the percentage of abnormal cells increased significantly if samples were kept at room temperature for the 24 hr before freezing (Morris et al. 2003). These findings indicate that inclusion of cold packs during overnight shipment would be necessary to ensure sample stability for this assay. On the other hand, an assay measuring chromosome breakage in sperm gave comparable results in fresh semen and semen stored at room temperature for 24 hr (Young et al. 2003).

Other approaches range from the relatively advanced Bio-Tranz (Zavos Diagnostic Laboratories, Inc., Lexington, KY; http://www.zdlinc.com/index.htm) shipping system (Zavos et al. 1998), for shipping semen at low temperature in protective medium to allow clinical diagnosis of infertility, to a simple process in which semen was frozen in condoms by study participants and later collected by the study organizers (Arbuckle et al. 1999). Although such simplified storage and collection procedures reduce the number of fecundity markers that can be measured in fresh, whole semen, they can still be used to measure the presence of pesticide metabolites and other stable biochemical molecules.

Although none of these home-collection systems have been thoroughly characterized for their ability to maintain the integrity of all the numerous analytes that can be measured in semen, they have clearly proven useful for measuring a number of useful parameters (e.g., certain sperm measures and pesticide levels). There is clearly a trade-off between the cost and complexity of the transportation system and the number of end points that can be measured in the laboratory. These considerations will need to be weighed in relation to study purpose to determine their ultimate applicability for field-based research.

Other Potential Biospecimens for Home Collection

Biospecimens with the potential for home collection need to be informative, accessible, and easy to produce. Saliva, milk, hair, hair follicles, nail, and buccal cells may also prove to be viable alternatives, depending on the biological marker being measured, and have been used with varying degrees of success.

Saliva. Steroid hormones normally are measured in urine and blood. However, several can also be measured in saliva, including dehydroepiandrosterone (DHEA), dihydrotestosterone, testosterone, estradiol, estrone, progesterone, and androstenedione cortisol. The use of saliva as a biospecimen offers several advantages:

- Ease of use: Saliva specimens can be collected anywhere at any time and at a much lower cost than blood collection. For example, collection of saliva samples from highly mobile flight attendants also was reported to produce high-quality samples for analysis (Whelan et al. 2002).
- Saliva collection is noninvasive and less stressful than venipuncture and thereby less likely to alter markers responsive to physiologic/psychologic stress.
- Saliva collection is more feasible when collection at timed intervals is desired (e.g., early morning).
- Hormones in saliva are exceptionally stable. They can be stored at room temperature for at least a week without loss of activity.

The choice of a saliva collection method should be tailored to the individual hormones to be quantified, as studies have shown that certain types of collection methods, such as the cotton-based Salivette system (Sarstedt, Newton, NC; http://www.sarstedt.com/php/main.php?SID=c1a82144d94e2efe0f11ec7ba74b3f72&language=en), can produce artificially elevated levels of certain hormones, including DHEA, testosterone, and estradiol (Granger et al. 1999a, 1999b; Shirtcliff et al. 2000, 2001). With this in mind, several methods are available currently for home collection of saliva. The first is to request study participants to expectorate up to 3 mL of saliva into a wide-mouthed container over a period of 10 min (Riad-Fahmy et al. 1987). The second is to ask participants to chew on a 6-inch cotton dental roll (Hertsgaard 1992). A portion of the saturated roll is subsequently placed into a needleless syringe and the saliva expressed into vials for analysis. The most common method, however, is to use the commercially available Salivette collection system.

Study participants are asked to chew on a polyester roll for 3 min before placing it into a plastic tube for shipping and analysis. Once the sample arrives at the laboratory, the tube is centrifuged to recover the saliva. Although three different versions of the Salivette system are available, studies have shown that the polyester insert form without citric acid crystals is generally the most appropriate for research purposes (Lamey and Nolan 1994; Schwartz et al. 1998), as the use of citric acid crystals to stimulate saliva flow can interfere with certain assays by lowering the pH of the sample (Schwartz et al. 1998). Salivette samples will begin to mold after 4–7 days; thus, it is recommended that they be stored at –20°C, if possible. Samples can later be shipped (on dry ice) to the testing facility via regular mail.

Of course there are also limitations to using saliva. The number of hormones that can be measured in saliva is fewer than can be measured in blood, and unlike blood, saliva does not provide live cells for other types of studies such as RNA expression analysis.

Breast milk. Research on chemical contaminants in breast milk spans several decades and dozens of countries. Results indicate that a wide range of chemical contaminants may enter breast milk, including organochlorine pesticides, PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), polybrominated diphenyl ethers, metals, and solvents (Solomon and Weiss 2002). These findings have highlighted gaps in current knowledge about this postnatal route of exposure, including the lack of information on the nature and levels of contaminants in breast milk and the lack of consistent protocols for collecting and analyzing breast milk samples. Breast milk contaminants are of particular interest where breast-fed infants are concerned, as many of the contaminants identified thus far have developmental effects in rodent models. Developmental effects in humans are not well characterized, and there is a general lack of data on health outcomes that may be produced in infants by exposure to chemicals in breast milk. However, in the studies conducted thus far, there is evidence that exposure to PCBs both pre- and postnatally through breast milk does have subtle negative effects on neurologic and cognitive development of children up to school age (Vreugdenhil et al. 2002; Walkowiak et al. 2001). Reproductive effects may also occur. For example, Blanck et al. (2000) found that *in utero* and lactational exposures to polybrominated biphenyls were associated with an earlier age of menarche.

Despite such studies, there remains a general paucity of data on outcomes related to infant exposure via breast-feeding, particularly those with a time-dependent nature. This information is necessary for performing exposure assessments without heavy reliance on default assumptions. Landrigan et al. (2002) thus called for "a carefully planned and conducted national breast milk monitoring effort in the United States" to provide the information needed to assess infant exposures through breast-feeding and to develop scientifically sound information on the benefits and risks thereof.

Collection of milk in the home environment for contaminant analysis is relatively simple. In a recently completed prospective pregnancy study that recruited women from 16 counties upon stopping birth control, women who later gave birth and initiated breast feeding were asked to provide at least one breast milk sample using a standardized protocol (100% compliance). Mothers successfully collected and shipped fresh milk samples along with a freezer pack to a toxicologic laboratory via Federal Express (Buck et al. 2002). Frozen breast milk samples have been used to analyze levels of PCBs, PCDDs, polychlorinated dibenzofurans, and numerous organochlorine-based compounds (Hooper et al. 2002). Further information on the collection and archiving of human milk may soon become available from the U.K. Department for Environment, Food and Rural Affairs. The department is currently cofunding a pilot project for establishing a U.K. human milk archive of representative samples that will be available for chemical analysis for up to 10 years (H.M. Government Department for Environment, Food and Rural Affairs 2002). The study aims to develop methods for recruiting participants, establish robust procedures for collection, transport, storage, and analysis of breast milk, and perform initial analysis for PCBs, dioxins, and phytoestrogens. Data from this study will provide information on temporal trends of environmental contaminants and an assessment of infant risk from exposure via human milk.

