Biomarkers in population based surveys: Promising tools for policy making

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Introduction

Population based surveys provide information of great value for policy-making. Among many others, they collect socio-demographic and fertility indicators, estimates of illnesses and mortality, and health care seeking behavior and utilization. The Demographic and Health Surveys (DHS) initiative, an USAID-funded follow-on project of the World Fertility Surveys that started in the 70s ensured a wide range of data was collected for use by a myriad of health, urban planning and program development officers, both in the public and private sector, as well as by the research community. Results of these national-level surveys have helped design programs and initiatives of large community impact, such as population and family planning, antenatal care, oral rehydration therapy for diarrhea-affected children and breastfeeding and nutrition supplementation programs.

On the other hand, biomarker data have found increasing utility over time. Biomarkers are biological and physiological measures of health conditions. The application of biomarker measurements to population surveys opened up an array of possibilities to enhance data collection. Variation in respondents recall and self reports, coupled with the lack of knowledge or symptoms of initial or established diseases or conditions severely limited the accuracy of their estimates. As technology advanced, collection of sophisticated biomarkers in surveys has become increasingly feasible and is helping to better understand current health conditions and diseases in the population and their relationship with social and economic determinants, as well as with individuals' behaviors.

In this paper we will describe some of the actual biomarker data collection carried out by the DHS over the last 20 years and the efforts required to include such data in the surveys, such as data collectors' training, laboratory assessment and upgrade and overall field work methodology. As part of implementation aspects we will include a section on ethical considerations emerging with biomarker data collection and reporting. This will be followed by a section of some illustrative findings depicting household situations such as iodized salt in the household, and anemia and HIV infection in the case of individual data. The final section will present some policy implications of findings using biomarkers and highlight the potential for wider use and application of this information to design more appropriate interventions in the developing world.

Biomarkers in Population-based Surveys

Health Examination Surveys (HES) were first conduct biomarker testing in the developed countries. National Health Examination Survey (NHES I) was first conducted in 1960-62. This survey focused primarily on chronic diseases of adults in the age group of 18-79 years. Growth and development of children (6-11 years) was the focus of the second phase of NHES (1963-65). The third phase from 1966-70 concentrated on the growth and development of youth (12-17

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years). National Health and Nutrition Examination Surveys (NHANES) conducted by the National Center for Health Statistics (NCHS) of the U.S. Centers for Disease Control and Prevention is the most prominent model of HES (Fisher et al, 1996). NHANES survey examines nationally representative sample of about 5000 persons of all ages each year. Four phases of NHANES have already been conducted, viz., NHANES I (1971-74), NHANES II (1976-80), NHANES III (1988-84), NHANES IV (1998-2002). Health Examination Surveys adds the physical examination and laboratory component (biomarker testing) to the interviews to have greater comparability in the data collected.

Biomarkers are used for screening and for monitoring (repeated surveys can measure changes in disease prevalence). Population groups "at risk" are identified by deviations from normal to mean values for biomarkers tested. Recent technological advances have made it feasible to include biomarkers in surveys conducted in less developed countries and in diverse situations.

In the case of the DHS, biomarkers have been measured for nearly 20 years. Since 1986, DHS has measured the height, weight of women and children and the existence of iodine in household salt. This has made possible to obtain the prevalence of and factors associated with international nutrition indicators such as the Body Mass Index (a ratio of weight-to-height) for mothers and underweight (weight-for-age), wasting (weight-for-height) and stunting (height-for-age) for children less than 5 years. For household iodized salt, see Table 1 below.

In the face of the epidemiological transition in the developing world (i.e. addition of chronic, metabolic and malignant conditions in rapidly growing urban segments of the population in a context of prevailing infectious and emerging infectious diseases), as well as the lack of precise estimates of diseases and conditions, more sero-prevalence data was needed. Hence, to these more traditional and standard indicators, anemia testing was added since 1995, when a survey in Kazakhstan showed that respondents were comfortable providing blood specimens for the test. Since then 19 more biomarkers have been added to the arsenal of tests and testing conducted in about 37 surveys. In less than a decade, about 30 surveys have conducted anemia testing and about 15 surveys have collected bio-specimens for HIV testing.

The different biomarkers tested in DHS cover a wide range of health problems in a population. These include hemoglobin (indicator of iron deficiency anemia), serum retinol (indicator of vitamin A deficiency), blood pressure, cholesterol, triglycerides, HDL and LDL (indicator of the health of the cardiovascular system), glycosylated hemoglobin (proxy indicator for diabetes), hepatitis B (indicator of vaccine coverage, liver disorder and STI), Tetanus and Measles (indicator of vaccine coverage), HIV, bacterial vaginosis, chlamydia, gonorrhea, HSV 2, syphilis and trichomonas (indicator of STIs), and lead (indicator of environmental exposure). See list of biomarkers by country in Appendix 1 (**(omitted for uploading the paper)**.

Collecting large quantities of biological specimens in widespread and remote areas, and analyzing them properly and consistently in a short period of time requires a level of commitment and organization little known to the public. The following sections briefly review field and laboratory requirements, as well as some organizational and training issues to ensure the success of this complex operation.

Specimen collection and field procedures

In any given survey, the type of specimen collected and collection technique depends on the technology available for the biomarker to be tested, the feasibility of collection in the field, the easiness of transfer of samples to a laboratory and the testing protocol. Specimen collected in these surveys includes whole blood, serum, plasma, dried blood spots, oral mucosal transudate (saliva), and vaginal swabs. Techniques of specimen collection in a given country also vary depending on the type of biomarker and number of biomarkers to be tested simultaneously.

For example, hemoglobin and lead testing in DHS is done onsite (at the household) using capillary blood. For determination of hemoglobin, blood collection requires relatively less equipment and supplies, and its relatively simpler procedures allow for less specialized personnel and quicker training than with other specimens. Capillary blood is collected via finger prick with a small retractable safety lancet. Typically a drop of blood is collected via a microcuvette and placed inside a small portable battery operated "reader" (Hb 201+, HemoCue) to measure the concentration of the hemoglobin. Results are typically obtained in less than a minute and given back to the respondent right in the respondent's household (see Figure 1). Similarly, testing of lead involves similar procedure for blood draw and measurement in yet another battery operated equipment called ESA lead analyzer (ESA Inc.)

