

National Health and Nutrition NHANES 1999-2014

Examination Survey Biospecimen Program: NHANES III (1988-1994) and



July 2015



Copyright information

All material appearing in this report is in the public domain and may be reproduced or copied without permission; citation as to source, however, is appreciated.

Suggested citation

McQuillan GM, McLean JE, Chiappa M, Lukacs SL. National Health and Nutrition Examination Survey biospecimen program: NHANES III (1988–1994) and NHANES 1999–2014. National Center for Health Statistics. Vital Health Stat 2(170). 2015.

Library of Congress Cataloging-in-Publication Data

National Health and Nutrition Examination Survey biospecimen program: NHANES III (1988-1994) and NHANES 1999-2014 / U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics.

p. ; cm. — (Vital and health statistics, series 2, data evaluation and methods research ; number 170)

Includes bibliographical references and index.

ISBN 0-8406-0671-0 (alk. paper)

I. National Center for Health Statistics (U.S.), issuing body. II. Series: Vital and health statistics. Series 2, Data evaluation and methods research; no. 170.

[DNLM: 1. National Health and Nutrition Examination Survey (U.S.) 2. Biological Specimen Banks--organization & administration--United States. 3. Health Surveys--United States. 4. Program Development--methods--United States. 5. Specimen Handling--methods--United States. W2 A N148vb 170 2015]

RA409 614.4'273--dc23

2015022324

For sale by the U.S. Government Printing Office Superintendent of Documents Mail Stop: SSOP Washington, DC 20402–9328 Printed on acid-free paper.

Vital and Health Statistics

Series 2, Number 170

National Health and Nutrition Examination Survey Biospecimen Program: NHANES III (1988–1994) and NHANES 1999–2014

Data Evaluation and Methods Research

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Centers for Disease Control and Prevention National Center for Health Statistics

Hyattsville, Maryland July 2015 DHHS Publication No. 2015–1370

National Center for Health Statistics

Charles J. Rothwell, M.S., M.B.A., *Director*Nathaniel Schenker, Ph.D., *Deputy Director*Jennifer H. Madans, Ph.D., *Associate Director for Science*

Division of Health and Nutrition Examination Surveys

Kathryn S. Porter, M.D., M.S., Director

Contents

Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals	Acknow	vledgment
Development of Biorepositories 2 Establishing the Serum, Plasma, and Urine Biorepositories. 2 Establishing a DNA Biorepository. 2 Ethics, Privacy, and Confidentiality 2 Informed Consent 3 Biospecimen Operations 3 Biospecimen Operations 3 Blood Collection for Serum or Plasma 3 Urine Collection 3 Pristine Biospecimens 3 Surplus Biospecimens 4 Access to Biospecimens 4 Access to Biospecimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Text Table 2 Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 7 II. Flow chart of NHANES biospecimen operations 7	Introdu	ction
Establishing the Serum, Plasma, and Urine Biorepositories. 2 Establishing a DNA Biorepository. 2 Ethics, Privacy, and Confidentiality. 2 Informed Consent 3 Biospecimen Operations 3 Blood Collection for Serum or Plasma 3 Urine Collection 3 Pristine Biospecimens 3 Surplus Biospecimens 3 Surplus Biospecimens 4 DNA Specimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Fext Table 2 Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 7 II. Flow chart of NHANES biospecimen operations 7 II. Flow chart for DNA specimen request proposals and Research Data	NHAN	ES III (1988–1994) and NHANES 1999–2014
Establishing a DNA Biorepository. 2 Ethics, Privacy, and Confidentiality 2 Informed Consent 3 Biospecimen Operations 3 Blood Collection for Serum or Plasma 3 Urine Collection 3 Pristine Biospecimens 3 Surplus Biospecimens 4 DNA Specimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Text Table 2 Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 2 II. Flow chart of NHANES biospecimen operations 7 II. Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals 8		
Ethics, Privacy, and Confidentiality 2 Informed Consent 3 Biospecimen Operations 3 Blood Collection for Serum or Plasma 3 Urine Collection 3 Pristine Biospecimens 3 Surplus Biospecimens 4 DNA Specimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Text Table 2 Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 7 II. Flow chart of NHANES biospecimen operations 7 II. Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals 8		
Informed Consent 3 Biospecimen Operations 3 Blood Collection for Serum or Plasma 3 Urine Collection 3 Pristine Biospecimens 3 Surplus Biospecimens 4 DNA Specimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Text Table 2 Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 2 List of Appendix Figures 7 I. Flow chart of NHANES biospecimen operations 7 II. Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals 8		
Biospecimen Operations 3 Blood Collection for Serum or Plasma 3 Urine Collection 3 Pristine Biospecimens 3 Surplus Biospecimens 4 DNA Specimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Text Table 14 Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 2 L. Flow chart of NHANES biospecimen operations 7 II. Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals 8		
Blood Collection for Serum or Plasma 3 Urine Collection 3 Pristine Biospecimens 3 Surplus Biospecimens 4 DNA Specimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Text Table 2 Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 2 L. Flow chart of NHANES biospecimen operations 7 II. Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals 8		
Urine Collection 3 Pristine Biospecimens 3 Surplus Biospecimens 4 DNA Specimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Text Table Derview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 1 Li Flow chart of NHANES biospecimen operations 7 III Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals 8		
Pristine Biospecimens 3 Surplus Biospecimens 4 DNA Specimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Iext Table Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 1 Li		
Surplus Biospecimens 4 DNA Specimens 4 Access to Biospecimens 4 Research Proposal Review Process for Serum, Plasma, and Urine Specimens 4 Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Iext Table Deverview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 1 Linked Figures 1 Linked Genetic Data Sets 5 Linked Genetic Data Sets 6 Linked Genetic Data Sets 7 Link		
DNA Specimens		
Access to Biospecimens		
Research Proposal Review Process for Serum, Plasma, and Urine Specimens	DNA	A Specimens
Research Proposal Review Process for DNA Specimens 5 Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Text Table Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 15 II. Flow chart of NHANES biospecimen operations 7 III. Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals 8		
Linked Genetic Data Sets 5 Summary 6 References 6 Appendix I. Supporting Figures and Tables 7 Appendix II. Glossary 14 Fext Table Overview of NHANES biospecimens, by survey cycle 2 List of Appendix Figures 15 II. Flow chart of NHANES biospecimen operations 7 III. Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals 8		
Summary		
References	Link	ed Genetic Data Sets
Appendix I. Supporting Figures and Tables	Summa	ıry
Appendix II. Glossary	Referer	nces
Fext Table Overview of NHANES biospecimens, by survey cycle	Append	dix I. Supporting Figures and Tables
Overview of NHANES biospecimens, by survey cycle	Append	lix II. Glossary
Overview of NHANES biospecimens, by survey cycle	Text Ta	able
Flow chart of NHANES biospecimen operations		
Flow chart of NHANES biospecimen operations	List of	Annendix Figures
Flow chart for DNA specimen request proposals and Research Data Center genetic data proposals		
List of Appendix Tables I. Pristine and surplus serum specimens available, from NHANES III		

