



**OSSERVATORIO EPIDEMIOLOGICO CARDIOVASCOLARE  
HEALTH EXAMINATION SURVEY  
ITALY**

**POST-OPERAM  
MANUAL OF OPERATIONS  
(Version 11 – May 2016)**

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## 1. AIMS AND PURPOSE OF THE SURVEY

### Background

Cardiovascular diseases (CVD) are the leading causes of death and hospitalization in both genders in nearly all countries of Europe. The magnitude of the problem contrasts with the paucity, weak quality and comparability of data available on incidence and prevalence of CVD.

In Italy, the last 30 years witnessed the implementation of longitudinal studies providing baseline data on numerous risk factors collected from random samples of the adult general population followed up for all-cause mortality, fatal and non fatal cardiovascular diseases, cancer and other chronic diseases. Among these, the CUORE Project was launched in 1998 by the Italian Ministry of Health and coordinated by the National Institute of Health (ISS), Unit of Epidemiology of Cardiovascular Diseases (Head of Unit S. Giampaoli) of the National Centre of Epidemiology Surveillance and Health Promotion (CNESPS) with the follow aims to:

1. implement a surveillance system of coronary and cerebrovascular events (National Population-based Registry of coronary and cerebrovascular events);
2. describe the risk factors in the Italian population (Osservatorio Epidemiologico Cardiovascolare/Health Examination Survey - OEC/HES);
3. evaluate cardiovascular risk of the Italian adult population and prepare tools to predict 10-years risk of major fatal and non-fatal cardiovascular diseases (acute myocardial infarction and stroke)

From 2005, the project has included additional objectives:

4. to train general practitioners on the use and application of risk assessment tools (risk chart and risk score) to transfer research findings into clinical practice;
5. to explain the decline in coronary heart disease mortality;
6. to update national cardiovascular risk charts.

Detailed information of this project are reported in *www.cuore.iss.it*.

The OEC/HES, the research line n.2 of the Italian CUORE Project, resulting from a collaboration between the ISS and the National Association of Hospital Cardiologists - Heart Care Foundation (ANMCO-HCF), represents the major source of information for CVD risk factors, prevalence of high risk conditions and of CVD and other chronic degenerative

diseases at national level, thanks to the examination of the adult general population and the adoption of standardized methodologies and procedures in data collection and measurements. The aims of the OEC/HES were to describe some individual characteristics recognized as risk factors, lifestyles (diet, physical activity, alcohol consumption, smoking habit), risk conditions (hypertension, dyslipidemia, obesity, diabetes) as well as to identify cardiovascular diseases requiring action in terms of prevention, diagnosis, therapy and assistance and to monitor temporal trends of risk factors distribution and CVD prevalence ([www.cuore.iss.it/ehes](http://www.cuore.iss.it/ehes)).

The added value of the CUORE Project is the horizontal approach of the standardized data collection, enabling the collection of information on health and its determinants: health status, health determinants, personal characteristics, health care. The simultaneous collection of data through different sources of information (population based registry, longitudinal observational studies, CVD risk assessment of the adult population by the Italian risk score, cross-sectional surveys) produces a global picture and time trend of the health of the population, identifying priority areas for treatment and prevention. In addition, the periodic collection of data within the OEC/HES permits to monitor changes in health and effects of health policies and interventions.

To assure data completeness and reliability, the results are supplemented by information collected periodically by the Italian Institute of Statistics (ISTAT) through health interview surveys (HIS), and compared with more objective data coming from cancer registries or routine statistics in order to develop an increased investment in health promotion, prevention, rationalization on health care and expenditure, thus providing a powerful framework for a rational policy decision-making process.

In the National Prevention Plan for 2005-2008 launched by the Ministry of Health, cardiovascular prevention ranked first among the four main objectives of the Plan and included: 1. the assessment of cardiovascular risk in the general adult population using the risk charts of the CUORE Project; 2. the management of diabetes; 3. the prevention of complications of stroke and coronary events; 4. the prevention of obesity. Within this framework, an agreement between the Ministry of Health and the Regions was signed on March 2005 and financial support was allocated to implement risk assessment in clinical practice and to support a national training programme for general practitioners (GPs) on the use and application of cardiovascular risk charts and score. GPs were encouraged to collect data on risk factors and 10-year-cardiovascular risk score using an ad hoc software, cuore.exe,

downloaded, free of charge, from the Cuore project website. Collected data are sent to the Cardiovascular Risk Observatory (CRO), which is a web-accessible tool to monitor the global absolute cardiovascular risk in the general population aged 35-69 years. The aim of the CRO was to demonstrate feasibility and effectiveness of the application of the risk score and charts in clinical practice and to evaluate the 10-year risk assessment as first step to implement preventive lifestyle actions at individual level ([http://www.cuore.iss.it/Osservatorio/DistribuzioneRegionale\\_en.aspx](http://www.cuore.iss.it/Osservatorio/DistribuzioneRegionale_en.aspx)).

An important goal in the recent history of the Italian public health was achieved with the “Guadagnare salute: rendere facili le scelte salutari” (“Gaining health: make healthy choices easy choices” [http://www.ccm-network.it/en\\_Gaining\\_Health](http://www.ccm-network.it/en_Gaining_Health)) action plan of the Ministry of Health in agreement with the Independent Regional and Provincial Governments. “Gaining health: make healthy choices easy choices” is an integral part of the chronic disease prevention and control strategies for the “Gaining health” promoted by the WHO in autumn 2006 and aims to change unhealthy behaviours in order to reduce incidence of chronic diseases. The strategy of “Gaining health” is based on the four changeable risk factors and major determining factors for the most frequent chronic diseases: smoking, unhealthy nutrition, alcohol abuse and physical inactivity. The action plan “Gaining health” was underwritten on May 2007 when the agreements were signed between the Ministry of Health and the representatives of trade union, business and association entities.

### **Definition of the scope and objectives of the OEC/HES survey**

The objectives of the OEC/HES were to assess the prevalence of risk conditions and more frequent chronic diseases, to assess mean levels of risk factors, to describe lifestyles and treatment indicators in representative samples of the Italian general adult population.

More specifically, in the general population ages 25-79 years, the OEC/HES project aims at:

1. describing the distribution of life-styles (diet, in particular saturated fats, salt, alcohol, - physical activity and smoking habits);
2. describing the distribution of common risk factors (blood pressure, body mass index-BMI, total and HDL-cholesterol, vital capacity, forced expiratory volume, central obesity)
3. assessing the prevalence of risk conditions (hypertension, dyslipidemia, overweight and obesity, diabetes);

4. assessing the prevalence of chronic diseases (ischemic heart disease, myocardial infarction, angina pectoris, left ventricular hypertrophy, atrial fibrillation, stroke, TIA, diabetes, chronic kidney disease, BPCO, cancer, osteoporosis);
5. monitoring national healthy lifestyle campaigns, in particular evaluating if salt consumption among the general population decreases over time as a result of the programme 'Gaining Health';
6. identifying diseases, risk factors and other conditions in different socio-economic status and in different groups, such as women, older people, migrants, which require more intensive actions in terms of prevention, diagnosis, treatment and social assistance;
7. studying time trends of risk factors and chronic diseases;
8. contributing to the update of risk charts and individual risk score for the assessment of 10 year prediction of major fatal and non-fatal cardiovascular diseases (coronary and cerebrovascular);
9. contributing to the European HES (EHES) through collection and measurements of health determinants following standardized procedures and method recommended by Feasibility of a European Health Examination Survey (FEHES) Project, giving the Italian contribution to the pilot phase of the EHES to built a surveillance system for monitoring health status at European level.

To achieve OEC/HES objectives, it was necessary to enrol and examine a sample of more than 9,000 individuals aged 25-79 years, randomly extracted from the general population (220 individuals per 1.5 million residents) in 1998 and 2008. This numerosity was assessed as sufficient to evaluate disease time trends and identify possible changes in the distribution of risk factors, risk conditions and some chronic diseases.

The project was approved by the Ethic Committees of the ISS on 11 March 2008 and 11 November 2009 and is part of the 'Gaining Health Program - make healthy choices easy choices - ' of the Ministry of Health ([www.salute.gov.it](http://www.salute.gov.it)).

## **2. ORGANIZATION AND MANAGEMENT OF THE ITALIAN HEALTH EXAMINATION SURVEY**

The OEC/HES was recognized in 2009 as part of the Joint Action of the European Health Examination Surveys (EHES – Measuring the Health of Europeans <http://www.ehes.info/>) Projects funded by DG SANCO within the Health Monitoring Programme.