Figure 1: Steps in the Collection and Processing of Blood for Anemia Testing (omitted for uploading the paper)

In the case of blood collection for HIV testing, though the method of data collection is similar, processing requires more sophisticated testing methods and equipment, such as immunoassays and spectrophotometers (e.g. for ELISA or Western Blot tests), thus specimens are taken back to central laboratories for analysis. Consequently the handling of these specimens is quite different. Drops of blood collected through capillary blood as described. Drops of blood from the finger are placed in the pre-printed circles on a special filter paper (Schleicher & Schuell 903 TM) and left to dry in a drying box, to produce dry blood spots (DBS). These DBS are wrapped with a glassine paper and stored properly in low gas permeable plastic zip lock bags containing desiccants and a humidity indicator card can be safely transported to a central laboratory for analysis (see Figure 2).

At the lab, the samples are stored at 4°C for up to 90 days and at -20°C if the samples need to be stored for longer duration.

Figure 2: Collection and storage of Dry Blood Spots for HIV Testing (omitted for uploading the paper)

If the number of tests increases or if a biomarker can be analyzed only by use of serum or plasma, it necessitates drawing blood using venipuncture. This obviously complicates the field operation, from the hiring of more specialized health personnel, their training (see below) and certainly the specimen collection method and handling until arriving at a more central laboratory. Both capillary and venous blood have been successfully collected and processed from children and adults in DHS surveys.

Testing technologies (kits, equipment, software, rapid tests)

The use and feasibility of biomarkers have increased in the DHS surveys because of state-of the-art technology. Computerization has lead to conversion of manual to automated procedures and the development of small equipments and rapid tests, hence taking the testing from lab to the field or household. The lead analyzers and HemoCue for hemoglobin testing are examples of such portable equipment, which are battery operated and eliminate the need to process blood specimens. Blood drops are collected through finger-pricking with a retractable lancet, and results obtained and reported to the respondent on site within a few minutes. In case of low levels of any biomarker tested in the field, respondents are generally informed about potential repercussions and given referral slips to visit the nearby health facility for further diagnosis and treatment.

State-of-the-art technology also offers the benefit of rapid diagnostics. Rapid test strips -of high sensitivity and specificity, based on qualitative immunoassays have been used in DHS surveys. The Zambia DHS and the Uzbekistan Health Examination Survey used the Determine® test strips for Syphilis (in conjunction with Rapid Plasma Reagin test) and the Hepatitis B surface antigen respectively. Results were reported back to participants in both surveys. In Zambia, Uganda and Madagascar respondents who tested positive for syphilis (only RPR) were also treated- an excellent example of survey-to-service continuum. There is a great potential for use of such test strips in future surveys.

In addition to rapid diagnostics use of dried blood spots (DBS) and oral specimens have also increased in recent years and successfully collected in DHS surveys. These are useful techniques of sample collection in the developing countries and remote areas where there is no electricity or refrigeration. Samples collected in these surveys are sent to the labs at room temperature without jeopardizing their integrity. In DHS surveys, DBS samples have been used in testing for various biomarkers such as HIV (using ELISA, Western Blot and PCR techniques), hepatitis B, HSV 2, serum retinol, measles and tetanus. On the other hand, a cold chain is required for specimens such as vaginal swabs, as collected in Tashkent, Uzbekistan. These samples were sent to the lab in a cold box with ice packs. Depending on the biomarker tested some surveys have used battery operated refrigerators to keep the specimens at lower-than-room temperatures. However for specimens that require sub-zero temperatures and longer storage, a portable dry-shipper or liquid nitrogen tank can be used. These do not require electricity and are charged with liquid nitrogen that keeps the samples at -70°C or lower. Venous blood samples thus stored have been successfully transferred from remote sites to laboratories in various DHS surveys (Zambia, Uganda, Uzbekistan etc) for testing of different biomarkers.

Lab procedures (algorithms)

The choice of analytical method depends on what is the purpose of an assessment. For example, if the purpose of the test might be to measure antibodies to HIV [in adults?] or maternal antibodies to HIV in children. Based on the purpose either an ELISA or PCR test is done in the lab. In addition, a testing strategy is also based on sensitivity and specificity of the test and HIV prevalence in the population/country tested. Various test kits of international standards that are validated and recommended by CDC and WHO have been used in these surveys. Testing algorithms and methods have been carefully developed and adapted for each biomarker in order

to deal with discordant provisional results and yield the true prevalence of a given indicator in the population.

Validation and Quality Control

How well a diagnostic test correlated with the disease/condition of interest is referred to as the accuracy or validity of the test. The process of selection and validation of a diagnostic test require careful consideration of the sensitivity and specificity of biomarker for a given condition. If the sensitivity of the test is increased, it will result in decreased specificity of the test. Similarly, if the specificity of the test is increased, the sensitivity will decrease. Diagnostic tests use din the surveys should have very high sensitivity and specificity. For example, the ELISA test kits used in the DHS surveys have sensitivity and specificity of 98%-100%. The higher the sensitivity and specificity the more accurate the test is at diagnosing the disease.

Validation process is also considered for establishing the accuracy, precision and quality assurance and control of the analytical procedure. Since most of the ELISA test kits are designed for use either with serum or plasma and not the whole blood or DBS samples, the DBS samples have to be validated on the kits selected for HIV testing in the survey. The EIA testing of DBS samples is integral to quality assurance of HIV testing in the DHS surveys. Serum aliquots from venous blood and matched DBS samples are processed for each ELISA test . Serum samples are first categorized as either HIV positive or HIV negative by using EIA's tests selected for the survey. The corresponding DBS samples are also used to evaluate the performance of these EIA's.