Acknowledgment

The authors gratefully acknowledge the assistance of Dr. Kathryn Porter in the preparation of this report.

Abstract

Background

The National Health and Nutrition Examination Survey's (NHANES) biospecimen program was formed to manage the collection of biospecimens (including serum, plasma, urine, and DNA) from NHANES cycles, the storage of biospecimens in NHANES biorepositories, accessing of biospecimens by researchers, and the providing of resulting data to future researchers. Data from biospecimen research can be combined with existing NHANES data.

Objective

This report provides background on the development of NHANES biorepositories and describes the collection, processing, and storing of biospecimens; ethical considerations and informed consent; and the proposal process for accessing biospecimens and resulting data. The number and types of biospecimens collected in each survey cycle from NHANES III (1988-1994) through NHANES 1999-2014 are discussed so that researchers can understand what biospecimens are available if they are considering using NHANES biospecimens in their research.

Keywords: DNA • serum • plasma • urine

National Health and Nutrition Examination Survey Biospecimen Program: NHANES III (1988–1994) and NHANES 1999–2014

by Geraldine M. McQuillan, Ph.D., and Jody E. McLean, M.P.H., National Center for Health Statistics; Michele Chiappa, B.A., Harris Corporation; and Susan L. Lukacs, D.O., M.S.P.H., National Center for Health Statistics

Introduction

The National Health and Nutrition Examination Survey (NHANES) is one of a series of health-related surveys conducted by the Centers for Disease Control and Prevention's (CDC) National Center for Health Statistics (NCHS). NHANES collects data on the health, nutritional status, and health behaviors of the civilian noninstitutionalized resident population of the United States (1). The survey is unique in that it combines a personal interview with a standardized physical examination that includes a blood draw and urine collection for laboratory analysis.

This report describes the origins and characteristics of the NHANES biorepositories, which store biospecimens obtained during NHANES III (1988–1994) and NHANES 1999–2014, as well as the process for obtaining access to the biospecimens for testing and use in research. Blood and urine specimens will continue to be collected in NHANES 2015–2016. The NHANES website (http://www.cdc.gov/nchs/nhanes/genetics/stored_specimens.htm) will provide the most current information about the biospecimen program.

Appendix I contains supporting figures and tables, and Appendix II provides a glossary of key terms used in this report.

NHANES III (1988–1994) and NHANES 1999–2014

NHANES III was conducted from 1988 through 1994 and included persons aged 2 months and over. As with previous NHANES cycles, NHANES III focused on health and nutrition. Data were also collected on environmental exposures, to examine the effect of environment on overall health (2). NHANES III gathered data on 30,000 survey participants across the United States. It was conducted in two phases—Phase I (October 1988—October 1991) and Phase II (October 1991—October 1994)—each nationally representative of the U.S. population (2).

In 1999, NHANES became a continuous annual survey. Approximately 5,000 participants of all ages are examined each year, and data are released in 2-year cycles (3). The focus of NHANES 1999–2014 was to provide prevalence data on selected diseases and risk factors for the U.S. population; monitor trends in selected diseases, behaviors, and environmental exposures; explore emerging public health needs; and maintain a national probability sample of baseline information on health and nutritional status (3).

Development of Biorepositories

Biorepositories were created to store NHANES participants' blood (serum or plasma), urine, and DNA for future research. Resulting research data can be combined with existing NHANES data. One biorepository stores pristine serum, plasma, and urine that have not undergone freezing or thawing; a second stores surplus serum, plasma, and urine that have undergone freezing and thawing; and a third stores DNA specimens (see Table).

Establishing the Serum, Plasma, and Urine Biorepositories

During NHANES III, either 1 or 2 ml of serum from each participant, depending on age group, were aliquoted into four vials in the NHANES Mobile Examination Center (MEC) and sent to NHANES' serum biorepository in Lawrenceville, Ga. There, the biospecimens were re-aliquoted into 0.5-ml vials and stored as pristine sera, even though they had undergone one freeze-thaw cycle. This biorepository for pristine sera was established in 1988 at the start of the survey.

In 1989, the laboratories that were testing the serum specimens as part of NHANES informed NCHS' Division of Health and Nutrition Examination

Surveys (DHANES) that they could no longer keep residual biospecimens in their laboratory freezers and that there was a sufficient volume of serum in each vial that could be made available for future testing. This was due to changes in laboratory methodology that required less specimen per individual laboratory test and because few tests needed to be repeated. At that point, DHANES contracted with a commercial biorepository and in 1989 began to store the surplus serum that was returned from the various laboratories.

In 1999, the biorepositories expanded to include both pristine and surplus serum, plasma, and urine. Pristine serum specimens were aliquoted in the MEC into 0.5-ml vials that have never undergone a freeze-thaw cycle.

Establishing a DNA Biorepository

In 1991, NHANES developed a biorepository to maintain DNA specimens for future studies. This would allow genetic information to be combined with other NHANES data to improve knowledge of human disease. Prior to establishing the DNA biorepository, blood specimens were collected from participants during NHANES III Phase I to validate a DNA processing and extraction method. A molecular genetic technique using crude cell lysates from cell lines was used to extract the DNA. Small samples of cells obtained from 8 ml of blood collected from participants

aged 12 and over were sent to CDC's National Center for Environmental Health (NCEH) molecular biology laboratory for extraction. With the knowledge gained, blood was then collected from participants during NHANES III Phase II, DNA was extracted, and specimens were stored for future genetic research (4). Starting in 1999, blood specimens for isolating DNA from whole blood were collected from participants during NHANES 1999–2002, 2007–2008, 2009–2010, and 2011–2012.