Therefore a manual of operations was developed according to recommendations provided by EHES Project and provided guidelines to implement a Health Examination Survey (HES) in Italy (Manual of operations Version 10 – December 2011 - <http://www.cuore.iss.it/fattori/manualeOec-Hes.asp>).

Two specific studies were associated to the OEC/HES: the CARHES study (CARDiovascular risk in Renal patients of the Italian Health Examination Survey), supported by the Association of Nephrologists with the main aim of assessing the chronic kidney disease prevalence among Italian adult general population and the MINISAL-GIRCSI project (Gruppo di lavoro Intersocietario per la Riduzione del Consumo di Sale in Italia), supported by the Centre of Disease Control of the Ministry of Health with the main aim of assessing the dietary intake of sodium and potassium in the Italian adult general population.

This manual covers the entire survey process: planning, preparation of budget, sampling and recruitment of participants, ethical, legal and data confidentiality issues, fieldwork staff training, selection and execution of measurements, blood sample handling, transport and storage, laboratory analyses, quality control methods, data management, some general indications on statistical analysis and dissemination, and provides steps we had covered and difficulties crossed during the 4 years of the two surveys.

### **Survey management**

The objectives, the schedule, the allocation of resources and the scope and methodology of the survey were approved by the Steering Committee composed by several experts. At present there are two project leaders: Simona Giampaoli, of the Istituto Superiore di Sanità and Diego Vanuzzo, designated by the ANMCO-HCF. They were and are still responsible for the organization of the survey, by allocating responsibilities and resources, managing the survey process by making decisions, giving guidance, providing and acquiring assistance, motivating team members and solving possible conflicts, day-to day monitoring and evaluating the survey process, schedules and budget and making adjustments to these when needed, planning statistical analysis and dissemination of results.

A core group have assisted the project managers in different subareas of the survey: the field work coordinator (Cinzia Lo Noce) who has prepared the field work logistics, training and day to day data collection activities; at present she is responsible of the follow-up of the population examined (vital status and mortality); two data managers (Chiara Donfrancesco and Luigi Palmieri) who were responsible for the computer system, programs, data management, sampling and at present are responsible of data analysis; the laboratory specialists (Licia Iacoviello and Amalia De Curtis) who were responsible of biological determinations; the expert in privacy and ethic issues (Virgilia Toccaceli) and the expert in communication (Eva Benelli); the responsible for coding ECG by Minnesota Code and disease-ICD (Francesco Dima). All the persons mentioned were part of the ISS staff, except Licia Iacoviello and Amalia De Curtis who during the period of the survey worked at the Laboratory of Genetic and Environmental Epidemiology of the Catholic University in Campobasso, and Eva Benelli, of the ZADIG, Salute Scienza e Ambiente.

Local authorities of each centre involved in the survey (General-Director, Director of the Health Unit, Regional assessors, Mayor) have received the manual of operations and the protocol of the study, the approval letter from the Ethics Committee of the ISS, the letter of presentation of the project, and the information notice of the project. The submission of the study to the local Ethic Committee, which has the only function to give an opinion, was at discretion of the Region. The survey has involved also a local health authority as the director of cardiology unit (as the OEC/HES survey was carried out in cooperation with ANMCO) or the director of prevention unit or the director of general medicine unit as local scientific responsible. They actively cooperate with ISS staff in order to facilitate the screening procedures.

The OEC/HES survey has included: blood pressure and pulse rate measurements, blood collection for lipid assays, fasting blood glucose and haemachrome, anthropometric measurements (height, weight, waist and hip circumference), examinations (ECG, spirometry, CO measurement, bone densitometry), questionnaire on health status, physical activity, smoking habits, personal and family history for cardiovascular diseases; a self-reported food frequency questionnaire (EPIC questionnaire), Activity of Daily Living-Instrumental Activity of Daily Living (ADL-IADL) self reported questionnaire filled by the participants during the survey, 24-hours (24h) urine collection; for persons 65 and older the Mini-Mental State examination of Folstein (MMSE) was administered. Biological samples of serum, plasma,

buffy coat, red cells and urine were stored and are preserved at the CNESPS biobank (<http://www.iss.it/biobankcnesps/>).

### **Time schedule**

The planning and preparation of the survey required more than one year before the fieldwork starting. The first survey was conducted between March 1998 and December 2002; the second survey between March 2008 and July 2012. The next table shows the time schedule of the OEC/HES 2008-2012.



Months	Friuli Venezia Giulia	Molise	Sicilia	Emilia Romagna	Calabria	Basilicata	Sardegna	Lazio	Piemonte-Veruno	Marche	Umbria	Veneto-Noale	Veneto-Motta di Livenza	Campania	Lombardia	Piemonte-Torino	Liguria	Lombardia-Montedcano	Puglia	Trentino Alto Adige	Valle d'Aosta	Toscana	Abruzzo	
April 2010										X														
May 2010										X														
June 2010										X	X													
July 2010											X													
August 2010											X													
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May 2012																					X	X		
June 2012																						X	X	
July 2012																								X

### **3. TIMING OF THE SURVEY**

#### **Periodicity**

EHES recommendation is to repeat the core measurements about every five years, while some additional measurements may be repeated less frequently (e.g. every 10 years). More frequent surveys usually do not reveal interpretable measurement changes. They can be considered only if there is a need to closely follow trends related to potential effects of specific health promotion activities.

The Italian OEC/HES represents a system of periodically data collection: the survey lasted three-four years and between one survey and the following there was a five-six years break (1<sup>st</sup> OEC/HES: 1998-2002; 2<sup>nd</sup> OEC/HES: 2008-2012). Collected data are used to monitor time trends of risk factors distribution and prevalence of lifestyles, high risk conditions and chronic diseases. This system has allowed to build a permanent survey team in the ISS with great expertise on organizing surveys, training local fieldwork staff and conducting surveys, analyzing and disseminating data.

#### **Length and timing of fieldwork period**

There is no general EHES rule for the optimal duration of data collection. In the Italian experience a four year period is the minimum time to examine 10.000 adult people using two sets of standard instruments with one team travelling across the country for involving local personnel, training and assessing quality control.

The 1998-2002 OEC/HES was conducted in 51 centres, one every 1.5 million inhabitants, each examining a random sample of 200 persons ages 35-74 years; therefore many regions had more than one centre to involve; a complete set of instruments was bought for each centre (blood pressure device, balance, tape for height, refloton for glucose and cholesterol tests). Local staff was involved; nurses and doctors were trained in Florence. Before starting the fieldwork each centre received the visit of the central coordinator to organize the work, repeat the training and assess the quality control, organize the shipment of the biological sample to the central laboratory and the data transfer to the ISS. The mean time for the fieldwork was about one month in each centre.

In 2008-2012 OEC/HES, the age range was extended to 79 years old and in order to improve the organization of the field-work and save money, it was decided to select only one centre in all regions, each examining a random sample of 220 persons for every 1.5 million inhabitants, guarantee one sample for each region with a smaller population and selecting an additional centre for some regions with a greater population.

The survey was planned to assess the health status of the population and more examinations were included; to reduce the variability two complete sets of instruments were bought and used in all centres (spirometer, ECG, bone densitometry, mercury sphygmomanometer, statimeter, balance beam scale, CO measurement instrument). The duration of the screening in each centre was due to the number of persons to be examined.

Since 20 persons a day were examined, for a sample of 220 persons, usually the screening lasted 12-15 working days, 6 days per week (including Saturday for those persons who couldn't participate during the working week); while a sample of 660 persons required at least three months.

If the survey lasted more than one month, particular attention was paid to regular quality control, re-testing and re-training of the fieldwork staff. It was also recommended to distribute the days of examination of all population subgroups (defined by age and sex) evenly over the whole survey period. Centres with a short survey duration usually needed a relatively large temporary staff, with consequently more problems related to training standardization and quality control.

The following table shows the number of persons examined in each region and the time period of the survey in each region for the OEC/HES 2008-2012.