Quality control checks are implemented not just for the laboratory testing and data recording, it is also instituted at every step including supply and equipment, collection of samples in the field, storage of samples in the field, transport of samples from the field to the lab. In addition to the supervisor for each team in the DHS surveys, highly trained and experienced supervisors from the central office in country and from Macro supervises every aspect of data collection including, identification of eligible respondents, interviewing, field editing, specimen collection, field testing and transport of samples.

Quality control checks in the laboratory consist of reliability checks between technicians or a referral lab, analysis of QC material, and blinded rechecking.

Training and capacity building

Adding biomarkers to the DHS is technically feasible however it raises training, logistical issues and ethical concerns. Firstly, depending on the biomarker tested it calls for training of medical and non-medical personnel for appropriate and accurate sample collection, processing and testing, and for waste disposal. Inclusion of biomarkers in a given survey increases the burden on the interviewer or a health technician and lengthens the time spent by the survey team in a given household. Thus training for biomarkers is a separate category within DHS. It involves field staff (e.g. for collection and storage/transport of specimens, recording of procedures) and lab personnel (e.g. for handling of specimens, analysis, recording and further storage).

In countries where biomarkers testing will be included as part of a survey, efforts are required to build the necessary technical and laboratory capacities to deal with both the field and the actual testing rigors. This is the objective of the first phase of biomarker work in the DHS. An expert visits the country to assess the status of the selected laboratory(ies) in preparation for the survey. DHS has developed several checklists to assess the baseline capacity of a lab, in order to determine the equipment, supplies and training required to bring it to the level required for the survey. The assessment looks into the qualification, training, experience and quantity of lab personnel for the activity. This is followed by verifying the types of samples (whole blood, serum, plasma, saliva, etc.) processed in the facility and whether there's any pre-treatment of the sample (e.g. centrifugation, heat inactivation).

Other assessment items for the lab are the existence of quality control procedures (internal and external), validation of kits, algorithms, manuals and guidelines in place, and a track record of actual tests conducted in last 6 months. Previous to any training and standardization of procedures, selected laboratories with deficiencies are provided with equipment and supplies such as generators, ELISA readers and washers, refrigerators or freezers, computers and printers, tubes, gloves, pipettes and reagents as required, in order to ensure basic capacity and a smooth operation of all tests.

On the second phase of technical assistance, contracts are signed with the organization representing the selected labs to ensure internal and external quality control procedures are followed during the survey.

Other aspects of capacity building include transferring the acting laboratory the means to minimize transcription errors. This is done by using electronic media for the reading of test results. CSPro, a software program developed between Macro International and the US Bureau of Census and Surveys (BUCEN) is used to generate computerized data from test readers and thus minimize errors coming from manual transcription of results. This software is installed in the lab and local staff are also trained on its use. Additionally, in the case of the HIV testing algorithm, for example, the software automatically and incrementally generates the 10 percent sample of negatives that will be re-tested as part of quality assurance procedures.

Once involved laboratories have been upgraded and their capacities increased, DHS staff ensure technicians and other personnel taking part of the survey receive the training appropriate for the survey.

Training parallels that occurring for the questionnaire application of the survey, that is, there is a pre-test phase followed by a main training phase. The pre-test training may include 10-30 people. During the pre-test, the field staff is trained on how to introduce the biomarkers aspect of the survey to the respondent, how to apply informed consent, the protocol and procedures for collecting the biospecimen (e.g. blood) and the recording of procedures in the questionnaire. Training is a mix of theoretical information and extensive practicum. Only surveyors who become proficient in sample collection are provided certificates allowing them to collect the necessary specimen. In the case of anemia collection in children, extra training is required for heel puncture. This may be done at a local under five clinic/or in nurseries/pre-school centers.

As to the laboratory component, during the pre-test the lab receives a final assessment of its capacity and refinements are done to bring all components to shape (e.g. ensuring all equipment, reagents and other supplies are in place, protocols at hand, and procedures well established). All test kits are opened and validated, using the procedures described above. Samples are collected from volunteer respondents in local institutions (e.g. non-sampled clinics) and all lab personnel conduct sufficient testing to achieve proficiency and confidence. As with the field component, only lab personnel who have showed mastery of the standard procedures (including their appropriate recording) are given the "green light" to operate in the main survey.

During pre-test all forms are revised for accuracy and relevance; all procedures tested for non-redundancy and validity. The DHS lab director reviews all components and makes the necessary changes to ready the lab and its personnel for the main training.

The laboratory main training is conducted at the same time as the questionnaire training, usually 4-6 weeks after the pre-test. The main training may include 20-150 people, depending on the size of the sample and the number of biomarkers collected. At that time all activities of the pre-test are replicated, with a "reality" dimension added. Successful technicians/personnel trained at the pre-test will rapidly achieve proficiency and some may become supervisors of newly trained personnel. Usually field work starts right after completion of the main training.

Safety precautions

During the field work or in the lab, universal safety precautions are followed at every stage from specimen collection, storage, processing and disposal of biohazard wastes. In general the precautions include using gloves for each blood draw and/or testing. Retractable safety lancets (for finger or heel pricks), and single use syringes (for venous blood draw) are used for blood draw. Collection of specimen generally takes place in plastic microcuvette, or mylar covered capillary tubes or on a filter paper card. Biohazard bags and sharp containers are used for appropriate disposal of biohazard waste. Precautions are also taken during transport of samples from the field to the lab. For example, a separate vehicle for picking up samples in a liquid nitrogen tank. In the laboratory too, technicians also follow the universal safety precautions by wearing adequate protection, using automated pipettes and are required to work under the hood.

Ethical considerations in biomarker testing

In addition to the above challenges and issues, there are ethical concerns that arise when adding biomarkers in a survey. The National Center for Health Statistics/CDC has set of out clear standards for ethical collection and analysis of a wide variety of biomarkers in population based surveys. The ethical issues which have particular relevance to health examination/population based biomarker surveys include: report of the findings of the test to the respondent, the use of stored samples, and the informed consent.