Ethics, Privacy, and Confidentiality

The NCHS Ethics Review Board (ERB) protects the rights and welfare of NHANES participants. The NHANES protocol, and the protocol to make DNA available for research, comply with the U.S. Department of Health and Human Services' Policy for Protection of Human Research Subjects (45 CFR 46), available from: http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46. html (3). Additionally, three federal laws protect information collected from individuals:

- Privacy Act of 1974 (5 USC 552a)
- Section 308(d) of the Public Health Service Act (42 USC 242m)
- Confidential Information Protection and Statistical Efficiency Act (CIPSEA) (PL 107–347, enacted in 2002) (2).

The Public Health Service Act stipulates that "...no information may be used for any purpose other than the purpose for which it was supplied unless ...[a] person consented to its use for such other purpose, and further that it cannot be released or published in a form that the particular ...person supplying the information or described in it is identifiable unless such ...person has consented...." These strict prohibitions, forbidding even unintentional unauthorized disclosures, guide the behavior of all NCHS staff and contractors (3).

In addition to scientific review, all research protocols proposing to use

Table. Overview of NHANES biospecimens, by survey cycle

			;	Specimen typ	е		
_		Pristine ¹			Surplus ²		
NHANES cycle	Serum	Plasma	Urine	Serum	Plasma	Urine	DNA
III (1988–1994)	Х			Х			Х
1999–2000	X	X	X	X	X	X	X
2001-2002	X	X	X	X	X	X	X
2003-2004	X	X	X	X			
2005-2006	X	X	X	X			
2007-2008	X	X	Χ	X			X
2009-2010	X	X	X	X			X
2011-2012	X		X	X			X
2013-2014	Χ		Χ	Χ			

^{...} Category not applicable

SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey, 2015.

¹Biospecimens immediately frozen for storage, did not undergo laboratory testing.

²Biospecimens were surplus after laboratories had completed testing.

NHANES biospecimens are reviewed by the NCHS Confidentiality Officer for disclosure risk and by the NCHS Human Subjects Contact and ERB for protection of the rights and welfare of NHANES participants, including vulnerable populations such as children, pregnant women, and older Americans (3).

Once a protocol is approved, it is reviewed annually by the ERB. After 5 years of annual continuations, if there is need for continued use of biospecimens to complete the study protocol, a new protocol is required. If at any time after approval a researcher wants to change the protocol, an amendment to the protocol is required and undergoes the same review process as the initial protocol.

Informed Consent

During NHANES III, participants aged 12 and over signed a form consenting to storage of a sample of their blood for future research (a parent or guardian also signed the consent form if the participant was under age 18); genetic research was not specifically mentioned. Beginning with NHANES 1999, participants aged 7 and over signed a form consenting to have blood and urine specimens stored for use in future studies (a parent or guardian also signed the consent form if the participant was under age 18). On the same form, participants aged 20 and over could consent to have blood stored for future genetic studies (3).

Beginning in 2007, these consents were separated into two forms and participants signed one consent form for storing blood and urine for future nongenetic studies and a different form for storing blood for use in future genetic studies. The consent form for nongenetic studies highlights the need for research using stored biospecimens to help find new ways to prevent, treat, and cure many diseases. The genetics consent form informs participants that their DNA will be used for genetic research to help understand genetic links to medical conditions. On all consent forms from 1999 through 2014, participants were informed that there was no plan to contact them with the results of future tests.

Biospecimen Operations

This section describes the collection, processing, storing, and shipping of biospecimens during the NHANES cycles. All biospecimens are collected and processed at the MEC by a laboratory team consisting of a phlebotomist and medical technologists who are certified in accordance with the guidelines of the American Society for Clinical Pathology (3).

Blood Collection for Serum or Plasma

Blood is collected from NHANES participants aged 1 year and over by a phlebotomist. Prior to blood collection (venipuncture), the participant (or parent or guardian) completes a questionnaire to screen for conditions that would exclude him or her from the blood draw, such as hemophilia, having received chemotherapy in the previous 4 weeks, and various reasons that would preclude the use of the participant's arms for a blood draw. The amount of blood drawn varies by age and survey year, ranging from 7 ml in participants aged 1 to 3 in NHANES III to not more than 128 ml in participants aged 12 and over in NHANES 1999-2014 (2,3). Blood is processed by centrifuging and separating serum or plasma from the appropriate collection tube, and then aliquoting the specimens into vials. As a biospecimen vial is processed, it is barcoded with a label for the participant and given a laboratory number. All vials are verified for correct labeling. Vials are stored under appropriate cold (4°C–8°C) or freezing (-20°C) conditions in preparation for shipment to the testing laboratories (2,3).

In each NHANES cycle, more than 25 laboratories receive NHANES biospecimens for testing. Biospecimens are packed in wet or dry ice, depending on the analyte to be tested, and shipped at least once a week, again depending on the analyte. Each shipment contains a log transmittal form or shipping manifest. Extra serum or plasma are aliquoted

into cryovials for long-term storage. These biospecimens are considered pristine and are directly stored in vaporphase liquid nitrogen (–80°C or below) and shipped to the CDC and Agency for Toxic Substances and Disease Registry (ATSDR) Specimen Packaging, Inventory, and Repository (CASPIR) for long-term storage.

Urine Collection

Starting in 1999, a random urine specimen was collected by having participants aged 6 and over void into a sterile container (2,3). Urine specimens are processed by aligoting varied amounts of urine into vessels appropriately sized for specific laboratory assays. During NHANES 1999-2014, a 5-ml aliquot of extra urine was placed in a cryovial for long-term storage. In NHANES 2009–2012, participants aged 6 and over also provided a first morning void urine specimen from home. Preprinted bar code labels for each participant are affixed to the urine vessels. The vessels are stored in specific containers, frozen, and shipped weekly or at the completion of a survey location. Each shipment contains a log transmittal form or shipping manifest.