<b>Region</b>	<b>Center</b>	<b>Number</b>	<b>Time period</b>
Friuli Venezia Giulia	Udine	222	March-May 2008
Molise	Campobasso	253	June-July 2008
Sicilia-	Caltanissetta	705	September-November 2008
Emilia Romagna	Modena	670	November 2008-January 2009
Calabria	Cittanova	253	January-February 2009
Basilicata	Potenza	208	March-April 2009
Sardegna	Loceri	230	June-July 2009
Lazio	Roma	632	October 2009-January 2010
Piemonte	Veruno	680	October 2009-January 2010
	Torino	193	September-October 2011
Marche	Camerino	235	April-June 2010

<b>Region</b>	<b>Center</b>	<b>Number</b>	<b>Time period</b>
Umbria	Perugia	226	June-September 2010
Veneto	Motta di Livenza	679	October-December 2010
Veneto	Noale	200	October-November 2010
Trentino Alto Adige	Borgo Valsugana	236	February-March 2012
Lombardia	Brescia	651	May-July 2011
	Montescano	207	January-February 2012
Toscana	Empoli	417	April-June 2012
Valle d'Aosta	Aosta	221	April-May 2012
Campania	Benevento	881	February-October 2011
Puglia	Bitonto	663	January-March 2012
Liguria	Arenzano	221	October-November 2011
Abruzzo	Atessa	228	June-July 2012

### **Time of the year**

High seasonal variation has been identified in several health determinants as well as in biological measures.

In the OEC/HES, the fieldwork was spread into several months in order to minimize the effect of seasonal variation to measurement results. Usually, fieldwork activities stopped in December, during Christmas holiday, in March or April, during Easter and August for summer holidays. When analyzing results of examinations, impact of seasonality on physical activity patterns, food consumption and quality of life was taken into account.

### **Weekdays and time of day**

In the OEC/HES it was important to include Saturdays and Sundays (usually morning only) in the survey period to facilitate people who work and raise willingness to participate. This was feasible thanks to the availability of the local staff involved in the screening.

Although both morning and evening appointments were scheduled to allow easy participation for working people, participants were usually recommended to show up at the screening centre between 8 am and 9 am as blood tests required a fasting period of at least 12 hours. The length of time from the last meal (in hours) was reported on the questionnaire. After blood pressure measurements, pulse rate for a minute and blood withdrawal, participants underwent examinations starting from 10 am until 6 pm; in some centres until 8 pm to facilitate people at work.

Thirty persons were invited every day in order to examine at least 20 persons per day, which was the maximum number of persons to be examined in one day. Out of 20 persons, 12-14 persons were usually examined in the morning, the others in the afternoon. The average duration of the complete set of examinations (except venipuncture) depended on the age of the participant and ranged from 45 to 75 minutes.

### **Order of measurements**

The following requirements were taken into account to ensure valid measurements and comparability between surveys.

#### *Clinical measurements*

The order of measurements are determined by:

1. Importance of the measurement: measurements that require 12 fasting hours were organized early in the morning. The EHES core measurements (blood pressure, blood collection, anthropometric measurements, questionnaire) were conducted first, before additional measurements.
2. Sensitivity of questions: uncontroversial questionnaires occurred early in the interview to allow participants to become relaxed and comfortable with the procedures.
3. Stressfulness of procedure: blood pressure measurement preceded venipuncture to avoid effect stress induced by blood collection and was measured in a quiet room with a comfortable temperature.

In the OEC/HES 2008-2012, to follow the order of the measurements of the previous survey (1998-2002), the following order of measurements was kept: reception of the participant, blood pressure and pulse rate measurements, blood collection. The subsequent phases (anthropometric measurements, ECG, spirometry, CO measurement, bone densitometry, lifestyle and clinical history questionnaires, food frequency questionnaire, ADL-IADL, MMSE and 24h urine collection) were performed according to local organization.

### **Questionnaires and interviews**

The questionnaires were administered during the examination (between measurements) or after the examination:

The face-to-face interview on lifestyle and clinical history was completed during the examinations. The interviewer had the responsibility to put the participant at ease in order to gain the correct information and avoid influences due to the examinations results.

The ADL-IADL questionnaire was self-administered and filled during the waiting time.

The self-administered food frequency questionnaire was filled out during the waiting time or at home after the examination, and then returned together with 24h urine container.

#### **4. TARGET POPULATION AND SAMPLE SIZE**

##### **Target population and sample size**

For the European Health Examination Survey the following definition for the target population is suggested:

- the set of all persons aged at least 25 years and at most 64 years and having permanent residence in the country;
- the eligible age group can be extended with a lower bound of 18 years and with no limitation for the upper bound.

The sampling frame consists of two stages: 1<sup>st</sup> stage, the possible cities (primary sampling units) for carrying out the survey can be selected; 2<sup>nd</sup> stage, the eligible persons. The first stage can be taken once at the start of the survey, the second stage should be taken from the registry of residents as close as possible to the time of the screening.

The EHES suggests a minimum of 4000 persons to be invited in each country, aged 25-64 years, with eight age-gender groups (25-34, 35-44, 45-54, 55-64) with at least 500 representative each. The sample size calculation is based on a participation rate of 70%. This minimum size relates to the requirements for statistical power when testing differences between countries for age-gender domains.

It was not recommended to leave out population groups which are difficult to contact or institutionalized persons (hospitals, nursing homes, elderly homes, children homes, military barracks, jails and monasteries) or migrants.

In the OEC/HES 1998-2002, 51 centres homogeneously distributed throughout the Italian territory were selected to participate in the study; the main challenge of each centre was to randomly enrol and examine 200 individuals aged 35-74 years per 1.5 million residents, also in demographically smaller regions. 9712 persons (4908 men and 4804 women) were examined and their distribution by age and sex is reported in table 1. That numerosness was estimated sufficient to evaluate distribution of risk factors and prevalence of cardiovascular high risk conditions for macro areas and assess time trends. Participation rate was not calculated in each of the 51 centres. In the total sample examined a mean participation rate of 50% was assessed.

**Table 1. OEC/HES 1998-2002: number of persons examined per age and sex**

<b>Age range</b>	<b>Men</b>	<b>Women</b>	<b>Total</b>
35-44	1147	1141	2288
45-54	1218	1232	2450
55-64	1276	1235	2511
65-74	1267	1196	2463
Total	4908	4804	9712

In the OEC/HES 2008-2012 survey, the target population included adult men and women aged 35-79 years having permanent residence in the country (Table 2)

**Table 2. OEC/HES 2008-2012: Estimate of the Italian resident population sample size required by age range and sex**

<b>Age range</b>	<b>Men</b>	<b>Women</b>	<b>Men sample size</b>	<b>Women sample size</b>
35-44	4.430.050	4.411.271	1.025	1.025
45-54	3.742.872	3.831.769	1.025	1.025
55-64	3.305.689	3.545.676	1.025	1.025
65-74	2.668.585	3.240.213	1.025	1.025
75-79	919.691	1.377.730	410	410
Total	15.066.887	16.406.658	4.510	4.510
Italy all ages	27.841.050	29.577.830		

To achieve the objectives, it was necessary to examine a sample of more than 9,000 individuals, randomly selected from the list of residents and distributed across the Regions. This numerosness has provided a complete and useful picture of the whole country and estimates for North, Centre, South and Islands, and was sufficient to assess time trends and geographical gradients.

One centre for each region was selected, for a total of twenty centres: this choice (different from that of the OEC/HES 1998-2002 survey, in which 51 centres were included) was done to improve the organization, reduce the observer variation, improve the quality control of collected variables, reduce the cost of the survey and facilitate the transfer of biological samples to the centralized laboratory for blood tests and to the ISS for storage of biological specimens. Each centre was

required to examine 220 individuals aged 35-79 years per 1.5 million residents, also in demographically smaller regions (e.g. in Lazio, the centre of Rome was selected, 660 persons were examined as the resident population in the Lazio region in the age range 35-79 years was approximately 4.500.000; in Umbria region, where the population was less than 1 million people, a sample of 220 individuals was examined), stratified by age and sex (25 persons of each sex in each age decade between the ages of 35-74 years plus 10 persons of each sex in the last 5-year age group 75-79 years every 220 units).

The access to the register of residents has permitted to know name, surname, date of birth, sex and address of each eligible person and then to send the invitation letter. The EHES Project recommends to enrol a population sample in the age-range 25-64 years, therefore, in the regions of Veneto and Piemonte, it has been decided to involve two additional centres to cover the missing age range 25-34 years. Two samples of 200 persons aged 25-64 have been examined in the municipalities of Noale and Torino following standardized procedures. Both the OEC/HES interviewer-administered questionnaire and the EHES self-administered questionnaire, translated into Italian, have been used in Noale and Torino. In total 911 persons were examined, 4555 men and 4556 women, ages 25-79 years in 23 centres, at least one in each region.