All surveys including biomarkers, DHS obtains approval from Macro's Institutional Review Board (IRB). In the country where the survey is being conducted, additional IRB approval is sought from the implementing agency's IRB and Ministry of Health in the respective countries, if required. As part of the survey, health investigator or the interviewer obtains the informed

consent of the respondent. In case a respondent is not capable of giving an informed consent, the proxy consent of a responsible parent or guardian is taken. It is made sure that the respondent has adequately understood the information and is entitled to choose freely whether to participate in the survey. The informed consent obtained in the DHS surveys safeguard the respondent's freedom of choice to participate and respects the respondent's autonomy.

Macro International ensures that they are protected from harmful effects. Macro International Inc. complies with the US Department of Health and Human Services regulations for the protection of human research subjects (45 CFR 46). As part of this compliance, an Institutional Review Board (IRB) reviews all research involving human subjects. The IRB is required to review any research project brought before it, or proposed changes to an existing project, before human subjects may be involved. As part of its activities, the IRB is required to submit documentation of its reviews and approvals to the Federal government.

Keeping in mind the general ethical principles (respect for respondents, beneficence, and justice) for some biomarkers such as anemia, lead, and syphilis, DHS survey teams report the result back to the respondent in their own household. Conditions such as syphilis that are treatable by an injection of penicillin are relatively easier to treat in the field under the supervision of a nurse or medical personnel. Iron deficiency anemia can be treated by providing iron and/or folate tablets with or without multivitamins or deworming tablets. However, in circumstances where the sample needs to be tested in a central laboratory, or the treatment of a condition requires confirmation of a test result or requires a complex regime, the respondent is referred to a nearby health facility for further diagnosis, treatment or counseling. In addition to treatment and referrals, informational brochures on the condition tested are also given to the respondent in their local language.

Survey research must provide respondents' with result of a test if the survey can identify the person, therefore, addition of biomarkers such as HIV and other sexually transmitted diseases raises more ethical concerns in terms of informed consent and reporting of results back to the respondent. One of the options for HIV testing includes "anonymous HIV testing". The DHS surveys collect specimens in an anonymous fashion in which reporting of the test results to the respondent is not possible because no link can be made between the respondent and the specimen collected. However, the national AIDS control organizations' in country work in conjunction with the surveys offer and provide additional voluntary counseling and testing (VCT) to participants. In addition, DHS surveys have taken extra steps to protect the identity of a survey respondent and the confidentiality of the survey data. In case of HIV testing using DBS, no unique identifiers such as name, sex, age, or cluster number are attached to the sample collected. Instead, a barcode is pasted on the filter paper card. After all of the sample testing is completed in the lab, the results can be linked –still anonymously to other data collected from the respondent.

If a survey sample is stored for future testing for an unknown test, the survey respondent is clearly informed and a separate consent is obtained for their willingness to have the sample stored.

For HIV testing, Macro provides explanation of potential risks to human subjects. For example, the potential (though extremely low) risk of infection from the actual finger pricking procedure in the field is addressed in the protocols submitted. However, the bigger issues are a) ensuring the voluntary participation of subjects in the data collection; b) protecting the anonymity of respondents and c) whether respondents who provide blood specimens should be provided with results and other benefits, such as free counseling.

As to the first issue, Macro and the DHS strictly adhere to the application of informed consent procedures. Respondents are fully informed of the link between HIV and AIDS, the cleanliness of finger-pricking procedures and their right to refuse testing without negative consequences, including any compromise to their eligibility for regular health care (see a model of an Informed Consent statement below).

CONSENT STATEMENT

Hello, my name is _____. I'm from the Ministry of Health and collaborating with the Central Bureau of Statistics. As part of this survey, we are studying HIV among women and men. As you know, HIV is the virus that causes AIDS. The government is trying to find out how common HIV and other sexually transmitted diseases are, so that they can develop programs to prevent HIV and care for those who have it.

We request that you participate in this test by giving a few drops of blood from a finger. For this test, I will use clean, sterile instruments that are completely safe. Blood will be tested later in the laboratory.

To ensure the confidentiality of this test result, no individual names will be attached to the blood sample; therefore, we will not be able to give you the result of your test and no one will be able to trace the test back to you. If you want to know whether you have HIV, I can tell you where you can go to get tested.

Do you have any questions?

I hope you will agree to participate in the HIV/STD testing. But if you decide not to have the test done, it is your right and I will respect your decision and this will not affect in any way the health services you normally receive.

Will you accept to participate in the HIV/STD test? GO BACK TO COLUMN (51). CIRCLE THE APPROPRIATE CODE AND SIGN.

IF RESPONDENT IS AGE 15-17, ASK PARENT/GUARDIAN: Now, will you tell me if you accept for (NAME OF YOUTH) to participate in the HIV/STD test? GO TO COLUMN (50). CIRCLE THE APPROPRIATE CODE AND SIGN. IF PARENT AGREES, READ THE PRECEDING PARAGRAPHS TO YOUTH FOR HIS/HER CONSENT AND RECORD IN COL. (51).

NOTE FOR THE INTERVIEWER:
THE RESPONDENT HAS THE RIGHT TO REFUSE THE HIV TEST, AND THEREFORE SHOULD NOT BE FORCED.

Regarding anonymity, DHS guarantees through a number of procedures that there is no individual identification of samples and results. In the case of HIV testing using DBS, no unique identifiers such as name, sex, age, or cluster number are attached to the sample collected. Instead, a barcode is pasted on the filter paper card. Bar codes guarantee confidentiality once the number identifier is scrambled and de-linked from subject identification, and provide a practical way of storing data electronically. Three to four bar code labels are produced for each test

sample. At the initial stage, one bar code label is used to label the sample itself, and the other is attached to the questionnaire to identify the respondent.

The bar codes are in the form X8Y9Z where X,Y,Z represent upper case letters, and 8,9 represent digits between 0 and 9. The first four characters provide a unique identifier, with 67600 possible combinations. The last letter is a check digit, calculated from the previous four letters and digits. When the bar codes are entered or scanned into DHS data entry systems, the check digit is used to verify that the bar code has been entered correctly. For this reason it is almost impossible to mis-key a bar code.