Pristine Biospecimens

Pristine biospecimens from NHANES III and NHANES 1999-2014 were shipped from the MEC and stored at CASPIR. On average, six vials of 0.5to 1.0-ml serum per vial per participant aged 3 or over (and one 0.5-ml plasma per vial from participants aged 6 and over from NHANES 1999-2010) are stored in vapor-phase liquid nitrogen (-80°C or below). Pristing NHANES III biospecimens underwent one freeze-thaw cycle (for aliquoting only) before storage; pristine biospecimens from NHANES 1999–2014 have not undergone any freeze-thaw cycles (3). Pristine urine specimens from NHANES 1999-2014 (one or two vials of 5-ml urine per participant aged 6 or over) are stored at CASPIR in vapor-phase liquid nitrogen (-80°C or below). Additionally, two aliquots of 5-ml urine collected from first void home urine specimens from NHANES 1999–2012 participants,

processed at the University of Minnesota, were sent to CASPIR for storage. See Table.

Surplus Biospecimens

After laboratories complete their testing, surplus serum, plasma, and urine specimens are shipped to the NHANES biorepository (a commercial biorepository under contract to NCHS) for long-term storage. These biospecimens have been through at least two freeze-thaw cycles prior to being shipped to the biorepository. At the biorepository, surplus serum or plasma specimens are stored at -80 °C. During NHANES 1999-2002, surplus urine specimens (one or two vials of 0.2- to 5-ml urine per participant aged 6 or over) were shipped from the testing laboratories to the NHANES biorepository for long-term storage. See Table.

DNA Specimens

Blood collection for the extraction of DNA (NHANES III Phase II and NHANES 1999-2002, 2007-2008, 2009-2010, and 2011-2012) followed the same NHANES screening procedures described previously for blood collected for serum and plasma. Blood specimens for DNA extraction were shipped to CDC's NCEH Division of Laboratory Sciences for processing and long-term storage. For NHANES III Phase II, DNA was extracted from crude lysates of cell lines from participants aged 12 and over. For these specimens, DNA concentrations vary and are estimated to range from 7.5 to 65 ng/µl, with an average of approximately 4 µg in 100 µl (4). For NHANES 1999-2002, 2007-2008, 2009-2010, and 2011-2012, DNA was purified from the blood of consenting participants aged 20 and over. DNA was isolated from two 10-ml aliquots of whole blood per participant. A master set of DNA specimens per survey cycle was stored at -80°C. A partial second DNA set from the second aliquot of blood was also frozen and stored at -80°C. Additionally, aliquots of purified DNA were normalized to concentrations of approximately 50 ng/µl (4).

Forty microliters of each DNA specimen were supplied to researchers who had an approved project. To prepare a complete DNA set for researchers, a 96-well plate map format is created to document the location of each DNA sample and to incorporate blanks and blind duplicates for quality control. For all survey cycles, each 96-well plate is barcoded and labeled with a readable identifier. These plate replicates are sealed in bags for shipping and frozen at -20°C. To prepare for shipping, plate replicates are retrieved from storage, the manifest listing is verified, and plates are packaged with appropriate shipping labels. Each shipment contains a shipping manifest

For a flow chart of NHANES biospecimen operations, see Figure I in Appendix I.

Access to Biospecimens

Research Proposal Review Process for Serum, Plasma, and Urine Specimens

All NHANES serum, plasma, and urine specimens from NHANES III and the 2-year survey cycles from NHANES 1999–2014 (Appendix I, Tables I–IV) are available to researchers. Those interested in using serum, plasma, or urine specimens for research must submit a proposal to the DHANES Biospecimen Program Project Officer (5). The proposal includes specific aims, background and public health significance, research design and methods, clinical significance of results, qualifications of the principal investigator, period of performance, and proposed or received funding. All proposals are evaluated by a technical panel for scientific merit, public health significance, and clinical significance. The panel consists of scientists from within and outside NCHS.

Because NHANES participants receive the results of their survey laboratory tests 12–16 weeks after examination at the MEC and have no further contact with NHANES staff,

there is no plan to contact participants with results of future tests resulting from research on biospecimens. Therefore, researchers wishing to access biospecimens cannot test for analytes that could have clinical significance for participants. A laboratory analyte is considered clinically significant if laboratory tests are performed by a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory and therefore deemed valid, the findings have significant implications for the participant's health, and a course of action is readily available to treat the associated health concern (5).

After a proposal is approved by the technical panel, it is evaluated by the NCHS Confidentiality Officer for disclosure risk, and by the NCHS Human Subjects Officer and the ERB for any potential human subjects concerns. The technical panel, Human Subjects Officer, and ERB also evaluate whether the test has clinical significance. The ERB reviews the proposal even if it was previously approved by the researcher's institutional review panel. Researchers can request changes to the protocol only through an ERB amendment process.

After 5 years of annual continuations, if there is need for continued use of biospecimens to complete the protocol study, a new protocol is required. This protocol is reviewed by the technical panel to assure the ERB that the sole purpose of the new protocol is to complete the original study. More information on the proposal process is available on the biospecimen program website at http://www.cdc.gov/nchs/nhanes/genetics/stored_specimens.htm and on the Federal Register website (5).

Once their proposal is approved, researchers complete and sign the Material Transfer Agreement (MTA) and send payment for the biospecimens. After the MTA and payment are received, the request to send samples to the researcher is authorized. If the researcher is within the federal government, biospecimens are provided after receipt of a signed Interagency Agreement rather than an MTA. If an approved project is unable to obtain funding, the project is canceled.

The researcher confirms the approved study population for testing with the DHANES Biospecimens Project Officer, who ensures that the requested biospecimens are available at CASPIR or the contract biorepository. The project officer places the researcher in contact with the biorepository to schedule overnight delivery of biospecimens.

After laboratory testing is complete, all results are sent to NCHS to be linked to the sequence number (SEQN) ID (the linking identifier on the public-use data files), and a data file is prepared. The data file, linked to NHANES public-use data, is returned to the researcher for quality control testing. See the NHANES biospecimen program website (http://www.cdc. gov/nchs/nhanes/genetics/stored specimens.htm) for updated guidelines. The researcher has up to 90 days to complete this testing, after which the data are released to the public on the NHANES website.