### **General requirements for centres participating in OEC/HES 2008-2012**

To be eligible for participation in the OEC/HES, each centre had to meet the following requirements:

- easy access to local registry office for sample selection;
- availability of local general practitioners to collaborate recommending their patients to participate at the screening;
- availability of local authorities to collaborate supporting the costs of local premises (not necessarily an hospital) and laboratory blood tests;
- availability of local survey personnel, possibly nurses and secretaries, to support fieldwork activities (prepare letters of invitation, arrange telephone calls to invite recruited persons, schedule appointments, welcome participants, perform laboratory tests and interviews, enter data);

- availability of local medical personnel for reading ECG, laboratory tests and other responses to be communicated to the participants;
- availability of well-equipped rooms, in particular: a big room where participants were welcomed and waited for interview/examinations; a relaxed room for questionnaires administration; a quiet room with comfortable temperature for performing blood pressure measurement; a room for performing ECG, anthropometric measurements, bone densitometry, spirometry and CO measurement; a room for blood collection and laboratory tests, with a centrifuge, a -30°C or -80°C freezer for blood samples storage and containers for special waste;
- materials for blood collection: vacutainers for serum, plasma and buffy coat with EDTA, needles and a small container for special waste collection.

A local scientific responsible was nominated to actively cooperates with ISS staff and facilitate screening procedures.

The information notice and the letter of presentation of the project was sent to the local general practitioners (GPs) for encouraging enrolled patients to participate in the study. In small centres, it was also recommended to inform the parish priest, local pharmacies, elderly clubs, as well as local press and TV, although a widespread survey publicity could increase the request for participation from persons not enrolled in the study.

Once the approval was obtained, the local register office was contacted to obtain the list of residents of the centre in the age range 35-79 years, possibly in electronic format. The random selection of the sample was performed at local level or by the ISS after receiving the list of residents.

All fieldwork activities followed the indications reported in the manual of operations of the OEC/HES and any other extra screening examinations were performed only after the established set of screening procedures.

Some centres were selected as they have a surveillance system of coronary and cerebrovascular events (National Population-based Register of Cardiovascular Events) and the collection on a regular basis of risk factors in these areas is of great importance. The Register exists in the following regions, covering the whole region or only specific areas: Friuli Venezia Giulia, Veneto, Lombardia, Emilia Romagna, Sicilia, Campania and Toscana. Up to now, Friuli Venezia

Giulia, Sicilia (Caltanissetta area), Emilia Romagna (Modena area) agreed to participate in the survey.

## 5. SAMPLING PROCEDURES

The sampling frame suggested by the EHES consists of two stages: 1<sup>st</sup> stage, the possible cities (primary sampling units) for carrying out the survey can be selected; 2<sup>nd</sup> stage, the eligible persons. The first stage could be taken once at the start of the survey, the second stage should be taken from the register of residents as close as possible to the time of the screening.

In Italy it was not possible to use a two-stage sample as within each Region the centre was selected only if it met the general requirements reported in the study protocol and manual of operations and necessary to implement screening procedures. The survey was sustainable if there was the support at local level: personnel, screening centres, laboratory. Once the municipality has been selected, the sample was stratified by age and sex and was randomly selected from the list of residents so that every eligible person had the same probability of being sampled. Only in regions where the sample size to examine was too large (Lombardia, Veneto and Piemonte), the survey was conducted in two centres.

Upon suggestion of Johan Heldal, expert in statistical methods and standards and member of the EHES Reference Centre (RC), our country has provided the RC with the population resident, by age and sex, of all Italian municipalities (8.100) stratified by Region to evaluate the probability for each individual of being sampled using the recommended EHES two-stage sampling design. Table 3 shows the target population (35-79 years) for each municipality and Region and the number of persons to be examined in each centre and the participation rate reached in each region, except Calabria, in which it was not possible because data were collected in a population enrolled for another study.

**Table 3. Regions, selected municipalities, total population in the municipalities, total population in the regions, sample size required in each region and participation rates**

<b>Region</b>	<b>Selected Municipality</b>	<b>Total population (35-79 years) municipality</b>	<b>Total population (35-79 years) in the Region</b>	<b>Sample size</b>	<b>Participation rate %</b>
Valle d'Aosta	Aosta	21,085	76,514	220	85.3
Piemonte	Veruno	1,049	2,640,080	660	63.0
	Torino	1,281,158		200	45.6
Liguria	Arenzano	7,234	1,000,527	220	60.5
Lombardia	Brescia	663,051	5,635,571	660	42.9
	Montescano	261		220	50.4
Trentino-Alto Adige	Borgo Valsugana	4,001	556,105	220	68.8
Veneto	Noale	9,092	2,787,342	200	57.0
	Motta di Livenza	5,665		660	60.8
Friuli Venezia Giulia	Udine	322,302	735,978	220	64.9
Emilia Romagna	Modena	390,516	2,520,047	660	73.6
Toscana	Empoli	28,134	2,180,859	440	53.1
Marche	Camerino	4,115	888,561	220	60.4
Umbria	Perugia	373,923	511,144	220	52.9
Lazio	Roma	2,362,845	3,202,752	660	40.3
Abruzzo	Atessa	6,043	746,660	220	48.9
Molise	Campobasso	129,851	179,621	220	43.8
Campania	Benevento	144,060	2,985,133	880	42.9
Basilicata	Potenza	213,428	323,889	220	50.1
Puglia	Bitonto	30,606	2,180,435	660	52.2
Calabria	Cittanova	5,387	1,067,084	220	---
Sicilia	Caltanissetta	141,761	2,659,893	660	52.4
Sardegna	Loceri	718	955,958	220	71.2

The population sample was extracted from the list of residents. Sample extraction was performed in the selected municipality or by the ISS upon receipt of the list of residents. If the municipality covered a large area (e.g city of Rome), the sample was extracted in the district of the screening centre. To develop the list of the eligible persons, it was necessary to create a sequence of random numbers according to the number of residents for each age decade, for men and women separately; then listed and numbered the name of residents in alphabetical order and extract the

person corresponding to the random number. To ensure that the numerosness required was reached independently of participation rate, the number of persons randomly extracted from the resident list of each centre participating in the screening was two times greater than the one originally established.

Sample extraction procedure included the following consequential steps:

- age decade was determined by subtracting the year of birth to the current year;
- age decades (35-44; 45-54; 55-64; 65-74) and last quinquennium of age (75-79) were determined by subtracting the year of birth of each individual to the current year;
- the individuals of each single decade/quinquennium were listed by alphabetical order and numbered progressively;
- a sequence of random numbers corresponding to two times as many persons as required for each decade (25 of each sex) and quinquennium (10 of each sex) was created for every 1.5 million residents;
- the individual corresponding to the first random number was extracted and so on.

To make an example, in a sample of 220 residents, 50 persons of each sex in each age decade (25 x 2) plus 20 of each sex in the last 5-year age group (10 x 2) were randomly extracted.

The individuals extracted were invited by letter to participate in the study.

The invitation letter, usually was sent 15 days prior to the beginning of screening procedures. If the first contact attempt was non successful for recruiting the selected person (the person did not show up to the scheduled appointment, the person has not refused invitation or the letter did not come back for unknown person), a second letter was sent. If also this attempt failed, a telephone call was made. After three failed attempts (two invitation letters and one telephone call), the first individual in the next group of 25 was invited in order to reach the required numerosity of 220 men and women per 1.5 million residents stratified by age and sex (25 persons of each sex in each age decade between the ages of 35-74 years plus 10 persons of each sex in the last 5-year age group 75-79 years).

The sample included resident immigrants defined as those persons born outside Italy. They represent 5% of the whole examined population (4.3% men and 5.8% women). It was necessary to work as much as possible to make them participate and understand the best they could the

questions included in the questionnaire. In the case they showed language understanding difficulties, it was possible to ask other immigrants to help them understand questions.

Regarding approaches to getting people to come to the screening, no monetary incentives were used, but participants received results of instrumental and laboratory examinations and also detailed lifestyle recommendations (tips for healthy eating and physical activity).

## **6. LEGAL, ETHICAL AND DATA CONFIDENTIALITY ISSUES**

### **General recommendations on the ethical conduct of a EHES**

In the HES it is important to obtain informed consent, to respect privacy and confidentiality, to avoid harm and to maintain well-being of participants.

Before conducting a HES, it is important to find out if there are any ethical restrictions to be considered.

All fieldwork staff members and all persons working at the Central Office need to be well informed of the reasons for which the informed consent is needed and that it is both a legal and ethical obligation. They should also know which topics are covered in the informed consent form and why they are important.