The procedure to deal with barcodes and personal identifiers is the following: At the time of programming for data entry, the data processing (DP) specialist sets up files to relate the barcode with cluster, HH number, region and residence data from the questionnaire, in order to calculate weights and select cases for analysis. As soon as that link is done, the DP person deletes the cluster, HH number and line number variables, to create the "anonymous" data file with weights calculated in it. Only after this is done will this file be taken to the lab to join the test results file for the preliminary report tabulations. At the country level the specialist will further sort the new data set in different ways from the original order of the cluster, and merge this dataset with the barcode data, after correcting for any mismatches. Similar weighting is done with the HIV tests datafile. In the end, the merged file (named "DHSANONY") will contain new sequential numbers that will make impossible to determine the identification of original respondents.

Linking biomarker data to respondents' demographic and behavior variables can provide very useful information. For example prevalence of HIV with respect to urban and rural areas, province, age, sex, condom use, number of partners and other crucial variables can provide a better insight into the HIV epidemic. We will illustrate below some HIV results from surveys.

On the issue of benefits from the intervention, the purpose of DHS surveys is to provide aggregate data that will inform appropriate policy making, rather than individually identifying affected individuals. Since DHS does not have the programmatic mandate nor the financial capacity to counsel and treat affected individuals, the main benefit from measuring the prevalence of HIV and its determinants in a country derives from providing policy makers with accurate nationwide and regional data with which to plan and implement sound preventive and treatment programs for the affected population. However, where feasible (including availability of resources and systems) DHS can set up a referral system (with vouchers or coupons) or follow-up mobile teams for the conduct of free counseling and individual testing among respondents wishing to know their HIV status. This was done in the case of Kenya and Uganda. For example, in the Uganda National HIV/AIDS Sero-Behavioral Survey, the survey team interviewed and colleted blood for HIV, HSV-2, Hepatitis B, and Syphilis from the eligible respondents. All eligible respondents were given a free VCT voucher. In addition to the survey team, another VCT team also followed the survey team in the same cluster. The VCT team would make small make-shift VCT center in the same cluster in a health facility. The purpose of having a VCT team tagged with every survey team was to have the VCT services available to all the eligible respondents right in their cluster/village.

Illustrative Results of biomarker testing in DHS

A. Iodized salt in households

In an effort to combat the existence of Iodine Deficiency Disorders (IDD), DHS started collecting information from households on the iodine content of the salt used for cooking. Since 1995 this information has been collected in a variety of countries around the world, from Guatemala, to Nepal to Niger. Table 1 provides an estimate of countries where test kits found "negative" or below 15 or 25 parts per million (ppm) iodine readings in the salt provided by the household for testing.

Table 1
Percentage of households with Negative or Low Readings for Iodine in domestic Salt

COUNTRY, YEAR OF DHS	Negative or Below Cut-off[#]	Number
Sub-Saharan Africa		
Benin, 1996	21	4,184
Cameroon, 1998	6	4,082
CAR, 1994-95	72	4,448
Chad, 1996-97	38	6,083
Ethiopia, 2000	72[2]	13,812
Gabon, 2000	82	5,279
Ghana, 1998	72[2]	5,877
Guinea, 1999	86	4,356
Madagascar, 1997	21	6,551
Malawi, 2000	47[1]	112,844
Niger, 1998	26	5,129
Rwanda, 2000	9[1]	8,736
Tanzania, 1999	33[2]	3,492
Uganda, 2000	5[1]	7,046
Zambia, 1996-97	4	6,252
North Africa/West Asia/Europe		
Egypt, 2000	72[3]	16,940
Yemen, 1997	12	4,761
Central Asia		
Kazakhstan, 1999	80[1]	5,616
South/Southeast Asia		
Cambodia, 2000	86	12,107
India, 1998-99	50[1]	90,586
Nepal, 1996	35[1]	8,063
Philippines, 1998	86[1]	11,540
Latin America/Caribbean		
Bolivia, 1998	9	11,291
Brazil, 1996	6	12,854
Dominican Republic, 1996	86[2]	7,985
Guatemala, 1995	8[2]	10,824
Haiti, 2000	88[1]	8,817
Nicaragua, 1997-98	7[2]	10,658
Peru, 2000	3	27,851

[#] There are two different cut-off points, see below.
[1] Below 15 ppm; [2] Below 25 ppm; [3] Below 26 ppm

The table shows the countries with the largest needs (e.g. Haiti, Cambodia, Dominican Republic, Philippines and Guinea) for designing salt iodization programs. Since data can be broken down by major regions/provinces, it can be seen how this information can further assist policy-makers prioritize action and target scarce resources to areas with critically low levels of iodine.

B. Children's nutritional status

Weighing and measuring children's heights has provided for many years the possibility of obtaining three distinct relational measures indicating children's nutritional status: weight-for-height, height-for-age and weight-for-age. Figure 3 for example presents the prevalence of undernutrition (underweight or less than 2 SD of the weight-for-age WHO reference) for children under 5 years of age for Sub-Saharan Africa. The graphic allows a quick comparison between countries and the division between countries where a third or more of their children suffer malnutrition, compared to others with lower figures. There is obvious utility of this type of information, within countries (again, where figures allow the breakdown by regions and other segments of the population subject to prioritized planning), and for regional food and agricultural aid programs by multilateral agencies and other funding bodies.

Figure 3: Percentage of Children under Five who are Underweight
DHS – Sub-Saharan Africa (omitted for uploading the paper)

Further analysis of the data provides more information for decision-making. For example, selecting the case of Mali in the 2001 survey, Figure 4 presents the three indicators of malnutrition by age of the child.

Figure 4: Malnutrition in Children Under 5 by Type of Indicator, Mali 2001 DHS (omitted for uploading the paper)

The importance of continued breastfeeding and adequate environmental sanitation transpire from the visualization of the fact that levels of malnutrition rise quite sharply after 6-8 months of life, when a number of mothers will reduce or stop breastfeeding. But more importantly, at this age the child is exposed to the dangers of infection through the introduction of complementary feeding. The combination of these circumstances result in stark contrasts: while at 5 months only approximately 6 percent of children under 5 are underweight in Mali, just six months later up to 40 percent of them will be in danger of dying because of a low weight for their age. With stunting, the rise is also rapid, however high levels at or over 50 percent continue to exist at every age until 5.