Hair/nail. The main advantage of using hair and nail is that is that they can be collected in a safe and noninvasive manner. However, being keratinous in nature and containing no living cells, hair and nail are not often considered of high value for many epidemiologic and exposure studies. Nevertheless, they may be useful biospecimens under certain circumstances. Hair, for example, has been used for decades as a timeline for exposure to heavy metals. Many heavy metals impact fertility and fecundity, either through direct toxicity in the reproductive organs, adversely affecting the endocrine system, or both. Lead, chromium, and cadmium, for example, reduce human semen quality (Li et al. 2001; Telisman et al. 2000). Female exposure to mercury alters estrous cyclicity in rats (Davis et al. 2001), and recently, blood lead levels were negatively associated with puberty milestones in girls (Selevan et al. 2003; Wu et al. 2003). Hair analysis can indicate exposure to numerous toxic metals including mercury, lead, arsenic, aluminum, and cadmium, and numerous other metals including calcium, zinc, manganese, cobalt, iron, potassium, sodium, and titanium. Although a useful tool for detecting chronic exposures, hair is not suitable for detecting very recent metal exposures (a blood test is required for this).

The clipping, storage, and transportation of nail and hair is clearly a simple task that can be conducted by most individuals. If the hair is long enough, it is generally taken from the nape of the neck. About an inch in length of the hair closest to the skin is needed in a quantity approximating a heaped teaspoon full. For bald men or those reluctant to donate head hair, a viable alternative is to take samples from the under arm or pubic area.

A little-tested use of hair and nail is gene sequencing. Mitochondrial (mt)DNA can be found in hair and nail (Anderson et al. 1999; Schreiber et al. 1988). Some groups have successfully tested hair and nail as an alternative to blood DNA for genotyping of polymorphic drug-metabolizing enzymes (Tanigawara et al. 2001).

One possible disadvantage of using hair and nail is that they can be easily contaminated with extraneous biological and chemical material (e.g., dirt under the nails; shampoo and dye residues in hair) that can sometimes complicate tissue analysis. Hair chemicals, including dyes, can contain lead that will attach to the hair and may contaminate the sample. The most accurate results thus come from hair that has not been chemically treated for at least 2 months.

Hair follicles. An aggressive pluck of a human hair will usually remove the root follicle along with the hair. In about 90% of cases, this specimen (trichogram) will be of a hair in the actively growing phase (anaphase), and therefore likely to yield sufficient quantities of good-quality RNA to support gene expression analysis. RNA is notoriously quick to degrade in samples once they are detached from a body. Therefore, to maintain the integrity of the RNA, such samples normally need to be processed quickly. Ambion, Inc. (Austin, TX; http://www.ambion.com/) has recently developed a storage product called RNALater, an aqueous, nontoxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA (Ambion 2003). Once in RNALater, RNA is stable for 1 week at room temperature, 1 month at 4°C, or indefinitely if frozen. These properties could be used to develop a simple kit that permits the home collection, storage, and shipping of hair follicles without specialized protocols or equipment.

Previous studies have found the yield of RNA from such human hair follicles to be in the region of 0.9 μg per whole follicle (Mitsui et al. 1997). This is sufficient for small numbers of limited gene expression profiling analyses

using reverse transcription-PCR (RT-PCR). RT-PCR–based gene expression profiling carried out on RNA extracted from hair follicles has yielded information on gene expression of growth factors (Mitsui et al. 1997) and enzymes (Chang et al. 1997). Unfortunately, RT-PCR assays are a rather limited form of gene expression analysis in that they can measure expression of only a few genes at a time. The recent development of DNA arrays (see reviews by Rockett and Dix 1999, 2000) has overcome this problem. Such arrays can detect the expression of many tens of thousands of genes simultaneously. Unfortunately, most require more RNA than can be obtained from a few hair follicles. The solution may be to incorporate a preamplification step prior to labeling and hybridization of the RNA sample. Fink et al. (2002) used this approach successfully in carrying out microarray analysis of RNA extracted from laser capture microdissection samples. Although the parity between array data from preamplified and regular RNA samples has yet to be fully established, early indications are that the gene expression patterns are fairly comparable. Alternatively, rolling circle amplification of the bound probe following hybridization to the microarray (Nallur et al. 2001) may prove a viable alternative.

In the future such studies using RT-PCR or gene array analysis may provide information on exposures through the identification of certain gene expression patterns. However, this approach is currently in embryonic form and will probably be without practical application for 5–10 years.

Buccal cells. Perhaps the most useful application of buccal cells (the epithelial cells lining the inside of the cheeks) is as a source of DNA for genotyping studies. However, buccal cells have also been used as a source of material for immunoassays (Byrne et al. 2000) and appear to be a good source of tissue for monitoring human exposure to inhaled and ingested occupational and environmental genotoxicants. Results of a study by Burgaz et al. (2002) suggested that occupational exposure to organic solvents may cause cytogenetic damage in buccal cells and that use of exfoliated buccal cells appears appropriate to measure exposure to organic solvents.

These studies together demonstrate how the same samples can sometimes be used to measure markers of both exposure and effect, thus helping to maximize the amount of useful information that can be obtained from a sample and improving the cost-benefit ratio.

Exfoliated buccal cells can be collected quickly, easily, and conveniently. Several methods of collection have been described, including the use of special cards (Harty et al. 2000a), cytobrushes (Garcia-Closas et al. 2001), cotton swabs (Koletzko et al. 1999), saline rinses (Hayney et al. 1995), and mouthwash. Lench et al. (1988) originally demonstrated that sufficient human DNA for gene analysis can be isolated from buccal cells obtained by mouthwash. Lum and Marchand (1998) later confirmed this, showing that good-quality DNA suitable for PCR-based genotyping could be obtained from 10 mL undiluted commercial mouthwash swilled in the mouth for 60 sec, then expelled into a collection container.

For home collection of buccal cells (at least for DNA analysis purposes), the aforementioned studies and others have shown that collection using the mouthwash approach gives greater yields than other methods and is feasible for use in cohort studies. Lum and Marchand (1998) reported that storage of the unprocessed specimens at room temperature or at 37°C for 1 week (temperature conditions that may be encountered when mailing samples) did not affect the DNA yield or ability to PCR amplify the samples. Study workers for the National Birth Defects Prevention Study (2003) have successfully developed and used kits for the collection of buccal cells, which are sent to study participants through the mail (Rasmussen et al. 2002). The kit contains an informed consent form, simple instructions, materials for collecting the specimens, a small monetary reimbursement in the form of a money order, and a prepaid U.S. Mail packet for specimen return.