The following international reference documents should be consulted before applying for ethical approval of the survey, considered to be pillars of the ethical standards for biomedical research involving humans and also using human biological samples:

- Declaration of Helsinki, "Ethical Principles for Medical Research Involving Human Subjects", adopted in June 1964 (last rev. 2008);
- US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979 Belmont Report ("Ethical Principles and Guidelines for the Protection of Human Subjects of Research");
- Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine; The Oviedo Convention, 1997;
- Council of Europe, Committee of Ministers. Recommendation. Rec (1990) 3 concerning medical research on human beings;
- Council of Europe, Committee of Ministers. Recommendation. Rec (1994) 1 of the Committee of Ministers to member states on human tissue banks (adopted by the Committee of Ministers on 14 March 1994 at the 509<sup>th</sup> meeting of the Ministers' Deputies);
- Council of Europe, Committee of Ministers. Recommendation Rec (2006) 4 of the Committee of Ministers to member states on research on biological materials of human

origin (adopted by the Committee of Ministers on 15 March 2006 at the 958th meeting of the Ministers' Deputies).

- International Declaration on Human Genetic Data, 16 October 2003 (UNESCO).
- Guideline for Obtaining Informed Consent for the Procurement and Use of Human Tissues, Cells and Fluids in Research (WHO, 2003).

Moreover, the Italian legal norms and ethical recommendations taken into account for the setting of legal and ethical procedures were:

- Italian Legislative Decree n. 196, 30th June. Data Protection Code 2003.
- Italian Authority for Personal Data Protection: Authorization to Genetic Data Treatment, 22nd February 2007
- Linee Guida per l'Istituzione e l'Accreditamento delle Biobanche. Rapporto del Gruppo di lavoro. Comitato Nazionale per la Biosicurezza e le Biotecnologie. Presidenza Consiglio dei Ministri. 19 aprile 2006.
- Raccolta di Campioni Biologici a fini di Ricerca. Consenso Informato. Rapporto del Gruppo di lavoro. Comitato Nazionale di Bioetica e Comitato Nazionale per la Biosicurezza e le Biotecnologie e le Scienze della Vita. Presidenza Consiglio dei Ministri. 16/2/2009.

### **Ethic Committee of the OEC/HES 2008-2012**

The Ethic Committee of the ISS examined the documentation related to the OEC/HES (protocol of the study, informed consent, information notice and curriculum vitae of the responsible of the Project) and approved all aspects of the study on March 2008, including the protocol, the informed consent, the safeguarding of privacy and the use of data and biological materials. The request to participate with the Italian data in the EHES Project and then contribute to the development of an European surveillance system was approved by the Ethic Committee meeting on November 2009. The Italian data, held in an anonymous format, are recorded in a central database together with specific data for the EHES (data range 25-64 years).

To obtain approval for an HES, the following issues were presented to the Ethic Committee: 1) establishment and duration of maintenance of the biological bank; 2) criteria for the enrolment of people affected by serious senile dementia or mental disorders incapable to give informed

consent; 3) possibility to withdraw from the study and consequent destruction of the biological material; 4) procedure of data protection; 5) procedure of patient's health status updating given that clinical records cannot be accessed independently; 6) possibility to use data and biological materials for the purposes of future research; 7) possibility to provide patients with examination results and lifestyle counselling to prevent disease occurrences; 8) destruction of identification data from participants who have died during the survey period; 9) responsibilities of the survey researchers in relation to long-term storage and use of biological materials. All points were exhaustively clarified to the ISS Ethic Committee, by the responsible of the study before the beginning of the HES pilot phase. More precisely, the responsible of the study highlighted that: 1) 30 years was the minimum time period for the development of a consistent number of cerebro and cardiovascular events necessary to study the association/causality between risk factors and cardiovascular and other chronic diseases and follow disease trends; 2) physically and mentally disabled people incapable to give informed consent but willing to participate in the study should undergo examinations only if accompanied by a family member who signs the informed consent form; 3) at any point during the study the person may withdraw from the study, asking for the destruction of his/her biological materials; 4) all data from persons who accept to collaborate in the study and to be followed over time are kept strictly confidential. Collected data, stored in a computer database at the ISS and protected by two different passwords, can only be accessed by the researches who have conducted the study; individual records are kept anonymized in a separate file from that which includes names or other information essential to identify the participant. Biological samples were split into aliquots of small volume, paillettes, that were labelled with bar codes to respect the privacy of study participants; clinical information are linked to biological samples through a secure method. A specific software keeps track of all the stored samples and their location in the liquid nitrogen containers. If the person is somehow concerned about a possible violation of his/her privacy, he/she can contact the responsible of the study of whom detailed contacts are reported in the information notes; 5) the consent to an anagraphical research in the municipality was obtained from the participant in order to realize the follow-up (vital status, total and cause-specific mortality, collection and validation of acute and chronic events); 6) at the end of the screening, examination results were collected in a folder and given to the participant. The folder contained also explanations of the exams performed and lifestyle

recommendations, which represents the incentive for participating in the study; 7) identification data from participants who died during the survey period were destroyed to respect their privacy in case of future use of their clinical data, which become “irretrievably anonymized”; 8) biological samples were stored in the biological bank of the CNESPS of the ISS and only the biobank personnel can be provided with patient’s name and address or any other information that can be used, if necessary, to identify him/her. The results of the study can be published or presented at scientific meetings but the identity of the participant is protected as well as data should be always disseminated in aggregated form.

After receiving approval from the Ethic Committee, the next step was to identify the appropriate Ethic Committee in each single Italian Region involved in the survey and its requirement for opinion.

### **Safeguarding of privacy, data protection and subjects’ rights**

As stated in the Declaration of Helsinki, “Every precaution should be taken to respect the privacy of the subject and the confidentiality of the patient’s information...”, which has become increasingly important, given the progress made in information technology and the consequent ease of access to data. That privacy is safeguarded, is ensured through legislation (generally a “Data Protection Act”). Given that performing a HES includes collecting individual data and that these data constitute a particular type of personal data (i.e. sensitive data regarding health) all aspects of data protection should be covered, in particular: access to data, the exchange of data, record linkage, and anonymization procedures.

To understand better the concept of data protection, some commonly used terms were defined:

- *Personal Data*: information regarding an identifiable person, that is, one who can be directly or indirectly identified, in particular by reference to an identification number or to factors specific to his/her physical, physiological, mental, economic, cultural or social identity.
- *Processing of Personal Data*: any operation (automatic or not) performed on personal data, for example, collection, storage, adaptation or alteration, retrieval, linkage, destruction and dissemination.
- *Controller*: the person or entity that determines the purposes and means of the processing of personal data.

- *Processor*: the person or entity that processes personal data on behalf of the controller.
- *Personal Data Act* (or Data Protection Act): legislation for protecting the privacy of natural persons in the processing of personal data.
- *Sensitive Data*: personal data revealing racial or ethnic origin, political opinions, religious or philosophical beliefs, trade-union membership, criminal convictions, and data concerning health or sex life.
- *Right of Access*: the right of a human subject to consult the data collected on him/her.
- *Duty of Notification*: the obligation of the controller to notify the data protection authorities of the intention to perform data processing, including a description of the processing.

### **Informed consent**

Before performing any kind of research involving humans, informed consent must be obtained, which goes beyond merely getting an individual to sign a written form. It is a process of communication between the person and the health care professional who is conducting the study, with the goal of ensuring that the individual fully understands the scopes of the study, the methods adopted, and how the data will be used. This communication process is both an ethical and a legal obligation. The concept of consent is relevant to both the performance of the study itself and protecting the privacy of the individual. The first step in obtaining informed consent is to provide the study candidate with information, which must be complete and clear, given that the ultimate goal is to ensure that participants are truly informed. The way in which the information is presented could also influence the participation rate. The information is contained in the “information notice” and in the “informed consent”: both documents are important to obtain the participant’s signature.

The information notice was provided to the study candidate some time before the informed consent form, so that the candidate had sufficient time for reading and understanding the information before agreeing to participate. It was provided together with the “invitation letter”, which was used as an introduction to explain in general what the study was about, its importance, and how and when the candidate could be contacted. The invitation was brief and “appealing” (for more information on the invitation letter, see Chapter 7, “Recruitment of participants”).

### *Information notice*

The information notice was provided the enrolled person to make him/her truly informed about the objectives of the survey, the importance of the survey for improving public health, the required numerosity, information on sample selection, a brief description of instrumental and laboratory examinations, information on delivery of examination results, storage of biological samples, personal data treatment, ethical approval, personal data protection and future follow up with collection of morbidity and mortality data. Also the information notice report the name and signature of survey leader. Every morning, before the reception, the OEC/HES local leader was used to give a lecture, no more than 5 minutes, pointing out the key issues of the informed consent. The most common question from participants was: “why I was selected, can you take a member of my family who needs an health examination?” and the usually replay was: “this is a random sample, sorry we cannot take others”.

The participant was informed that the examinations were performed for his/her own interest and collected data from all participants were pooled for public health and research purposes.