Research Proposal Review Process for DNA Specimens

DNA specimens were stored in NCEH's molecular biology laboratory. In 1999, NHANES announced that it would provide the public with an anonymized set of genetic data (from the DNA specimens collected in NHANES III Phase II) with a limited number of demographic variables (i.e., age, race, and sex) for analyses. This minimized the research potential for genetic data by limiting access to the full array of NHANES data. In 2002, NHANES obtained approval from the NCHS ERB to allow researchers to request DNA samples for approved research. Resulting genetic data could be linked with other NHANES data through NCHS' Research Data Center (RDC).

In 2012, the ERB suspended the NHANES DNA Specimen Program from receiving new proposals due to concerns about reporting of incidental

findings from research results. As of March 2015, DHANES has received approval to reopen the program. Refer to the NHANES website

(http://www.cdc.gov/nchs/nhanes/genetics/genetic.htm) in late 2015 for the updated Federal Register notification (Table V).

From 2002 through 2012, researchers could submit a new research proposal on either January 1 or July 1 of each year to request DNA specimens (4). The proposal was first submitted to the DHANES Genetics Project Officer. It was then sent to the Genetics Technical Panel, comprised of experts in the genetics field, for review. When the comments and questions from the panel had been appropriately addressed by the requesting researcher, the approved proposal was sent to the NCHS Human Subjects Contact, Confidentiality Officer, and ERB for review and approval. Once a research proposal was approved—and after receipt of a completed and signed MTA and payment—the biospecimens were shipped to the researcher. If the researcher was within the federal government, biospecimens were provided after receipt of a signed Interagency Agreement rather than an MTA. For projects requiring funding, DNA specimens were not provided to the requesting researcher until the project had received funds. Requests from approved projects that were unable to obtain funding were canceled. For a flow chart showing the NHANES DNA request proposal process, see Figure II.

Following approval, researchers were shipped an approved and complete set of participants' samples, plus quality control samples that represented 5% of the complete set. Quality control samples are blinded to the researchers. All test results from DNA specimens are returned to NCHS for quality control review. The first step in quality control review is to test the percentage of the quality control specimens that were discordant with their corresponding specimens in the complete set; if more than 5% are discordant, then the genetic variable failed quality control (i.e., 95% or greater duplicate concordance is required). The second step is to assess deviations from HardyWeinberg proportions separately for each of the three main race and ethnicity groups (non-Hispanic white, non-Hispanic black, and Mexican-American) using chi-square goodness-of-fit tests. If two of the three race and ethnicity groups deviate from Hardy-Weinberg proportions at p < 0.001, the genetic variable fails quality control (6).

Test results that pass quality control review are linked to NHANES data by SEQN ID. After 6 months, the genetic data are deposited in the NHANES Genetic Data Repository and made available, by proposal, to other researchers.

Researchers depositing genetic data or researchers wanting to analyze secondary genetic data in the NHANES Genetic Data Repository are required to submit a proposal and access the data through the NCHS RDC. All proposals are reviewed for potential disclosure issues. All analyses are performed in the RDC at NCHS in Hyattsville, Md.; in the RDC on the CDC campus in Atlanta, Ga.; or through the NCHS remote access system. A fee is charged to use RDC services.

For more information on gaining access to NHANES' Genetic Data Repository for research, see http://www.cdc.gov/nchs/nhanes/genetics/genetic_access.htm. For a flow chart showing the RDC genetic proposal process, see Figure II.

Linked Genetic Data Sets

Linking of NHANES III Phase II, NHANES 1999–2002, and NHANES 2007–2008 public-use and restricted data with the genetic information available in the NHANES Genetic Data Repository provides researchers with the opportunity to conduct a vast array of outcome studies to investigate the association between a wide variety of health factors and genetic variation (7). The linkage process continues as more genetic variation information becomes available. Data in the repository can be accessed through the RDC. The majority of genetic data in the repository are single nucleotide polymorphisms (7).

Summary

This report has provided an overview of the collection, storage, and availability for research of biospecimens collected through NHANES. More detailed information is available on the NHANES website at http://www.cdc.gov/nchs/nhanes.htm.

References

- National Center for Health Statistics. About the National Health and Nutrition Examination Survey. Hyattsville, MD. 2014. Available from: http://www.cdc.gov/nchs/ nhanes/about_nhanes.htm.
- National Center for Health Statistics. Plan and operation of the Third National Health and Nutrition Examination Survey, 1988–94. Vital Health Stat 1(32). 1994. Available from: http://www.cdc.gov/nchs/data/ series/sr 01/sr01 032.pdf.
- 3. Zipf G, Chiappa M, Porter KS, et al. National Health and Nutrition Examination Survey: Plan and operations, 1999–2010. National Center for Health Statistics. Vital Health Stat 1(56). 2013. Available from: http://www.cdc.gov/nchs/data/series/sr 01/sr01 056.pdf.
- CDC. National Health and Nutrition Examination Survey (NHANES) DNA samples: Guidelines for proposals to use samples and cost schedule. Fed Regist 75 FR 32191– 5. 2010. Available from: https:// federalregister.gov/a/2010-13517.
- 5. CDC. National Health and Nutrition Examination Survey (NHANES) stored biologic specimens:
 Guidelines for proposals to use samples and proposed cost schedule. Fed Regist 74(138):35872–5. 2009.
 Available from: http://www.cdc.gov/nchs/data/nhanes/Fed_Reg_Serum.pdf.

- National Center for Health Statistics. National Health and Nutrition Examination Survey (NHANES) DNA quality control protocol. Hyattsville, MD. 2010. Available from: http://www.cdc.gov/nchs/data/ nhanes/genetics/Quality_Control_ Public.pdf.
- National Center for Health Statistics. NHANES genetic data. Hyattsville, MD. 2011. Available from: http:// www.cdc.gov/nchs/nhanes/genetics/ genetic.htm.