The main caveat of using buccal cells for DNA is that researchers should be aware of the likely presence of nonhuman DNA in the extracted specimens. This can originate from food residues and from microflora that live in the digestive system and respiratory tract. Such nonhuman DNA is normally not problematic for PCR and other hybridization studies as long as appropriate (i.e., specific) probes and primers are selected.

Vaginal swabs. Bacterial vaginosis (BV) is an alteration of the vaginal flora where the normally predominant *Lactobacilli* are replaced by a cocktail of other organisms, including those responsible for sexually transmitted diseases (STDs) (e.g., *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Trichomonas vaginalis*). BV has been associated with a number of adverse outcomes in pregnant women, including late miscarriage, premature rupture of membranes, preterm delivery, postpartum sepsis and postpartum endometriosis (Gravett et al. 1986; Hay et al. 1994; Jacobsson et al. 2002). Prevalence of BV varies among different groups of women, but recent studies suggest that between 4 and 61% may suffer from the disease, including as many as 20% of pregnant women and 12% of adolescent virgins (Priestley and Kinghorn 1996).

The diagnosis of BV normally requires the procurement of vaginal swabs. These can be used to identify agents of infection through microscopic observation, microbial culture, or nucleic acid amplification technologies. There is evidence to suggest that collection of vaginal swabs may be amenable to home-based strategies and have the added benefit of increasing participation. In an office-based study by Smith et al. (2001), participants were offered the choice of STD screening in the context of a traditional pelvic examination or the use of self-obtained vaginal swabs. All eligible participants chose the latter, suggesting that most female patients are comfortable obtaining such samples. In a different approach, clinical staff were successful in visiting the homes of community-based trial participants and collecting self-administered vaginal swabs (Wawer et al. 1998). Compliance with interview, sample collection, and treatment in this study was over 90%.

The home collection of vaginal swabs raises two issues. The first is the potential biohazard issue to other family members or visitors, although this could conceivably be overcome by the use of carefully designed containers. The second is that storage of samples or delay in getting them to the laboratory may adversely impact identification of infectious agents using standard microscopic or culture techniques. An appropriate method for the collection, storage, and transportation of self-administered vaginal swabs thus needs to be determined. Alternatively, nucleic acid amplification methods are available (Smith et al. 2001) that are both sensitive and adaptable to high-throughput assays.

Home-Based Analysis of Biospecimens

Recent increases in technology now permit participants to not only collect biospecimens at home, but also analyze them. Using commercially available kits, individuals are now able to track the timing of their fertile window, identify pregnancies, and estimate sperm concentrations in semen, all in the privacy of their own home. These new techniques offer promise for research purposes, as they allow investigators to collect information without the time and cost associated with having samples analyzed at a remote location.

Home fertility monitors. Home fertility monitors are now available that allow women to detect both the occurrence and timing of ovulation. Several kits are commercially available, including ClearPlan Easy (Unipath Ltd., Bedford, Bedfordshire, UK; http://www.unipath.com), Ovuquick One-Step (Quidel Corp., San Diego, CA; http://www.quidel.com/Home.php), and Surestep (Applied Biotech, Inc., San Diego, CA; http://www.abiapogent.com). The kits work by detecting the LH surge in urine with varying levels of sensitivity, ranging from 35 mIU/mL

(SureStep) to 50 mIU/mL (ClearPlan Easy) (Nielsen et al. 2001). The ClearPlan Easy kit offers an advantage over the other methods in that the system will store basic fertility data for up to 6 months. The information is stored on a data card that can be easily transported to the study site for download to a personal computer. The information stored includes the start date of each cycle, the cycle length, the date the LH surge, and the dates of intercourse. Other home-based ovulation detection systems, such as the Lady Free Biotester (TK Yun, South Korea) and the TCI OvuLook (TCI Optics, Inc., Kapaau, HI; http://www.ovulook.com/), detect ovulation based on salivary ferning (Barbato et al. 1993). The latter also has a built-in tracking system that allows women to see and refer back to their saliva patterns over time, thus providing a system that could be useful in epidemiologic studies.

Home pregnancy tests. In 1999 approximately 19 million home pregnancy test kits were sold in the United States (Lipsitz 2000). Among a sample of women with children, approximately 33% reported using a home pregnancy test prior to seeking care from a professional (Jeng et al. 1991). The currently available home pregnancy test kits use monoclonal antibodies to detect hCG in urine.

Although the manufacturers of home pregnancy test kits claim that they are 97–99.5% accurate, recent work suggests that the individual sensitivities and specificities vary somewhat by brand when the products are used in the general population (Bastian et al. 1998). Many kits instruct women to test their urine as early as the day that menses is expected. However, using women's self-reported average cycle lengths, Wilcox et al. (2001) estimated that the maximum possible sensitivity on the day that menses is expected is 90%. Only after 1 week was the sensitivity found to be about 97%. The high false-negative rate seen among home pregnancy tests has been attributed to two factors. Without the aid of a fertility monitor to pinpoint ovulation, women who ovulate late in their cycle may end up testing too early (i.e., before implantation has had time to occur). Comprehension of testing protocols needs to be established to ensure that study participants are using kits correctly and yielding valid data. We are aware of only one study that reported difficulties associated with understanding the instructions in home pregnancy tests. In this study Daviaud et al. (1993) reported that 230 of 478 positive pregnancy tests were falsely interpreted as negative, with difficulty in understanding the directions being cited as the primary reason for the error.

Although there may be some concern that home pregnancy tests are marketed to the general public as being highly sensitive and specific throughout testing, they remain an extremely valuable data collection methodology in a research setting. Home pregnancy test kits allow investigators to detect early losses in situations where the collection of urine for analysis at a remote location is not feasible. Through careful instruction, women can be shown how to use the kits in a manner that reduces the chances of generating false data.

At-home screening for male infertility. Recently, an at-home test kit became available (FertilMarQ*;* Embryotech, Wilmington, MA; http://www.embryotech.com) that allows men to evaluate one aspect of semen quality, sperm concentration, in the privacy of their home. According to the instructions, semen samples are to be collected by either masturbation or intercourse (with a special condom) following a 3-day period of abstinence. After allowing the semen to liquefy in the provided cup for at least 15 min, it is transferred to the testing well via dropper and combined with the appropriate reagents. A light blue color in the test well indicates that the individual has a sperm concentration of < 20 million/mL, which is consistent with the operational definition for oligospermia (Rowe et al. 1993). The test is to be repeated 3–7 days after the first test to confirm the finding. According to the manufacturer, the overall accuracy of the test is 78%. Although the test cannot provide a definitive answer regarding the presence of male infertility, it may serve as a useful screening tool in certain populations.