The person enrolled was free to decide whether or not to give the consent to the use of his/her data and donation of biological specimens.

The information notice was signed by the ISS responsible of the Study. All contact details of the person who decided to withdraw from the study were reported.

The information notice was complete, self-explanatory and easy to understand.

Below is provided the information notice sent in OEC/HES 2008-2012 to the recruited participants, to local authorities (Director-General, Health Director of the Health Unit and regional assessors) and to the general practitioners to inform about the initiative.

### **THE PROGETTO CUORE – Epidemiology and Prevention of Cardiovascular Disease - Information notice for participants – Osservatorio Epidemiologico Cardiovascolare/Health Examination Survey**

*Cardiovascular disease (CVD) represents the main cause of death, the most frequent cause of hospitalization and one of the leading causes of disability; CVD is also one of the main causes of disability and cognitive impairment in old age.*

*A great deal is nowadays known about cardiovascular risk factors, conditions associated with an increased probability of developing CVD. Most recent epidemiological studies have demonstrated the reversibility of risk, that is the possibility to reduce or postpone the occurrence of disease by reducing risk factors. Given the burden of CVD, significant public health efforts are focused on its prevention.*

*Periodic population surveys and CVD registers are important tools to monitor activities aimed at fighting CVD and to assess prevalence, attack rate, case fatality of most serious forms of ischemic heart disease and stroke, mean levels of cardiovascular risk factors, prevalence of high risk conditions and treatment indicators. For this reason, the aims of the Epidemiologic Cardiovascular Observatory/Health Examination Survey (OEC/HES) 2008-2012 are: 1) to describe, after 10 years from the first survey performed between 1998 and 2002, some individual characteristics recognized as risk factors, lifestyle habits (nutrition, physical activity, smoking habit) as well as the prevalence of risk conditions (hypertension, dyslipidemia, obesity, diabetes); 2) to identify pathology areas and other conditions requiring action in terms of prevention, diagnosis, therapy and assistance; 3) to monitor temporal trends of risk factors and CVD in statistical samples representative of general population aged 35-79 years.*

*The OEC/HES has represented the Italian reference point for CVD and other chronic degenerative diseases thanks to the adoption of standardized methodologies and procedures for data collection and measurements. Data are published and are available on the website of the CUORE Project for single regions, macro-areas and for the whole country ([www.cuore.iss.it](http://www.cuore.iss.it)).*

*Recently, the interest has been progressively focused on the need to include other determinants and indicators of chronic degenerative diseases. Also, the implementation of an health examination survey has become a real possibility and a chance to participate with Italian data in the European Health Examination Survey and then contribute to the development of an European surveillance system.*

*Health Examination Surveys are based on randomly enrolled population samples and are particularly suited for providing information on behaviours and health determinants: anthropometric, physiological, and clinical measures, hematochemical parameters, useful information to assess prevalence of chronic diseases, need for health services and access to them, functional capacity and nutritional status are collected through the direct examination of*

*population. To achieve OEC/HES 2008-2012 objectives, it is necessary to enrol and examine a sample of 9.020 individuals aged 35-79 years, randomly extracted from the general population (220 individuals aged 35-79 per 1.5 million residents). In random sampling, each individual has the same probability of being chosen, also those physically or mentally incapable to giving consent. In this case, study objectives and rights of participant are illustrated to a family member. This numerosity is sufficient to evaluate disease time trends and identify possible changes in the distribution of risk factors, risk conditions and cardio-cerebrovascular diseases.*

*One or two centres maximum are selected in each Region. In each centre, a number of individuals sufficient to cover the total sample established for the whole Region is extracted from the list of residents. The screening personnel are properly trained to perform physical examinations.*

*The following screening procedures are performed: questionnaire (including questions about anagraphical data, habits and lifestyle, in particular dietary habits, past history of disease, current therapies, need of health services, in particular hospitalizations, family history of coronary heart disease, cerebrovascular diseases, diabetes, hypertension and hypercholesterolemia), venipuncture for testing total, HDL and LDL cholesterol, triglycerides and fasting blood glucose, anthropometric measurements (weight, height, hip and waist circumference), blood pressure measurement, electrocardiogram (ECG), 24-hour urine collection (for sodium, potassium, iodine and urinary creatinine measurement), carbon monoxide assessment, bone densitometry, spirometry.*

*All procedures and methodologies used follow international recommendations and quality controls.*

*Biological samples are examined at the central Laboratory of Genetic and Ambiental Epidemiology of the Catholic University of Campobasso and at the Department of Clinical and Sperimental Medicine, Faculty of Medicine and Surgery, University Ferderico II of Naples.*

*Biological samples (serum, plasma, buffy coat and packed red blood cells) are stored at the biological bank of the National Centre of Epidemiology, Surveillance and Health Promotion of the National Institute of Health (ISS) for a minimum period of 30 years, which is the minimum time period for the development of a consistent number of cerebro and cardiovascular events*

*necessary to study the association between risk factors and cardiovascular and other chronic diseases and follow disease time trends.*

*At the end of the screening, examination results are collected in a folder and given to the participant. The folder contains also explanations of the exams performed on participant and lifestyle recommendations.*

*The ISS coordinates training, standardization, quality control and support activities and is responsible for data analysis.*

*The enrolled population is followed over time for total and cause-specific mortality and for fatal and non-fatal coronary and cerebrovascular events; the surveillance activity includes collection and linkage of mortality data and hospital discharge diagnoses of resident population.*

*To achieve the objectives of the event registration, the following information are collected: resident population in the study area for men and women and for 5-year age group, cause-specific death certificates for all residents in the study area, hospital discharge records from hospitals and clinics to which residents were admitted. Suspected events can be then identified and classified following the diagnostic categories of major international epidemiologic studies (MONICA Project, WHO).*

*The CUORE Project received approval from the Ethics Committee of the ISS on 15 March 2006, on 11 March 2008 and on 11 November 2009. The request to participate with the Italian data in the European Health Examination Survey (EHES) Project and then contribute to the development of an European surveillance system was approved at the last meeting in November 2009. The Italian data, held in an anonymous format, are recorded in a central database together with data from the HES conducted in other European countries. The CUORE Project is partially funded by the Centre for Disease Control of the Ministry of Health and is part of the programme 'GAINING HEALTH', aiming at:*

- 1. Gaining health making healthy diet the easiest choice (nutrition)*
- 2. Gaining health facilitating movement and physical activity (physical activity)*
- 3. Gaining health encouraging smoking cessation (fighting smoking)*
- 4. Gaining health discouraging excessive alcohol (fighting alcohol abuse)*

*The 'GAINING HEALTH' Programme is subscribed by:*

*Ministry of Health; Ministry of Agriculture, Nutrition and Forestry Politics – INRAN; Ministry of Family Affairs; Ministry of Public Education; Ministry of Economics and Finance; Internal Ministry; Ministry of Transportation; Ministry of Economic Development; Ministry of Young Politics and Sport Activities; Ministry of University and Research; Government; Producers and Managers of public services; Regions; Local Sanitary Units; Local Entities.*

*Data and urine samples collected within the OEC study are used in two specific studies: the CARHES study (CARDIOVASCULAR risk in Renal patients of the Italian Health Examination Survey), aiming at assessing the association between chronic kidney disease and cardiovascular risk, and the MINISAL-GIRCSI (Good practice on diet: assessment of sodium, potassium and iodine concentration in the diet of the Italian population) aiming at assessing average daily consumption of sodium, potassium and iodine in adult Italian population.*

## **PERSONAL DATA PROTECTION LAW**

*All data collected by this study will be handled in accordance with the Italian law on the protection of personal data (Legislative decree n.196/2003)*

*Your personal data will be stored and processed by an electronic system in accordance with privacy regulations and will be used solely for the purposes of this research study.*

*Clinical records will be kept strictly anonymized; after collection they will be kept in a separate file from that which includes anagraphical data and only the project Responsible and persons in charge of the data processing (researchers and technicians of the ISS) will be entitled to link them.*

*Personal data shall be communicated to third parties only if necessary for the purpose of the study.*

*You will be able to exercise all rights according to Article 7 of the Legislative decree nr. 196/2003 that we wholly reproduce for your convenience.*

*1. The interested party shall have the right to obtain confirmation as to whether or not personal data concerning him/her exist, regardless of their being already recorded, and communication of such data in intelligible form.*

*2. The interested party shall have the right to be informed:*

*a) of the source of the personal data;*

*b) of the purposes and methods of the procession;*

*c) of the logic applied to the procession, if the latter is carried out with the help of electronic means;*

*d) of the identification data concerning data controller, data processors and the representative designated as per Article 5, paragraph 2;*

*e) of the entities or categories of entity to whom or which the personal data may be communicated and who or which may get to know said data in their capacity as designated representative(s) in the State's territory, data processor(s) or person(s) in charge of the processing.*

*3. A data subject shall have the right to obtain:*

*a) updating, rectification or, where interested therein, integration of the data;*

*b) erasure, anonymization form or blocking of data that have been processed unlawfully, including data whose retention is unnecessary for the purposes for which they have been collected or subsequently processed;*

*c) certification to the effect that the operations as per letters a) and b) have been notified, as also related to their contents, to the entities to whom or which the data were communicated or disseminated, unless this requirement proves impossible or involves a manifestly disproportionate effort compared with the right that is to be protected.*

4. A data subject shall have the right to object, in whole or in part:

a) on legitimate grounds, to the processing of personal data concerning him/her, even though they are relevant to the purpose of the collection;

b) to the procession of personal data concerning him/her, where it is carried out for the purpose of sending advertising materials or direct selling or else for the performance of market or commercial surveys.