Appendix I. Supporting Figures and Tables

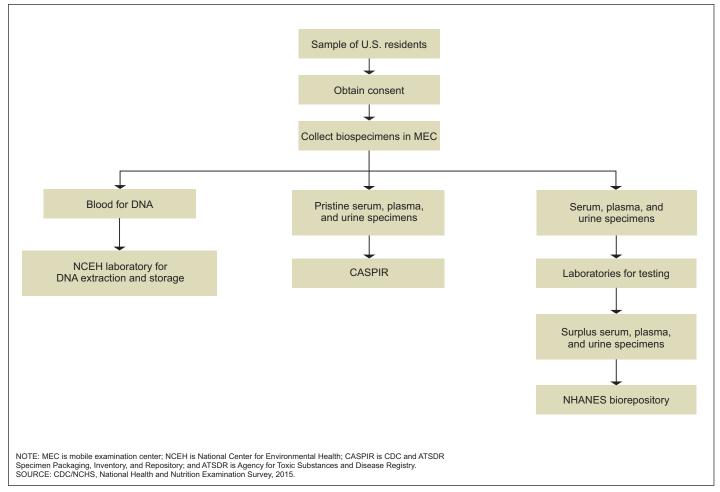


Figure I. Flow chart of NHANES biospecimen operations

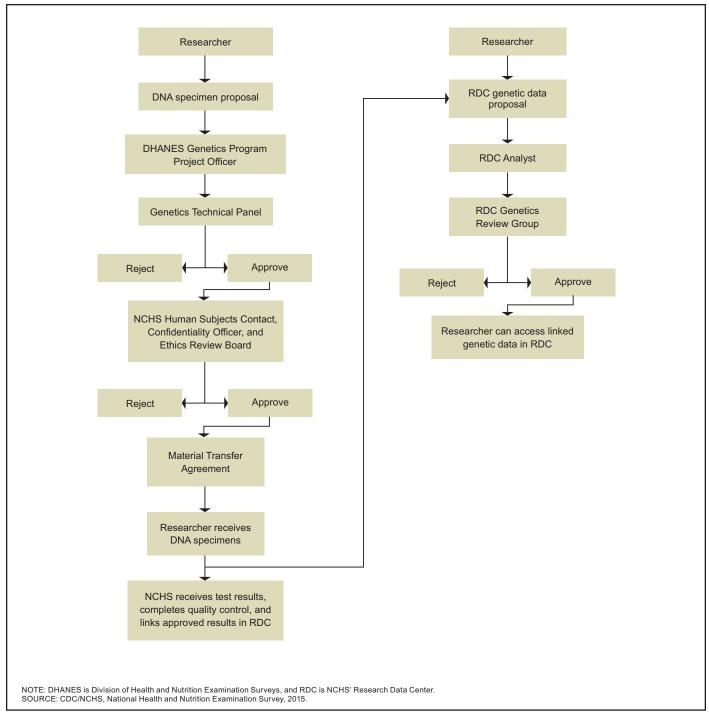


Figure II. Flow chart for DNA specimen proposals and Research Data Center genetic data proposals

Table I. Pristine and surplus serum specimens available, from NHANES III

Age group and specimen source	Pristine serum	Surplus serum
3–5 years		
Participants		1,381
Vials		2,955
6-11 years		
Participants	2,469	2,568
Vials	12,781	6,342
12–19 years		
Participants	2,586	2,918
Vials	16,308	16,667
20 and over		
Participants	15,029	16,035
Vials	140,806	103,706

^{...} Category not applicable.

NOTES: Specimens accessible for testing under approved protocols. Numbers are current based on data available at publication. Vials contain ≥0.2 ml.

SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey, 2015.

Table II. Pristine serum, plasma, and urine specimens available, from NHANES 1999–2014

Survey cycle, age group, and specimen source	Serum	Plasma	Urine
1999–2000			
G-11 years:			
Participants	705	730	788
Vials	2,381	730	1,492
2–19 years:			
Participants	1,846	1,825	1,919
Vials	20,701	1,825	3,577
20 and over:			
Participants	3,724	1,305	3,839
Vials	41,327	1,305	7,336
	,-	,	,
2001–2002			
-11 years:			
Participants	917	968	1,017
Vials	2,578	968	1,753
2–19 years:			
Participants	2,017	2,081	2,105
Vials	20,116	2,081	3,475
0 and over:			
Participants	4,398	4,527	4,511
Vials	43,259	4,527	7,950
2003–2004			
-5 years:	215		
Participants	315	•••	
Vials	315	•••	
i–11 years:	710	400	050
Participants	719	489	850
Vials	1,299	489	1,513
2–19 years:			
Participants	1,899	1,200	1,995
Vials	16,732	1,200	3,393
0 and over:			
Participants	4,244	2,545	4,260
Vials	37,106	2,545	8,383
2005–2006			
3-5 years:			
Participants	302		
Vials	302	•••	
i–11 years:	302		•••
Participants	789	845	935
Vials	2,773	845	935
2–19 years:	2,110	040	933
	1 004	1.050	1 000
Participants	1,884	1,859	1,899
Vials	16,534	1,859	1,899
0 and over:	4.050	4 454	4 400
Participants	4,252	4,151	4,160
Vials	36,706	4,151	4,812
2007–2008			
-5 years:			
Participants	329		
Vials	1,269	•••	
–11 years:	1,200	•••	•••
Participants	866	864	526
Vials	5,190	864	526
	5,190	004	526
2–19 years:	055	067	E00
Participants	955	967	532
Vials	9,815	967	532
0 and over:	4.000	4.6.46	
Participants	4,832	4,840	2,638
Vials	49,060	4,840	2,638

See footnotes at end of table.

Table II. Pristine serum, plasma, and urine specimens available, from NHANES 1999–2014 —Con.