Limitations of Home-Based Collection and Analysis of Biospecimens

Noncompliance. In many cases, home collection of specimens may seem to be an ideal solution to some of the limitations of clinic-based studies. However, it is not without its potential problems. Although the convenience factor may be an important consideration, it is prudent to recognize that the advantages of home-based sample collection are potentially offset by noncompliance with collection and storage instructions. For example, with respect to many sensitive reproductive end points such as hormonal profiles, the need for specimen collection timed to a menstrual cycle or to accommodate diurnal or other fluctuations can be more difficult (though not impossible) to capture successfully in the home.

Range of assays. Many potentially useful assays and experiments cannot be conducted on biospecimens collected and stored in the home. Home collection clearly lacks the clinical and/or scientific environment (i.e., equipment and specialized training) necessary to collect and process certain analytes. For example, if live cells are needed (e.g., blood cells for *in vitro* exposures or semen for sperm motility measures), the samples need to be collected in an environment where they can be processed quickly before the cells die.

Introduction of error. A further complicating factor is that many laboratory and health-care workers overlook the impact of specimen collection, storage, and transportation on medical errors. Specimen collection and transportation originating outside the laboratory can increase laboratory error rates. Indeed, 46–68% of laboratory errors occur in the pre-analytical rather than in the analytical and postanalytical phases (Boone et al. 1995; Plebani and Carraro 1997). Thus, when planning an epidemiologic study involving the collection of field specimens, the nature of the specimens and the types of measurements that will be derived from them must be carefully considered to determine the best way to obtain, store, and transport the specimens to maintain the integrity of the target parameters (e.g., steroid hormones, pesticide metabolites, DNA, RNA).

Transportation of biospecimens. Movement of samples from the homes of study participants to the clinic or analytical laboratory is perhaps the main factor when considering the suitability of home collection, as it usually involves the most time and expense and increases biohazard concerns. Transportation can take place via one of three modes:

- Conveyance to the clinical office by the study participant. Again, transportation is normally at ambient temperature. This approach may reduce costs slightly, facilitate more rapid transfer of the sample, and reduce the chances of the sample being lost or damaged in transit.
- Collection by study staff. This may facilitate maintenance of samples in a more controlled environment (e.g., frozen) but is more costly than the other two methods.
- Shipping by mail or courier service. In most cases, samples are shipped at ambient temperature. This is acceptable for many analytes. Where necessary (usually in warmer climates), cold packs can be included to maintain a cool temperature during overnight shipments and avoid sample degradation. However, when time is a critical factor (e.g., sperm motility as a part of semen analysis), a courier may be the only (and more costly) alternative.

In terms of biohazard risk, the main pathogens of concern are bloodborne pathogens such as HIV and hepatitis. However, the handlers of any human biospecimens can be at risk of exposure to these or other pathogens. The risk is minimized when the patient brings samples into the clinic. Risk is increased for study workers who visit the subject's home to obtain the sample (e.g., draw blood) and transport it. In these cases the safety of the study worker and participant

(or his/her family) is increased by following validated stepwise procedures designed to reduce the chances of sample exposure. The collection of liquid samples presents the greatest risk to those who collect and transport them, and there should be established protocols for carrying equipment and samples. For example, all equipment and samples should be transported in a sturdy lockable container with specimens inside sealed in a secondary container displaying a biohazard label.

The risk of accidental exposure becomes higher when biological samples, particularly in liquid form, are shipped via courier or postal service. Indeed, biological specimens should not be sent by courier or through the mail unless they are contained in an approved shipping container. In the United States, this means that the containers must comply with U.S. Postal Service regulations (USPS 1999), Centers for Disease Control and Prevention regulation 42 CFR72 (CDC 1999), and Department of Transportation regulation 67 FR 53118 (DOT 2002), all of which address the shipment of clinical specimens. Various companies produce such containers. Doxtech (Beaverton, OR; http://www.doxtech.com/), for example, produces a high security container for collection, transport and storage of specimens for "drug testing, forensic evidence, food samples and potable water samples." Quorpak (Bridgeville, PA; http://www.qorpak.com/) produces a two-part specimen mailer manufactured to meet government regulations for mailing liquids in glass or plastic. The outer container has a fiberboard body with a waxed inside liner, and the metal neck is securely crimped to the fiber body. The inner container, which holds the specimen, is a high-density polyethylene bottle with a polypropylene closure and polyethylene foam liner. The increasing number of biospecimens being sent through the mail has even prompted some postal agencies to design special single-use containers specifically for this purpose. The Royal Mail Service in the United Kingdom recently launched Safebox, a prepaid packaging concept for medical, veterinary, and pharmaceutical samples (First Filtration International, Banglamung, Thailand; http://www.firstfiltration.com/safebox.html). Safebox is a tough plastic container that is delivered by the Royal Mail as part of a normal postal delivery. It is opened at the laboratory by pulling off a tear strip and because the inner chamber is transparent, any leakage can be detected without risk of physical contact. If leakage does occur, it is absorbed by the Aqui-Pak system (First Filtration International), a protective packaging material for the transport of pathological specimens. Aqui-Pak complies fully with secondary packaging instructions issued by the USPS and Royal Mail. Finally, because the Safebox is designed for single use only, there is no risk of cross-contamination from packaging materials being reused.

In some cases field specimens may be originate outside the country where the analytical laboratory is based. Since the World Trade Center tragedy, the international transportation of hazardous or potentially hazardous agents has come under increased scrutiny. Given that all human samples are considered potentially hazardous, investigators are responsible for adhering to packaging and shipping standards issued by the International Air Transport Association (IATA). The IATA produces both regulation and training literature for dangerous goods, and various IATA-endorsed training programs are offered by companies specializing in the provision of such training.

Conclusions

Though there are a number of caveats, the use of home-based collection of biospecimens in epidemiologic studies, including those focusing on sensitive reproductive end points, should assist investigators in obtaining high participation rates and thereby minimize misclassification bias regarding either exposure or effect. This strategy, in addition to all the other advantages discussed, helps to ensure valid study conclusions while filling critical data gaps. For example, couple-based prospective pregnancy studies designed to assess effects of parentally mediated exposures before, at, or shortly after conception could benefit from home-based biospecimen collection, which would make it easier to obtain samples at specific times over the course of menstrual cycles, pregnancy, and lactation. Such approaches offer promise for ascertaining data to fill critical data gaps such as the toxicokinetics of environmental contaminants across pregnancy and lactation as well as their effects, if any, on sensitive markers of human reproduction and development.

Urine and blood continue to lead the way as the most popular biospecimens obtained in epidemiologic studies. This is mainly because they are both accessible and informative for a large number of clinically important parameters. The methodology for collecting, storing, and transporting these types of sample is also well established for measuring a number of parameters. Urine particularly appears to offer a good way forward, as it is relatively easy to collect from all age groups, and for many analytes the sample can be stored from room temperature to –20°C, conditions that can be accommodated in most residential dwellings. However, in terms of home-based collection, blood is somewhat problematic in that realistically it takes visits by trained phlebotomists to obtain samples. The only benefit this might offer is convenience for study participants, which might increase participation rates/decrease drop-out rates in epidemiologic studies.