Similarly, the anthropometric data collected in the NFHS-2 survey shows that mean-height-for-age z-scores or stunting is significantly associated with increase in the blood lead levels of children less than three years of age in Mumbai, India.

Figure 5: Mean height-for-age z-scores for children according to lead levels, Mumbai, NFHS-2 (omitted for uploading the paper)

C. Biomarkers for chronic diseases: Uzbekistan

Another type of biomarker is blood pressure. The Women's and Men's questionnaires for the 2002 Uzbekistan Health Examination Survey (UHES) included questions to determine if the respondent had been diagnosed as hypertensive and if she/he was taking medication to control blood pressure. Respondents were also asked if their blood pressure could be measured as part of the survey. Among the 5,588 women and 2,447 men eligible for blood pressure measurement, response rates were very high, at 98 and 95 percent, respectively. Table 2 presents a table from the final report concerning the rates found for men and differentials by several characteristics.

Table 2 Hypertension among men

Prevalence of hypertension among men and percent distribution of men by blood pressure status, according to background characteristics, Uzbekistan 2002

		Classification of blood pressure									
Background characteristic	Prevalence of hyper-tension[1]	Optimal	Normal	High normal	Blood pressure less than 140/90 mmHg with medication	Mildly elevated (stage 1)	Moderately elevated (stage 2)	Severely elevated (stage 3)	Missing	Total	Number
Age											
15-19	2.1	76.3	18.7	2.9	0.4	1.5	0.1	0.0	0.0	100.0	380
20-24	4.2	61.7	29.8	3.7	0.7	3.3	0.2	0.0	0.6	100.0	388
25-29	3.6	51.7	37.8	6.9	0.5	2.7	0.4	0.0	0.1	100.0	399
30-34	5.8	46.3	39.6	8.2	0.0	5.5	0.3	0.0	0.0	100.0	293
35-39	6.5	43.5	43.3	6.7	0.8	4.1	0.0	1.6	0.0	100.0	256
40-44	15.4	26.9	44.3	12.5	2.2	11.2	1.9	0.1	1.0	100.0	227
45-49	20.5	19.8	42.5	17.2	0.9	14.2	5.4	0.1	0.0	100.0	196
50-54	24.8	24.0	37.4	13.8	5.5	15.8	3.3	0.2	0.0	100.0	140
55-59	19.9	18.4	43.5	18.2	0.8	16.5	0.5	2.1	0.0	100.0	54
15-49	6.9	50.6	35.0	7.3	0.7	5.1	0.9	0.2	0.2	100.0	2,140
Residence											
Urban	8.9	44.9	35.6	10.3	1.4	6.2	1.1	0.2	0.3	100.0	916
Rural	7.8	50.5	35.1	6.4	0.8	5.9	0.9	0.3	0.2	100.0	1,417
Region											
Western	13.4	43.8	31.7	11.1	3.1	7.5	2.5	0.3	0.0	100.0	314
Central	9.4	60.2	23.6	6.8	0.0	7.3	1.6	0.5	0.0	100.0	510
East-Central	6.4	46.1	39.1	7.8	0.0	6.2	0.0	0.3	0.6	100.0	646
Eastern	5.5	46.8	42.0	5.6	1.6	3.1	0.8	0.0	0.1	100.0	665
Tashkent City	12.3	37.0	36.6	14.0	1.5	9.4	0.9	0.5	0.1	100.0	198
Over sampled areas											
Karakalpakstan	13.9	30.9	37.8	17.4	0.8	8.9	3.7	0.4	0.0	100.0	185
Ferghana Oblast	7.9	41.5	43.7	6.6	0.7	5.8	1.3	0.0	0.3	100.0	259
Education											
Primary/middle	6.2	61.0	27.8	4.2	0.0	6.1	0.2	0.0	0.8	100.0	188
Secondary	6.9	50.2	35.8	7.1	0.9	4.8	1.1	0.0	0.0	100.0	1,311
Secondary special	9.9	47.1	34.2	8.6	1.3	6.8	1.2	0.6	0.2	100.0	470
Higher	12.1	36.5	38.9	12.0	1.4	9.2	0.7	0.7	0.6	100.0	364
Ethnic group											
Uzbek	7.6	50.0	34.9	7.3	0.9	5.7	0.8	0.2	0.2	100.0	2,011
Other	12.3	37.8	38.1	11.8	1.8	8.0	2.1	0.3	0.0	100.0	322
BMI											

<18.5	2.5	76.3	17.8	3.4	0.3	2.2	0.0	0.0	0.0	100.0	82
18.5-24.9	5.8	54.1	34.0	6.2	0.7	4.3	0.7	0.1	0.0	100.0	1,482
≥25	14.4	33.0	40.4	11.9	1.8	10.2	1.7	0.7	0.3	100.0	725
Missing	1.9	53.2	30.4	9.1	0.0	1.9	0.0	0.0	5.4	100.0	44
Total	8.3	48.3	35.3	7.9	1.0	6.0	1.0	0.3	0.2	100.0	2,333

Note: When systolic and diastolic blood pressures fall into different categories, the higher category determines the individual's status.
[1]Blood pressure ≥140/90 mmHg or currently taking antihypertensive medication

The overall rate for men is 8 percent among the sampled population. Uzbekistan rates of high blood pressure for the age range 35-44 are lower than those of the United States (19 percent) and China (17 percent). Comparison of gender-specific rates of hypertension, restricted to the same age interval (age 15-49), indicates little difference between women (8 percent) and men (7 percent). Regarding age, the rates in men increased about tenfold from 2 percent (age 15-19) to 20 percent and higher (age 45-59). The age-specific rates of hypertension were lower for women than for men below age 35 and higher at older ages.