Survey cycle, age group, and specimen source	Serum	Plasma	Urine
2009–2010			
3–5 years:			
Participants	347		
Vials	772		
6-11 years:			
Participants	811	869	973
Vials	1,003	869	1,111
12–19 years:	.,000	000	.,
Participants	1,008	1,020	1,062
Vials	4,618	1,020	1,246
20 and over:	4,010	1,020	1,240
Participants	5,072	5,129	5,181
· · · · ·	25,026	5,129	6,137
Vials	25,026	5,129	0,137
2011–2012			
3–5 years:			
Participants	259		
Vials	461		
6-11 years:			
Participants	749		958
Vials	1,350		1,901
12-19 years:			
Participants	916		990
Vials	3,327		1,968
20 and over:			
Participants	4,273		4,470
Vials	16,195		8,886
0040 0044			
2013–2014			
3–5 years:	004		
Participants	334		
Vials	651	•••	•••
6–11 years:			
Participants	852		934
Vials	1,688	•••	1,719
12-19 years:			
Participants	1,026		1,085
Vials	4,013		2,000
20 and over:			
Participants	4,771		4,785
Vials	18,786		9,765

^{...} Category not applicable.

NOTES: Specimens accessible for testing under approved protocols. Numbers are current based on data available at publication. Vials contain ≥0.2 ml.

 ${\tt SOURCE:CDC/NCHS,\ National\ Health\ and\ Nutrition\ Examination\ Survey,\ 2015.}$

Table III. Surplus serum, plasma, and urine specimens available, from NHANES 1999–2012

	Serum	Plasma	Urine
1999–2000			
-5 years:			
Participants	351	240	
Vials	929	240	
-11 years:			
Participants	867	638	975
Vials	3,447	638	1,440
2–19 years:			
Participants	2,110	1,460	2,125
Vials	12,758	1,506	3,660
0 and over:	12,750	1,500	0,000
Participants	4,173	3,663	4,242
Vials			
viais	21,767	5,529	7,582
2001–2002			
–5 years:			
Participants	431	•••	
Vials	1,063		
-11 years:			
Participants	1,007		764
Vials	3,928		764
2–19 years:	, -		
Participants	2,203		2,281
Vials	17,990		2,375
0 and over:	.,,,,,,,,	•••	2,073
Participants	4,835	2,960	4,492
·		,	
Vials	36,743	3,200	4,925
2003–2004			
–5 years:			
Participants	444		
Vials	1,509		
-11 years:			
Participants	838		
Vials	3,784		
2-19 years:			
Participants	2,048		
Vials	12,078		
0 and over:	,		
Participants	4,473		
•	22,587		
Vials	22,307	•••	
2005–2006			
–5 years:	400		
Participants	482	•••	
Vials	1,783		
–11 years:			
Participants	926	•••	
Vials	4,505		
2–19 years:			
Participants	1,978		
Vials	12,532		
0 and over:	,	***	•••
Participants	4,490		
Vials	29,248	•••	
	20,270		
2007–2008			
Evenyer			
–5 years:	400		
Participants	406	•••	
	552		
Vials			
Vials	986		
-11 years:	1,477	•••	
–11 years: Participants	1,477		
–11 years: Participants Vials			
-11 years: Participants	1,074		
-11 years: Participants			
-11 years: Participants	1,074 4,429		
–11 years: Participants	1,074		

See footnotes at end of table.

Table III. Surplus serum, plasma, and urine specimens available, from NHANES 1999–2012 —Con.

Survey cycle, age group, and specimen source	Serum	Plasma	Urine
2009–2010			
3-5 years:			
Participants	323		
Vials	323		
6-11 years:			
Participants	887		
Vials	1,646		
12–19 years:	,		
Participants	1,177		
Vials	3,585		
20 and over:	0,000	•••	
Participants	5,708		
Vials	19,105		
	,		
2011–2012			
6-11 years:			
Participants	845		
Vials	898		
12–19 years:			
Participants	1,035		
Vials	1,510		
20 and over:	,		
Participants	4,605		
Vials	6,759		

^{...} Category not applicable.

NOTES: Specimens accessible for testing under approved protocols. Numbers are current based on data available at publication. Vials contain ≥0.2 ml. Total number of surplus specimens (participants and vials) for 2013–2014 was not available at time of publication. Updates are available on the NHANES website at https://www.cdc.gov/nchs/nhanes.htm.

SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey, 2015.

Table IV. Biospecimens available for participants aged 0–2 years, from NHANES 2003–2006

Specimen source ¹	Surplus serum
Participants	873
Vials	1,214

¹Specimens only available for 4 years combined.

NOTE: Additional specimens for 2002 available by special request.

SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey, 2015.

Table V. NHANES DNA specimens available

Survey cycle	Age group	Participants	DNA source
NHANES III	12 and over	7,159	Crude cell lysates
1999-2002	20 and over	7,839	Blood
2007-2008	20 and over	4,615	Blood
2009-2010	20 and over	3,898	Blood

NOTE: Total number of participants for 2011–2012 was not available at time of publication.

SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey, 2015.

Appendix II. Glossary

Biorepository—A place where biologic specimens are stored and made available for research purposes.

Biospecimens—Materials taken from the human body.

CASPIR—Centers for Disease Control and Prevention (CDC) and Agency for Toxic Substances and Disease Registry (ATSDR) Specimen Packaging, Inventory, and Repository for biospecimens.

Civilian noninstitutionalized population—Includes all people living in U.S. households and excludes those living in institutionalized group quarters and persons on active duty with the military.

DNA—Deoxyribonucleic acid, the hereditary material in humans and almost all other organisms.

Ethics Review Board—A committee within NCHS that performs ethical review of proposed research.

National Center for Health Statistics (NCHS)—The principal health statistics agency in the United States. NCHS compiles statistical information to help guide public health and health policy decisions to improve the health of the American people. It also designs, develops, and maintains a number of systems that produce data related to demographic and health concerns, including data on registered births and deaths collected through the National Vital Statistics System (NVSS), as well as data from the National Health Interview Survey (NHIS), the National Health and Nutrition Examination Survey (NHANES), the National Health Care Surveys (NHCS), and the National Survey of Family Growth (NSFG), among others. NCHS is part of the U.S. Department of Health and Human Services' Centers for Disease Control and Prevention.

Plasma—The pale yellow liquid component of nonclotted blood after the blood settles.

Pristine biospecimen—A biospecimen that, once frozen, has never been thawed.

Public-use data—An electronic data set containing participant records from a survey, with a subset of variables collected in the survey that have been reviewed by analysts within NCHS to ensure participant identity is protected.

Research Data Center (RDC)—An NCHS entity that provides researchers access to restricted data from NCHS surveys.