Although urine and blood have formed the mainstay of biospecimen collection in most field-based epidemiologic studies to date, a number of other accessible biological samples can provide useful and complementary information on a wide range of physiologic indicators and toxicant exposures. Those receiving the most attention include saliva and semen. Studies have shown that these can provide information on hormone levels (saliva) as well as information on testicular function and chemical exposures (semen). These samples are easy to collect and can be stored in the home environment.

Samples that are accessible and potentially informative but have received relatively little attention include breast milk, hair follicles, buccal cells, nail clippings, and vaginal swabs. Breast milk appears to be an obvious biospecimen for analysis in studies such as the National Children's Study that focus on child health. It can be used to monitor body burdens in reproductive-age women and it estimates *in utero* and nursing-infant exposures. However, the range of biochemical targets that can be robustly measured in milk, the sensitivity of milk-based assays, and the effects of storage on such sensitivity have only recently started to be properly assessed.

This situation is much the same for hair follicles and nail clippings, whose main use may be to provide nucleic acid for gene expression profiling and gene polymorphism analysis, respectively. It appears unlikely that nail clippings will displace buccal cells as convenient sources of DNA for sequencing and polymorphism analysis in studies of older children and adults, as a substantial body of literature suggests that buccal cells can be conveniently collected, stored, and transported from the home environment and provide high yields of good-quality DNA. However, for studies of infants and young children, the nail-clipping method may be an ideal alternative.

Though home collection of biospecimens appears to offer advantages over clinic-based studies in terms of participation rates and reduced costs in certain circumstances, definitive assessments of cost-benefit compared with clinic-based studies are needed to verify this assumption Also needed are formal assessments of collection, storage, and other quality control issues associated with home collection, particularly for the less-studied specimens such as nails, buccal cells, or vaginal swabs. When suitable operating procedures have been defined, the integration of a wider range of biospecimens than has been the case thus far has the potential to enhance the robustness of epidemiologic studies by helping to characterize the causes of adverse outcomes and facilitate new approaches to identifying biomarkers of exposure, effect, and disease development.

References

Abell A, Ernst E, Bonde JP. 2000a. Semen quality and sexual hormones in greenhouse workers. Scand J Work Environ Health 26:492–500.

Abell A, Juul S, Bonde JP. 2000b. Time to pregnancy among female greenhouse workers. Scand J Work Environ Health 26:131–136.

Ambion. 2003. From Patient to Result: Assure RNA Quality. A Revolutionary Tissue Storage System for RNA Stabilization. Available: http://www.ambiondiagnostics.com/rnalater/ [accessed 10 February 2003].

Anderson TD, Ross JP, Roby RK, Lee DA, Holland MM. 1999. A validation study for the extraction and analysis of DNA from human nail material and its application to forensic casework. J Forensic Sci 44:1053–1056.

Anderson TD, Ross JP, Roby RK, Lee DA, Holland MM. 1999. A validation study for the extraction and analysis of DNA from human nail material and its application to forensic casework. J Forensic Sci 44:1053–1056.

Aprea C, Colosio C, Mammone T, Minoia C, Maroni M. 2002. Biological monitoring of pesticide exposure: a review of analytical methods. J Chromatogr B Analyt Technol Biomed Life Sci 769:191–219.

Arbuckle TE, Schrader SM, Cole D, Hall JC, Bancej CM, Turner LA, et al. 1999. 2,4-Dichlorophenoxyacetic acid residues in semen of Ontario farmers. Reprod Toxicol 13:421–429.

Baird DD, McConnaughey DR, Weinberg CR, Musey PI, Collins DC, Kesner JS, et al. 1995. Application of a method for estimating day of ovulation using urinary estrogen and progesterone metabolites. Epidemiology 6:547–550.

Baird DD, Weinberg CR, Wilcox AJ, McConnaughey DR, Musey PI. 1991. Using the ratio of urinary oestrogen and progesterone metabolites to estimate day of ovulation. Stat Med 10:255–266.

Barbato M, Pandolfi A, Guida M. 1993. A new diagnostic aid for natural family planning. Adv Contracept 9:335–340.

Bastian LA, Nanda K, Hasselblad V, Simel DL. 1998. Diagnostic efficiency of home pregnancy test kits: a meta-analysis. Arch Fam Med 7:465–469.

Becan-McBride K. 2002. Avoiding specimen transportation errors. MLO Med Lab Obs 34:38–39.

Becker K, Kaus S, Krause C, Lepom P, Schulz C, Seiwert M, et al. 2002. German Environmental Survey 1998 (GerES III): environmental pollutants in blood of the German population. Int J Hyg Environ Health 205:297–308.

Becton, Dickinson and Company. BD Vacutainer Tube Guide. 2003.Available: http://www.bd.com/vacutainer/pdfs/plus_plastic_tubes_wallchart_tubeguide_VS5229.pdf [accessed 14 May 2003].

Benziger DP, Edelson J. 1983. Absorption from the vagina. Drug Metab Rev 14:137–168.

Blanck HM, Marcus M, Tolbert PE, Rubin C, Henderson AK, Hertzberg VS, et al. 2000. Age at menarche and tanner stage in girls exposed *in utero* and postnatally to polybrominated biphenyl. Epidemiology 11:641–647.

Boone J, Steindel SC, Herron R, Howanitz PJ, Bachner P, Meier F, et al. 1995. Transfusion medicine monitoring practices. Arch Pathol Lab Med 119:999–1006.

Buck GM, Vena JE, Greizerstein HB, Weiner JM, McGuinness B, Mendola P, et al. 2002. PCB Congeners and pesticides and female fecundity, New York State Angler Prospective Pregnancy Study. Environ Toxicol Pharmacol 12:83–92.

Burgaz S, Erdem O, Cakmak G, Erdem N, Karakaya A, Karakaya AE. 2002. Cytogenetic analysis of buccal cells from shoeworkers and pathology and anatomy laboratory workers exposed to *n*-hexane, toluene, methyl ethyl ketone and formaldehyde. Biomarkers 7:151–161.

Byrne TJ, Reece MT, Adams LA, Hoffman DE, Lane MA, Cohn GM. 2000. A rapid immunoassay predicts BRCA1 and BRCA2 mutations in buccal cells. Oncol Rep 7:1203–1207.

CDC (Centers for Disease Control and Prevention). 1999. Interstate Shipment of Etiologic Agents, 42 CFR Part 72. Available: http://www.cdc.gov/od/ohs/biosfty/shipregs.htm [accessed 24 June 2003].

———. 2003. Second National Report on Human Exposure to Environmental Chemicals. NCEH Publ No 02-0716. Available: http://www.cdc.gov/exposurereport/pdf/SecondNER.pdf [accessed 14 May 2003].