There were notable differences in the prevalence of hypertension by level of education; rates were higher among men with higher education (12 percent) than among those with less education (6 or 7 percent). Differentials in hypertension rates by urban-rural residence are modest. However, there is a notable difference in the distributions of urban and rural men between the optimal and high-normal categories. There are relatively fewer urban than rural men in the optimal category (45 versus 51 percent) and relatively more urban than rural men in the high-normal category (10 versus 6 percent). In Tashkent (the capital) the rate for men is higher than other regions (12 percent), except for the Western region (13 percent).

As expected, hypertension levels were higher among overweight/obese subjects compared with those of normal weight. The hypertensive rate among overweight/obese men (BMI ≥ 25) was 14 percent as compared with 3 and 6 percent, respectively, among men who were thin (BMI < 18.5) or normal weight (BMI 18.5-24.9).

Respondents who were found at higher than normal levels of blood pressure and who were not taking antihypertensive medication were advised to attend a health service to further check on their pressure. Differentials found (e.g. more urban areas, men) can be used by program officers to target these sub-populations for dietary, exercise and check-up schemes geared at controlling high levels and maintaining overall health.

D. Hemoglobin for Anemia

As explained above, the collection of blood drops from the finger of adults or heel of infants in the field and the use of a portable hemoglobinometer has made it possible to obtain levels of hemoglobin of good accuracy and precision,[2] which can be given back to the respondents at the point-of-survey. Table 3 provides levels of moderate to severe anemia for women and children in a number of DHS countries.

[2] With the new HemoCue Hb 201+ analyzer, research has shown a medium bias of only 0.1 g/dL compared to the international standard. For more details, see Bäck et al, 2004.

Table 3
Percentage of Women and Children with Moderate or Severe Anemia[#]
Selected DHS countries

COUNTRY, YEAR OF DHS	Individual	Anemia			Number
		Moderate	Severe	Moderate or Severe[#]	
Sub-Saharan Africa					
Madagascar, 1997	Women[1]	11	1	12	3,299
	Children[2]	45	8	53	2,308
Uganda, 2000	Women	7	1	8	7,222
	Children	35	6	41	5,624
North Africa/West Asia/Europe					
Egypt, 2000	Women[3]	5	0	5	7,609
	Children	11	0	11	4,630
Central Asia					
Kazakhstan, 1999	Women	8	1	9	2,269
	Children	18	2	20	570
Kyrgyz Republic, 1997	Women	9	2	11	3,767
	Children[4]	22	1	23	843
Uzbekistan, 1996	Women	14	1	15	4,333
	Children[5]	26	1	27	988
South/Southeast Asia					
Cambodia, 2000	Women	13	1	14	3,634
	Children	31	2	33	1,414
India, 1998-99	Women[6]	15	2	17	79,663
	Children[7]	46	5	51	19,943
Latin America/Caribbean					
Bolivia, 1998	Women	6	1	7	3,299
	Children	32	3	35	1,721
Haiti, 2000	Women	16	3	19	3,138
	Children	33	2	35	2,428
Peru, 2000	Women	6	0	6	6,184
	Children	25	1	26	2,150

[#] Corresponds to levels of hemoglobin below 10 g/dL.
[1] women with a child in past 3 years; [2] children 6-35 months; [3] ever-married women 5-49 years
[4] children 6-35 months; [5] children 6-35 months; [6] ever-married women 5-49 years; [7] children 6-35 months

Madagascar, Uganda, India, Bolivia and Haiti are countries where high percentages of severe anemia in women and/or children (over 2 percent) denote a serious public health problem. These countries have started to act upon these high levels, through food supplementation and fortification, and iron distribution programs. The national surveys also brought unexpected results, such as one of the world's highest prevalence figures for mild anemia in women (not shown) in Uzbekistan.

E. HIV

Perhaps the most crucial biomarker testing DHS has conducted is for HIV. Despite the challenges reviewed above (i.e., lab capacity, ethical issues, and testing methodologies) HIV testing has proved an indispensable commodity for countries widely affected by the disease.

Earlier debate about the advisability of household testing vs. sentinel surveillance has given way to an assurance about the feasibility of the operation and the accuracy of estimates. HIV testing through household sampling is now considered "the gold standard" for prevalence estimation (Boerma et al, 2003).

To date, DHS has provided HIV prevalence figures for 9 countries and 14 more are in several stages of design or completion. As with other indicators, prevalence of the infection is being analyzed further by respondents' characteristics, confirming initial estimates (e.g., increased prevalence in urban areas or among the relatively educated population), but also opening new debate or producing inconclusive results (e.g., relationship with circumcision) requiring further analyses.

A recently released report of the 2003-04 Tanzania HIV/AIDS Indicator Survey (THIS) revealed that 7 percent of Tanzanian adults are HIV positive. Differentials present a varied picture. For example, the rate for women is 7.7 while that of men is 6.3. Urban areas are more affected, at 11 percent (see Figure 3). The prevalence by age indicates that women are infected earlier than men, with a peak at 30-34 years for women and 40-44 for men. Women and men who are separated, divorced or widowed have a significantly higher rate of HIV infection than currently married or never married survey respondents.

Other characteristics also reveal important differentials. Thus, women and men with secondary or further education have higher prevalence rates (9 and 7 percent respectively) than those with no education (6 and 4 percent respectively). The pattern is even more contrasting by economic situation. Women at the highest wealth index quintile are four times more likely to be infected than those in the lowest quintile (11.4 vs. 2.8 percent). The pattern is similar but less striking for men (9.4 vs. 4.1 percent). Similar relationship between wealth and being infected with HIV has been found in Kenya DHS, 2003 (Figure 6).