Restricted-use data—An electronic data set containing participant records from a survey that contain some information that may, if released to the public, risk disclosure of individual participants. These data are available through the NCHS RDC and include data sets with (a) data items that were collected for an odd number of calendar years (1, 3, or 5 years); (b) data linked geographically to some other contextual data file (often supplied by the data user); and (c) data items that are determined to be too sensitive or too detailed to be released to the public due to confidentiality restrictions.

Serum—The clear liquid portion of blood after it clots.

Surplus biospecimen—A biospecimen that was used for other laboratory tests and is now available for future research.

Urine—Waste material secreted by the kidneys.

Vital and Health Statistics Series Descriptions

ACTIVE SERIES

- Series 1. Programs and Collection Procedures—This type of report describes the data collection programs of the National Center for Health Statistics. Series 1 includes descriptions of the methods used to collect and process the data, definitions, and other material necessary for understanding the data.
- Series 2. Data Evaluation and Methods Research—This type of report concerns statistical methods and includes analytical techniques, objective evaluations of reliability of collected data, and contributions to statistical theory. Also included are experimental tests of new survey methods, comparisons of U.S. methodologies with those of other countries, and as of 2009, studies of cognition and survey measurement, and final reports of major committees concerning vital and health statistics measurement and methods.
- Series 3. Analytical and Epidemiological Studies—This type of report presents analytical or interpretive studies based on vital and health statistics. As of 2009, Series 3 also includes studies based on surveys that are not part of continuing data systems of the National Center for Health Statistics and international vital and health statistics reports.
- Series 10. Data From the National Health Interview Survey—This type of report contains statistics on illness; unintentional injuries; disability; use of hospital, medical, and other health services; and a wide range of special current health topics covering many aspects of health behaviors, health status, and health care utilization. Series 10 is based on data collected in this continuing national household interview survey.
- Series 11. Data From the National Health Examination Survey, the National Health and Nutrition Examination Survey, and the Hispanic Health and Nutrition Examination Survey—In this type of report, data from direct examination, testing, and measurement on representative samples of the civilian noninstitutionalized population provide the basis for (1) medically defined total prevalence of specific diseases or conditions in the United States and the distributions of the population with respect to physical, physiological, and psychological characteristics, and (2) analyses of trends and relationships among various measurements and between survey periods.
- Series 13. Data From the National Health Care Survey—This type of report contains statistics on health resources and the public's use of health care resources including ambulatory, hospital, and long-term care services based on data collected directly from health care providers and provider records.
- Series 20. **Data on Mortality**—This type of report contains statistics on mortality that are not included in regular, annual, or monthly reports. Special analyses by cause of death, age, other demographic variables, and geographic and trend analyses are included.
- Series 21. **Data on Natality, Marriage, and Divorce**—This type of report contains statistics on natality, marriage, and divorce that are not included in regular, annual, or monthly reports. Special analyses by health and demographic variables and geographic and trend analyses are included.
- Series 23. Data From the National Survey of Family Growth—These reports contain statistics on factors that affect birth rates, including contraception and infertility; factors affecting the formation and dissolution of families, including cohabitation, marriage, divorce, and remarriage; and behavior related to the risk of HIV and other sexually transmitted diseases. These statistics are based on national surveys of women and men of childbearing age.

DISCONTINUED SERIES

- Series 4. **Documents and Committee Reports**—These are final reports of major committees concerned with vital and health statistics and documents. The last Series 4 report was published in 2002. As of 2009, this type of report is included in Series 2 or another appropriate series, depending on the report topic.
- Series 5. International Vital and Health Statistics Reports—This type of report compares U.S. vital and health statistics with those of other countries or presents other international data of relevance to the health statistics system of the United States. The last Series 5 report was published in 2003. As of 2009, this type of report is included in Series 3 or another series, depending on the report topic.
- Series 6. Cognition and Survey Measurement—This type of report uses methods of cognitive science to design, evaluate, and test survey instruments. The last Series 6 report was published in 1999. As of 2009, this type of report is included in Series 2.
- Series 12. **Data From the Institutionalized Population Surveys**The last Series 12 report was published in 1974. Reports from these surveys are included in Series 13.
- Series 14. **Data on Health Resources: Manpower and Facilities**—
 The last Series 14 report was published in 1989. Reports on health resources are included in Series 13.
- Series 15. Data From Special Surveys—This type of report contains statistics on health and health-related topics collected in special surveys that are not part of the continuing data systems of the National Center for Health Statistics. The last Series 15 report was published in 2002. As of 2009, reports based on these surveys are included in Series 3.
- Series 16. Compilations of Advance Data From Vital and Health
 Statistics—The last Series 16 report was published in 1996.
 All reports are available online, and so compilations of
 Advance Data reports are no longer needed.
- Series 22. Data From the National Mortality and Natality Surveys—
 The last Series 22 report was published in 1973. Reports from these sample surveys, based on vital records, are published in Series 20 or 21.
- Series 24. Compilations of Data on Natality, Mortality, Marriage, and Divorce—The last Series 24 report was published in 1996.
 All reports are available online, and so compilations of reports are no longer needed.

For answers to questions about this report or for a list of reports published in these series, contact:

Information Dissemination Staff National Center for Health Statistics Centers for Disease Control and Prevention 3311 Toledo Road, Room 5419 Hyattsville, MD 20782

Tel: 1-800-CDC-INFO (1-800-232-4636)

TTY: 1-888-232-6348

Internet: http://www.cdc.gov/nchs
Online request form: http://www.cdc.gov/info

For e-mail updates on NCHS publication releases, subscribe online at: http://www.cdc.gov/nchs/govdelivery.htm.

U.S. DEPARTMENT OF HEALTH & HUMAN SERVICES

Centers for Disease Control and Prevention National Center for Health Statistics 3311 Toledo Road, Room 5419 Hyattsville, MD 20782–2064

Tryattovino, WB 20702 2004

OFFICIAL BUSINESS PENALTY FOR PRIVATE USE, \$300

For more NCHS Series reports, visit: http://www.cdc.gov/nchs/products/series.htm.



FIRST CLASS MAIL POSTAGE & FEES PAID CDC/NCHS PERMIT NO. G-284