Chang YT, Hyland K, Mues G, Marsh JL. 1997. Human hair follicles as a peripheral source of tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA. Neurosci Lett 222:210–212.

Cherry N, Labreche F, Collins J, Tulandi T. 2001. Occupational exposure to solvents and male infertility. Occup Environ Med 58:635–640.

Curl CL, Fenske RA, Elgethun K. 2003. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. Environ Health Perspect 111:377–382.

Daviaud J, Fournet D, Ballongue C, Guillem GP, Leblanc A, Casellas C, et al. 1993. Reliability and feasibility of pregnancy home-use tests: laboratory validation and diagnostic evaluation by 638 volunteers. Clin Chem 39:53–59.

Davis BJ, Price HC, O'Connor RW, Fernando R, Rowland AS, Morgan DL. 2001. Mercury vapor and female reproductive toxicity. Toxicol Sci 59:291–296.

Davis RO, Katz DF. 1989. Computer-aided sperm analysis (CASA): image digitization and processing. Biomater Artif Cells Artif Organs 17:93–116.

Dempsey DA, Partridge JC, Jones RT, Rowbotham MC. 1998. Cocaine, nicotine, caffeine, and metabolite plasma concentrations in neonates. J Anal Toxicol 22:220–224.

DOT (Department of Transportation). 2002. Hazardous Materials—Revision to Standards for Infectious Substances; Final Rule 67 FR 53118. Available: http://www.cdc.gov/od/ohs/pdffiles/DOTHazMat8-14-02.pdf [accessed 24 June 2003].

Ehrenkranz JRL. 2002. Home and point-of-care pregnancy tests: a review of the technology. Epidemiology 13:S15–S18.

Embleton ND. 2001. Fetal and neonatal death from maternally acquired infection. Paediatr Perinat Epidemiol 15:54–60.

Evenson DP, Larson KL, Jost LK. 2002. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 23:25–43.

Fink, L, Kohlhoff S, Stein MM, Hanze J, Weissmann N, Rose F, et al. 2002. cDNA array hybridization after laser-assisted microdissection from nonneoplastic tissue. Am J Pathol 160:81–90.

Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, et al. 2001. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev 10:687–696.

Garry VF, Harkins ME, Erickson LL, Long-Simpson LK, Holland SE, Burroughs BL. 2002. Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environ Health Perspect 110(suppl 3):441–449.

Granger DA, Schwartz EB, Booth A, Curran M, Zakaria D. 1999a. Assessing adolescent dehydroepiandrosterone in saliva: a simple radioimmunoassay for use in studies of children, adolescents, and adults. Psychoneuroendocrinology 24:567–579.

Granger DA, Schwartz EB, Booth A, Arentz M. 1999b. Salivary testosterone determination in studies of child health and development. Horm Behav 35:18–27.

Gravett MG, Nelson HP, DeRouen R, Critchlow C, Eschenbach DA, Holmes KK. 1986. Independent association of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. JAMA 256:1899–1903.

Harty LC, Garcia-Closas M, Rothman N, Reid YA, Tucker MA, Hartge P. 2000a. Collection of buccal cell DNA using treated cards. Cancer Epidemiol Biomarkers Prev 9:501–506.

Harty LC, Shields PG, Winn DM, Caporaso NE, Hayes RB. 2000b. Self-collection of oral epithelial cell DNA under instruction from epidemiologic interviewers. Am J Epidemiol 151:199–205.

Hay PE, Lamont RF, Taylor-Robinson D, Morgan DJ, Ison C, Pearson J. 1994. Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. Br Med J 308:295–298.

Hayney MS, Dimanlig P, Lipsky JJ, Poland GA. 1995. Utility of a "swish and spit" technique for the collection of buccal cells for TAP haplotype determination. Mayo Clin Proc 70:951–954.

Hertsgaard L, Gunnar M, Larson M, Brodersen L, Lehman H. 1992. First time experiences in infancy: when they appear to be pleasant, do they activate the adrenocortical stress response? Dev Psychobiol 25:319–333.

Hill JB, Palmer P. 1969. Filter paper blood collection and punching as a means of quantification. Clin Chem 15:381–389.

H.M. Government Department for Environment, Food and Rural Affairs. 2002. Environmental Protection DG Research Newsletter 2002/03. Available: http://www.defra.gov.uk/environment/research/2002/06.htm [accessed 10 February 2003].

Hooper K, Hayward D, Chu M, Anderson M, Farland W, Lucier G, et al. 2002. Calux results correlate with GC/MS data from Kazakstan breast milk samples and support new TEF values. Available: http://www.dioxins.com/pdfdocs/BreastMilkStudy.pdf [accessed 25 September 2002].

Hu YA, Barr DB, Akland G, Melnyk L, Needham L, Pellizzari ED, et al. 2000. Collecting urine samples from young children using cotton gauze for pesticide studies. J Expo Anal Environ Epidemiol 10:703–709.

Inoue O, Kanno E, Yusa T, Kakizaki M, Ukai H, Okamoto S, et al. 2002. Urinary benzylmercapturic acid as a marker of occupational exposure to toluene. Int Arch Occup Environ Health 75:341–347.

Jacobsson B, Pernevi P, Chidekel L, Jorgen Platz-Christensen J. 2002. Bacterial vaginosis in early pregnancy may predispose for preterm birth and postpartum endometritis. Acta Obstet Gynecol Scand. 81:1006–1010.

Jeng LL, Moore RM, Kaczmarek RG, Placek PJ, Bright RA. 1991. How frequently are home pregnancy tests used? Results from the 1988 National Maternal and Infant Health Survey. Birth 18:11–13.

Kawai T, Mizunuma K, Okada Y, Horiguchi S, Ikeda M. 1996. Toluene itself as the best urinary marker of toluene exposure. Int Arch Occup Environ Health 68:289–297.

Kesner JS, Knecht EA, Krieg EF Jr. 1995. Stability of urinary female reproductive hormones stored under various conditions. Reprod Toxicol 9:239–244.

———. 1999. Measuring endocrine profiles of women in field studies. Scand J Work Environ Health 25(suppl 1):17–19.

Kesner JS, Knecht EA, Krieg EF Jr, Wilcox AJ, O'Connor JF. 1998. Detecting preovulatory luteinizing hormone surges in urine. Human Reprod 13:15–21.

Koch HM, Hardt J, Angerer J. 2001. Biological monitoring of exposure of the general population to the organophosphorus pesticides chlorpyrifos and chlorpyrifos-methyl by determination of their specific metabolite 3,5,6-trichloro-2-pyridinol. Int J Hyg Environ Health 204:175–180.

Koletzko B, Knoppke B, von Schenck U, Demmelmair H, Damli A. 1999. Noninvasive assessment of essential fatty acid status in preterm infants by buccal mucosal cell phospholipid analysis. J Pediatr Gastroenterol Nutr 29:467–474.