Figure 6: Adjusted odds ratio- relationship of wealth to probability of being HIV positive, Kenya, 2003 (omitted for uploading the paper)

However, the most puzzling finding seems to be arising from the relationships with circumcision status. Though it has been found in other studies that circumcision seems to be associated with lower prevalence rates, for Tanzania the figures are conflicting. Thus, by self-report, 17.7 percent of women and 69.4 percent of men were circumcised. HIV seroprevalence among circumcised women was half that of uncircumcised women, at 4.3 percent versus 8.4 percent [RR=0.51; 95% CI 0.38, 0.70]. Conversely, HIV seroprevalence was slightly higher (though not significant) among circumcised men in comparison to uncircumcised men, at 6.5 percent versus 5.6 percent [RR=1.16; 95% CI 0.91, 1.47] (Stallings, 2005). These and other findings –including results of multivariate analyses, will be presented in another panel at this same conference.

Results presented here illustrate the utility and diversity of information provided by the measurement of various biomarkers. Given its relative newness in the sphere of international health, results have more confirmed suspicions or surprised researchers than been understood and analyzed *at profundis* in order to derive informed policy from them.

Biomarker vs. recall information

Biomarkers have the potential of calibrating our estimates and redressing the balance of assumptions. This has been especially seen in the case of estimates of HIV prevalence. Generally, DHS survey results have lowered the estimates thought to exist in a number of countries (e.g. Kenya, Tanzania). Other examples abound. In the Uganda 2000-2001 DHS, night blindness was reported by 7 percent of women 15-49. However, upon testing, 52 percent of women had serum retinol levels below the cut-off point of normality. As with anemia, this apparent lack of association also indicates the need to interpret carefully biological findings with respondents' perception based on existence (or lack thereof) of clinical symptoms.

Another example comes in the case of measles in Madagascar. A recently completed survey included a response rate of 84 percent of children under 5 who provided a blood sample for tetanus and measles immunity testing. Tests found 64 percent of children 12-23 months of age immunized against measles, compared to 59 percent arising from mother's reporting based on memory or a vaccination card. This slight difference might indicate sub-reporting by mothers or an excess "natural" immunization occurring in children otherwise not vaccinated. Differences by province level generally reveal a small gap at most of 8 percentage points.

Curiously however, in one of the poorest province, Toliara, province figures are more contrasting: 65 percent of children are immunized according to levels measured by titrating in comparison with 32 percent by recall. The fact that in the recent past there had been an outbreak of measles in the population may seem to indicate the need to interpret findings in light of factors such as the natural immunization of children due to viral exposure in the community or actual acquisition of disease.

Other tests in Madagascar found two-thirds of children are anemic (34 percent moderately or severely). Almost 90 percent of eligible men and women were tested for syphilis there: 6 percent were found positive.

Biomarkers and Policy making

An important step in the DHS process is the dissemination of results and translation of data for policy making. Information collected on biomarkers and respondents' determinants are presented in ways they can influence programs and improve the health of populations.

Often times the task of tracking the aftermath of the release of a DHS dataset or the realization of a dissemination workshop is near to impossible. There is a timing factor, where results are not exerted upon until a time when the presence of Macro staff is no longer. Other times actors change and decisions might be made by officers in divisions (e.g. at the MOH) unknown to Macro staff. Still, a few examples exist of the relevance and currency of biomarker information to assist country programs and policies.

For example, the Uganda 2000 DHS found high levels of vitamin A deficiency in children and women of reproductive age. As a result of the information disseminated, recently the government is looking for ways to improve vitamin A levels in the population through food fortification

programs. With support from the USAID a pilot food fortification program was launched in July 2004 (New Vision, 2004).

DHS results in mid to late 1990s in Kazakhstan, Uzbekistan, and the Kyrgyz Republic found that about 50 percent or more of the sampled households lacked iodized salt. This has brought about the design and implementation of salt iodization programs, with the concourse of UNICEF. These findings were a major stimulant for salt iodization programs that have been initiated in all of the Central Asian republics with technical assistance from UNICEF. As of mid-2004, all of the Central Asian Republics have adopted or were in the process of adopting legislation mandating the iodization of salt used for human consumption. For example, following the 1999 Kazakhstan survey, in 2001, a five year national program to combat iodine deficiency disorders was approved.

Since 1995, the DHS surveys have been the only source of measurement of anemia levels in the Central Asian region. In four of the surveyed countries, high moderate-to-severe levels of anemia among children (0-3 years) and women (15-49 years) have led to the programs of iron supplementation and fortification of wheat flour. For example, following the 1997 Uzbekistan DHS and Turkmenistan DHS, a program of wheat flour fortification was begun in each country with the assistance from UNICEF and the Asia Development Bank.

In setting the goals for the Tenth Five Year Plan (2002-20007), India has used nutrition and child feeding data from the National Family Health Survey, 1998-99. One of the goals is to reduce severe undernutrition by 50% during the plan period from the baseline NFHS-2 estimate. Height boards (Shorr Inc.,) and Seca scales (UNICEF) have been used to measure height and weight of children in the DHS surveys.

HIV estimates from the Kenya 2003 DHS survey led government to adjust their official HIV prevalence rates figures for the country, from previously higher figures. In addition, the Kenyan investigators are also using the HIV prevalence and related information to analyze the impact of HIV/AIDS on contraception and fertility in order to ‘reposition family planning’.

Conclusion

DHS surveys have advanced an important tool for public health policy and research: the inclusion of biomarker measurements as part of the survey effort. The fact that results from these biomarkers can be linked with demographic and socioeconomic characteristics of women and men interviewed adds an almost endless potential for a much wider and deeper understanding of phenomena previously only speculated or known in limited areas.

As electronic and computerized technology brings more miniaturization of equipment and the use of light-weight materials add portability, more biomarkers will join the list of those readily available for field screening and mass application. In this paper we have reviewed some requisites to ensure quality testing in harsh conditions existing in developing countries. As conditions improve, a wider array of tests can be incorporated in the arsenal at our disposal, to match the challenge posed by new and emerging diseases and conditions, such as viral infections, malignant diseases, chronic and genetic disorders and others arising from changes in

lifestyle and behavior (e.g. obesity). DHS and other projects will research and adopt these new technologies, which should only benefit countries striving to obtain accurate data in the face of scarce resources and imperfect systems. Though demographic and routine statistics may one day replace questionnaire surveys, data derived from human or environmental biological markers should be always required and are here to stay.

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