In accordance to the above mentioned law, you will have the right to ask for the destruction of your biological sample (blood and urine) at any point during or after the study by making a written request to the responsible of the study.

### *Informed consent form*

Key elements in the informed consent can be defined as information, understanding, competence, voluntariness, and decision making.

The fieldwork staff members were recommended to illustrate in simple words the key points of the consent to the participant and checked if he/she had well understood the contents of the information notice, giving the possibility to ask questions or express doubts before signing the consent form.

The ‘partially restricted consent’ model was used in the OEC/HES 2008-2012. The following items were included in the informed consent form: declaration of having read, understood and approved the information provided in the information notice; assurance of data confidentiality; possibility to withdraw from the study at any time; future uses of data and biological samples; statement to contact subject for follow-up of morbidity and mortality; statement to consent to store and use biological samples for future research in the field of cerebro and cardiovascular disease; name of the person receiving the consent. The study candidate was asked to sign three copies of the informed consent form: one was given to the participant together with the results of instrumental and laboratory examinations, one is stored at local level and one is stored at the ISS.

The informed consent form used in OEC/HES 2008-2012 is shown below

## Cardiovascular Epidemiology Observatory - Informed consent form

### Section A. Consent to participate in the Study

I, the undersigned, Mr/Mrs/Miss: \_\_\_\_\_  
born in (place of birth) \_\_\_\_\_  
on (date of birth) \_\_\_\_\_

After reading the “Information Notice” and receiving a copy of it,

#### DECLARE

- that I have read and understood the Information Notice;
- that I have received clear and detailed information about objectives and procedures of the study;
- that I accept that biological samples (serum, buffy coat, red cells) extracted from my blood will be preserved in a **biological sample bank**;
- that I accept that the above-mentioned material will be stored for up to **30 years** in the biological bank of the **National Institute of Health**;
- that I have been informed and give permission to use my blood sample in **future research within the field of cerebro and cardiovascular disease** for the period specified above;
- that I have been informed and accept that results of screening and haematochemical examinations foreseen by the study protocol are stored magnetically, **kept strictly confidential and used exclusively for research purposes**;
- that I have been informed that the result of the research will be published only in **aggregate and anonymous form** exclusively for research purposes;
- that I have been informed that the study foresees regular **update** of vital and anagraphical status, as well as identification of cerebro and cardiovascular events using available sources of information mentioned in the study protocol (clinical records, GPs, registry offices, etc) and hereby I consent to this updating;
- that I have been informed that the study **received approval from the Ethics Committee of the National Institute of Health**;
- that I have been informed that my data, held in an anonymous format, will be recorded in a central database together with data from the health examination surveys (HESs) conducted in other European countries to contribute to the development of an **European surveillance system**;
- that I am aware that my **participation in this study is voluntary and that I may withdraw from the study** at any time and without giving any reasons. I know that refusal to participate or withdrawal from the study will involve no penalty, no discrimination or loss of benefits;
- that I know that for further information I can contact the Scientific Responsible of the study or any designated person.

*I declare that I have read and understood the explanation provided to me and I voluntarily agree to participate in this study.*

**/YES/**

**/NO/**

***I give permission for my blood sample to be stored in the biological bank of the National Institute of Health for up to 30 years and its use in future research in the field of cerebro and cardiovascular disease. I was informed that any future study will be published in the CUORE Project web site. I give also my permission to be re-contacted for any eventual follow-up.***

**/YES/**

**/NO/**

***Section B. Consent to the use personal data***

*I have read and understood the information on use of personal and sensible data (articles 7,8, 9, 13 of the Legislative Decree n. 196/2003) reported in the “Information Notice” .*

*I give permission to use my personal data for the purposes of this study and the management of biological samples.*

**/YES/**

**/NO/**

Date: \_\_\_\_\_

*Signature of the Participant*

*Signature of the local Responsible*

## **7. RECRUITMENT, SCHEDULING APPOINTMENTS AND MOTIVATING PARTICIPANTS**

This chapter provides suggestions for recruitment process, recruitment methods, definition of participation rate and non-participant data. The strategy and methods for recruitment OEC/HES samples were based on feasibility, budget, and regional cultural characteristics.

High participation rate is extremely important to the reliability and validity of the survey and depends directly on the success of recruitment. Since non-respondents tend to have different health characteristics from the rest of the sample, their omission often results in bias: some subjects refuse to come for examination because they feel fit and cannot be bothered, others because they feel ill and afraid. The amount of bias introduced depends on the frequency of the condition in the sample as a whole, the proportion of non-respondents, and the extent to which the non-respondents are atypical.

Since the likelihood of bias depends on the cause of non-response, the investigator numbered those who that fell into various categories were reported – for example, removed since census, on holiday, ill, dead, or refused to take part.

Personal contact (by nurse, physician or other local health professionals), convenient appointment and arrangements for time off from work were of help to elicit cooperation and overcome resistance to response.

### **Recruitment process**

#### *First contact attempt*

To obtain a high participation rate the first contact attempt was crucial. In the OEC/HES the first contact attempt was made by the personal invitation letter, accompanied by the information notice. The invitation letter was easy to understand, even by participants with slight linguistic or cognitive impairment; it stated that the examination was free and refusal to participate did not compromise any future health assistance.

The following information and instructions for the participants were included in the letter of invitation:

- objectives of the survey;

- importance of the survey for improving public health;
- importance of participation and benefits for participant;
- brief description of the measurements;
- details of the appointment: date and hour of appointment; exact location of the survey centre;
- contact information: name of the person to contact, telephone number and time to call. Contact information was important to re-schedule, confirm, cancel the appointment, or to ask for further information on the survey. Participants were asked to confirm their appointment. The opportunity to re-schedule the appointment on weekends or evenings and to obtain a certificate of absence from work was mentioned;
- indication for fasting: the minimum recommended fasting time was 12 hours (blood drawing was performed in the morning). The participant could drink water and take his/her regular medicines before the visit;
- identification: participants were recommended to bring sanitary card (including address and fiscal code), or any other valid identification card;
- medication: boxes of medicines/prescriptions were asked to bring to the examination visit;
- participants were recommended to bring eye glasses to fill in self-administered questionnaire

Sometimes it was necessary that the letter was signed by both the local responsible and the national coordinator to give more emphasis to the event. Website address for more information on the survey was reported ([www.cuore.iss.it](http://www.cuore.iss.it)).

The invitation letter was sent to the participant 15 days prior to the beginning of screening procedures: usually if recruited persons get the invitation too early they may forget the date of the appointment; on the contrary, if they get it too late, they may be unable to change their previous engagements.

The invitation letter sent to the participants in the OEC/HES 2008-2012 is reported at the end of this chapter.

The invitation letter was accompanied by the information notice.