Kumar R, Pant N, Srivastava SP. 2000. Chlorinated pesticides and heavy metals in human semen. Int J Androl 23:145–149.

Lamey P, Nolan A. 1994. The recovery of human saliva using the Salivette system. Eur J Clin Chem Clin Biochem. 32:727–728.

Landrigan PJ, Sonawane B, Mattison D, McCally M, Garg A. 2002. Chemical contaminants in breast milk and their impacts on children's health: an overview. Environ Health Perspect 110:A313–315.

Lasley BL, Overstreet JW. 1998. Biomarkers for assessing human female reproductive health, an interdisciplinary approach. Environ Health Perspect 106(suppl 4):955–960.

Lay MF, Richardson ME, Boone WR, Bodine AB, Thurston RJ. 2001. Seminal plasma and IVF potential. Biochemical constituents of seminal plasma of males from *in vitro* fertilization couples. J Assist Reprod Genet 18:144–150.

Lench N, Stanier P, Williamson R. 1988. Simple non-invasive method to obtain DNA for gene analysis. Lancet 1:1356–1358.

Li H, Chen Q, Li S, Yao W, Li L, Shi X, et al. 2001. Effect of Cr(VI) exposure on sperm quality: human and animal studies. Ann Occup Hyg 45:505–511.

Liaw LC, Nayar DM, Pedler SJ, Coulthard MG. 2000. Home collection of urine for culture from infants by three methods: survey of parents' preferences and bacterial contamination rates. Br Med J 320:1312–1313.

Lindbohm M. 1995. Effects of parental exposure to solvents on pregnancy outcome. J Occup Environ Med 37:908–914.

Lipsitz R. 2000. Pregnancy tests. Sci Am 283:110–111.

Lum A, Le Marchand L. 1998. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. Cancer Epidemiol Biomarkers Prev 7:719–724.

Macleod J, Rowsell R, Horner P, Crowley T, Caul EO, Low N, et al. 1999. Postal urine specimens: are they a feasible method for genital chlamydial infection screening? Br J Gen Pract 49:455–458.

McCann MT, Thompson MM, Gueron IC, Tuchman M. 1995. Quantification of orotic acid in dried filter-paper urine samples by stable isotope dilution. Clin Chem 41:739–743.

Mitsui S, Ohuchi A, Hotta M, Tsuboi R, Ogawa H. 1997. Genes for a range of growth factors and cyclin-dependent kinase inhibitors are expressed by isolated human hair follicles. Br J Dermatol 137:693–698.

Morris RA, Jeffay SC, Strader LF, Evenson DP, Olshan AF, Lansdell LW, et al. 2003. Evaluation of sperm chromatin structure assay (SCSA®) in human sperm after simulated overnight shipment [Abstract]. J Androl Suppl (March/April):54.

Mussalo-Rauhamaa H. 1991. Partitioning and levels of neutral organochlorine compounds in human serum, blood cells, and adipose and liver tissue. Sci Total Environ 103:159–175.

Nallur G, Luo C, Fang L, Cooley S, Dave V, Lambert J, et al. 2001. Signal amplification by rolling circle amplification on DNA microarrays. Nucleic Acids Res 29:E118.

National Birth Defects Prevention Study. 2003. Available http://www.health.state.ny.us/nysdoh/cmr/nbdps.htm [accessed 10 February 2003].

NCS (National Children's Study). 2003. Available: http://nationalchildrensstudy.gov/ [accessed 10 February, 2003].

Nielsen MS, Barton SD, Hatasaka HH, Stanford JB. 2001. Comparison of several one-step home urinary luteinizing hormone detection test kits to Ovuquick®. Fertil Steril 76:384–387.

Ostermeier GC, Dix DJ, Miller D, Khatri P, Krawetz SA. 2002. Spermatozoal RNA profiles of normal fertile men. Lancet 360:772–777.

Parkes J, Ray R, Kerestan S, Davis H, Ginsberg B. 1999. Prospective evaluation of accuracy, precision, and reproducibility of an at-home hemoglobin A1c sampling kit. Diabetes Technol Ther 1:411–419.

Perreault SD, Rubes J, Robbins WA, Evenson DP, Selevan SG. 2000. Evaluation of aneuploidy and DNA damage in human spermatozoa: applications in field studies. Andrologia 32:247–254.

Petrelli G, Figa-Talamanca I. 2001. Reduction in fertility in male greenhouse workers exposed to pesticides. Eur J Epidemiol 17:675–677.

Plebani M, Carraro P. 1997. Mistakes in a stat laboratory: types and frequency. Clin Chem 43:1348–1351.

Poirier MC. 1997. DNA adducts as exposure biomarkers and indicators of cancer risk. Environ Health Perspect 105 (suppl 4):907–912.

Priestley CJF, Kinghorn GR. 1996. Bacterial vaginosis. Br J Clin Pract 50:331–334.

Rasmussen SA, Lammer EJ, Shaw GM, Finnell RH, McGehee RE Jr, Gallagher M, et al. 2002. Integration of DNA sample collection into a multi-site birth defects case-control study. Teratology 66:177–184.

Reutman SR, Lemasters GK, Knecht EA, Shukla R, Lockey JE, Burroughs GE, et al. 2002. The potential for endocrine disruption by exposure to fuels and solvents. Environ Health Perspect 110:805–811.

Riad-Fahmy D, Read GF, Walker RF, Walker SM, Griffiths K. 1987. Determination of ovarian steroid hormone levels in saliva. J Reprod Med 32:254–272.

Rockett JC, Dix DJ. 1999. Application of DNA arrays to toxicology. Environ Health Perspect 107:681–685.

———. 2000. DNA arrays: technology, options and toxicological applications. Xenobiotica 30:155–177.

Rockett JC, Kavlock RJ, Lambright CR, Parks LG, Schmid JE, Wilson VS, et al. 2002. DNA arrays to monitor gene expression in rat blood and uterus following 17beta-estradiol exposure: biomonitoring environmental effects using surrogate tissues. Toxicol Sci 69:49–59.

Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ. 1993. WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple. Cambridge, UK:Cambridge University Press.

Royster MO, Lobdell DT, Mendola P, Perreault SD, Selevan SG, Rothmann SA, et al. 2000. Evaluation of a container for collection and shipment of semen with potential uses in population-based, clinical, and occupational settings. J Androl 21:478–484.

Sallmen M, Lindbohm ML, Kyyronen P, Nykyri E, Anttila A, Taskinen H, et al. 1995. Reduced fertility among women exposed to organic solvents. Am J Ind Med 27:699–713.

Sandberg F, Ingelman-Sundberg A, Ryden G, Joelsson I. 1968. The absorption of tritium-labelled prostaglandin E1 from the vagina of non-pregnant women. Acta Obstet Gynecol Scand 47:22–26.

Scheeres JJ, Chudley AE. 2002. Solvent abuse in pregnancy: a perinatal perspective. J Obstet Gynaecol Can 24:22–26.

Schreiber A, Amtmann E, Storch V, Sauer G. 1988. The extraction of high-molecular-mass DNA from hair shafts. FEBS Lett 230:209–211.

Schuhmacher M, Domingo JL, Agramunt MC, Bocio A, Muller L. 2002. Biological monitoring of metals and organic substances in hazardous-waste incineration workers. Int Arch Occup Environ Health 75:500–506.

Schwartz EB, Granger DA, Susman EJ, Gunnar MR, Laird B. 1998. Assessing salivary cortisol in studies of child development. Child Dev 69:1503–1513.

Scialli AR, Swan SH, Amler RW, Baird DD, Eskenazi B, Gist G, et al. 1997. Assessment of reproductive disorders and birth defects in communities near hazardous chemical studies. II. Female reproductive disorders. Reprod Toxicol 11:231–242.

Selevan SG, Rice DC, Hogan KA, Euling SY, Pfahles-Hutchens A, Bethel J. 2003. Blood lead concentration and delayed puberty in girls. N Engl J Med 348:1527–1536.

Senzolo C, Frignani S, Pavoni B. 2001. Environmental and biological monitoring of occupational exposure to organic micropollutants in gasoline. Chemosphere 44:67–82.

Sever LE, Arbuckle TE, Sweeney A. 1997. Reproductive and developmental effects of occupational pesticide exposure: the epidemiologic evidence. In: Occupational Medicine: State of the Art Reviews, Vol 12. Philadelphia:Hanley & Belfus, 305–325.

Shideler SE, Munro CJ, Johl HK, Taylor HW, Lasley BL. 1995. Urine and fecal sample collection on filter paper for ovarian hormone evaluations. Am J Primatol 37:305–315.

Shirtcliff EA, Granger DA, Schwartz E, Curran MJ. 2001. Use of salivary biomarkers in biobehavioral research: cotton-based sample collection methods can interfere with salivary immunoassay results. Psychoneuroendocrinology 26:165–173.

Shirtcliff EA, Granger DA, Schwartz EB, Curran MJ, Overman W. 2000. Assessing estradiol in biobehavioral studies using saliva and blood spots: simple radioimmunoassay protocols, reliability, and comparative validity. Horm Behav 38:137–147.

Smith AH, Patterson DG Jr, Warner ML, MacKenzie R, Needham LL. 1992. Serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels of New Zealand pesticide applicators and their implication for cancer hypotheses. J Natl Cancer Inst 84:104–108.

Smith K, Harrington K, Wingood G, Oh MK, Hook EW III, DiClemente RJ. 2001. Self-obtained vaginal swabs for diagnosis of treatable sexually transmitted diseases in adolescent girls. Arch Pediatr Adolesc 155:676–679.

Solomon GM, Weiss PM. 2002. Chemical contaminants in breast milk: time trends and regional variability. Environ Health Perspect 110:A339–347.

Sorokine-Durm I, Durand V, Le Roy A, Paillole N, Roy L, Voisin P. 1997. Is FISH painting an appropriate biological marker for dose estimates of suspected accidental radiation overexposure? A review of cases investigated in France from 1995 to 1996. Environ Health Perspect 105(suppl 6):1427–1432.

Spielberg F, Critchlow C, Vittinghoff E, Coletti AS, Sheppard H, Mayer KH, et al. 2000. Gross home collection for frequent HIV testing: acceptability of oral fluids, dried blood spots and telephone results. HIV Early Detection Study Group. AIDS 14:1819–1828.

Tanigawara Y, Kita T, Hirono M, Sakaeda T, Komada F, Okumura K. 2001. Identification of *N*-acetyltransferase 2 and CYP2C19 genotypes for hair, buccal cell swabs, or fingernails compared with blood. Ther Drug Monit 23:341–346.

Telisman S, Cvitkovic P, Jurasovic J, Pizent A, Gavella M, Rocic B. 2000. Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. Environ Health Perspect 108:45–53.

USPS (U.S. Postal Service). 1999. Hazardous, Restricted and Perishable Mail. Publ no. 52. Available: http://www.cargopak.com/resources/pub52.pdf [accessed 24 June 2003].

van Valkengoed IG, Morre SA, Meijer CJ, van den Brule AJ, Boeke AJ. 2002. Do questions on sexual behaviour and the method of sample collection affect participation in a screening programme for asymptomatic *Chlamydia trachomatis* infections in primary care? Int J STD AIDS 13:36–38.

Vine MF, Hulka BS, Margolin BH, Truong YK, Hu PC, Schramm MM, et al. 1993. Cotinine concentrations in semen, urine, and blood of smokers and nonsmokers. Am J Public Health 83:1335–1338.

Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. 2002. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr 140:48–56.

Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, et al. 2001. Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet 358:1602–1607.

Ward LS, Novis RB, Nascimento VT, Nobrega MS, Saad MJ. 1996. Determination of glucose levels using dried filter paper blood spots: new perspective in home monitoring. Rev Paul Med 114:1100–1103.

Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Paxton L, Berkley SA et al. 1998. A randomized, community trial of intensive sexually transmitted disease control for AIDS prevention, Rakai, Uganda. AIDS. 12:1211–1225.

Whelan EA, Grajewski B, Wood E, Kwan L, Nguyen M, Schnorr TM, et al. 2002. Feasibility issues in reproductive biomonitoring of female flight attendants and teachers. J Occup Environ Med 44:947–955.

Wilcox AJ, Baird DD, Dunson D, McChesney R, Weinberg CR. 2001. Natural limits of pregnancy testing in relation to the expected menstrual period. JAMA 286:1759–1761.

WHO (World Health Organization). 1999. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th ed. Cambridge, UK:Cambridge University Press.

Wu T, Buck GM, Mendola P. 2003. Blood lead levels and sexual maturation in U.S. girls: the Third National Health and Nutrition Examination Study, 1988-1994. Environ Health Perspect 111:737–741.

Yamamoto AY, Mussi-Pinhata MM, Pinto PC, Figueiredo LT, Jorge SM. 2001. Usefulness of blood and urine samples collected on filter paper in detecting cytomegalovirus by the polymerase chain reaction technique. J Virol Methods 97:159–164.

Young KE, Robbins WA, Xun L, Elashoff D, Rothmann SA, Perreault SD. 2003. Evaluation of chromosome breakage and DNA integrity in sperm: an investigation of remote semen collection conditions. J Androl 24:853–861.

Younglai EV, Foster WG, Hughes EG, Trim K, Jarrell JF. 2002. Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing *in vitro* fertilization. Arch Environ Contam Toxicol 43:121–126.

Zavos PM, Correa JR, Rodriguez F, Zarmakoupis-Zavos PN. 1998. Viability of human semen specimens cryostored and transported at 5°C using the Bio-Tranz shipping system. Prim Care Update Ob Gyns 5:170–171.