The following information were provided to the participant before starting the visit:

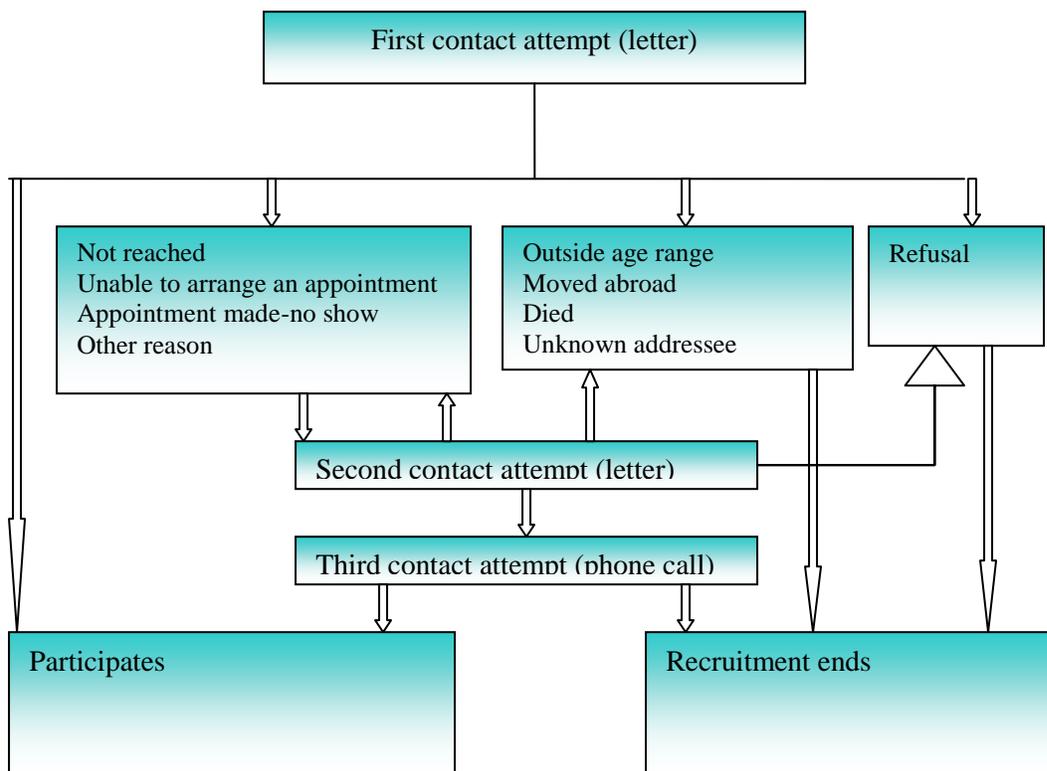
- smoking: the participant did abstain from smoking one hour before the examination (affects blood pressure measurements).
- vigorous physical exercise: the participant did abstain from vigorous physical exercise one hour before the examination (affects blood pressure measurements and blood samples).
- presence of family members, proxy: their presence could be allowed if needed e.g. in case of problems with understanding the language, speaking, hearing or cognitive capacity.

### **Re-contact attempt**

In the OEC/HES if the first contact attempt was non successful for recruiting the selected person (the person did not show up to the scheduled appointment, the person had not refused invitation, the letter did not come back for unknown person), a second letter was sent, checking accuracy, correctness and recentness of the contact information. If also the second attempt failed, a telephone call was made, although reaching people by home telephone is difficult as majority of people nowadays has only mobile phone, which is not included in telephone directories. Telephone directories often contain alphabetical lists of householders names, therefore it was not easy to obtain the number of married women. A personal approach by a phone call usually is more effective because allows to explain the objectives of the study, to stress the importance of participation and to schedule the appointment taking into account the requests or needs of the participant. It was important to state that all the participants were equally important to the survey regardless of their health condition. After three failed attempts (two invitation letters and one telephone call), recruitment attempts ended. The flow-chart shows different responses to contact attempts. The next in the list of sampled persons is invited in order to reach the required numerosity. Substitution of a non-contact with a neighbour or a person with similar characteristics was not acceptable. The number of persons invited to participate were recorded as they represent the denominator of participation rate. To calculate participation rate, the following categories were excluded from the denominator: persons who died between recruitment and invitation, persons who moved out of the sampling area; persons whose invitation letter has returned to sender for unknown address were considered unresolved if they were in the list of the residents. The enrolled populations were followed over time for total and cause-specific mortality; to achieve the objectives of mortality registration, the following information were

collected and stored also for non-respondents: name, surname, date of birth and residence. This allows linkage with total and cause-specific deaths and comparison between respondents and non-respondents.

**Figure 1. Flow-chart of contact attempts**



### Participation rate

The participation rate is essential to generalize results yielded from the sample to the general population. In the OEC/HES it was recommended to include almost one centre for each region.

It was recommended to perform all screening phases at one single centre, therefore people accepting to participate in the study normally went through all screening phases, except when they were unable to undergo examinations despite the given consensus (e.g difficulty in withdrawing blood, persons on dialysis, difficulty to measure weight/height for persons on wheelchair). Consequently we have no difference between participation rates and response rates

at each stage of data collection. Participation rates vary significantly across places: usually, they are higher in small towns and suburban areas than in metropolitan areas (see Table 3).

### **Definition**

To calculate the participation rate, the following categories were excluded from the original sample as not able to attend the screening: persons not receiving the invitation letter (undelivered letter), dead persons, persons moved out of the residence area after the sample selection. They were substituted with persons from the sample list.

Recruitment efforts were done towards obtaining the highest possible participation rate to be representative of the general population. The ideal participation rate was at least 70% but preferably higher; but during the last few years, only a few surveys have reached such a high participation rate; this is why special attention was given to increase participation rate.

It is known that non-participant are more often young men from lower socio-economic class; they have also worse health profile, more psychological disorders, they are more often smokers and have higher total and cause-specific mortality than participants.

### **How to increase participation rate**

Competence and motivated survey personnel played an important role during the recruitment process. They were trained to make enrolled persons feel valued and appreciated, part of the study and aware that their contribution to future research on chronic disease prevention was important. They were professional, friendly, respectful, showing caring manner towards participants. Proper training to manage telephone calls was also important: what were the correct answers to frequently asked questions, how to persuade uncertain participants in an acceptable manner, what options could be offered in case of difficulties in scheduling a visit (week-ends, evening hours), how to ask reasons for non-participation.

During the recruitment process there were several important issues that influenced the motivation to participate, such as multiple contact attempts; communication plan involving local press, local General Practitioners' and other health operators; possibility to schedule appointments on weekend; possibility to re-schedule an appointment and to withdraw from the study at any time

for any reason; prolonged opening hours; providing participants with examinations' results; offering certification of absence from work.

In addition, the letter of invitation was structured to favour participation in the study.

To facilitate participation of resident migrants showing language difficulties, they were assured that one member of the family (usually a younger component) or other migrants could help them to understand questions if necessary.

No monetary incentives were used to encourage attendance but examinations results and tailored detailed lifestyle recommendations (tips for healthy eating, physical activity and smoking cessation) may acted as incentives.

In addition to the recruitment process, motivation of the personnel was also important during the fieldwork. Issues that increase motivation at the examination site were:

- friendly atmosphere, so that participants felt welcome and appreciated;
- examination site near participants' home;
- comfortable facilities, e.g. room privacy is important;
- no waiting times or minimal waiting between measurements;
- possibility to interrupt visit and re-schedule it in a later time;
- possibility to be accompanied by a family member or a proxy, if needed.

Also the importance of keeping promises and adjusting to different situations (e.g. special needs) were important issues for the motivation.

Partnership and collaboration with local organizations, professionals and communities helped to raise awareness of the importance of the survey, and to arrange easy access to the examinations.

Pre-notification prior to survey to local government and health authorities of each centre involved in the survey was an effective way to favour participation; they were notified to ensure the community's understanding and support; they received the manual of operations and the protocol of the study, the approval letter from the Central Ethic Committee, the letter of presentation of the project, and the information notice of the project explaining that the survey aimed at giving a complete picture of the Italian population's health status; an overview of the health status facilitates the planning and evaluation of future preventive programmes. Local health authorities, in particular GPs, were notified to encourage patients enrolled to participate in the study. In small

centres, parish priest, local pharmacies, elderly clubs, departments of prevention, as well as local press and TV, received information about the study. Cooperation with regional or local hospitals, research centres and universities increased the interest in participation.



## THE PROGETTO CUORE – Epidemiology and Prevention of Cardiovascular Disease

### Letter of invitation

Dear Mr/Mrs/Ms,

you have been enrolled to participate in the Progetto CUORE - Epidemiology and Prevention of Cardiovascular Disease.

This project aims at assessing the distribution of cardiovascular risk factors in the Italian population, the number of persons at high risk of cardio-cerebrovascular diseases and of those who have already experienced an event, in order to prevent the occurrence of these diseases, which represent the main cause of mortality, morbidity and disability in our country.

The scientific protocol of the study, of which you find enclosed the information notice, foresees:

- an accurate collection of your clinical data and of those of your relatives in a questionnaire administrated by an health operator; information on lifestyles, nutrition and physical activity will be collected;
- a medical examination with weight, height, waist, hip measurements; pulse rate and blood pressure measurements;
- the execution of electrocardiogram, bone densitometry and spirometry;
- a venipuncture for assessment of fasting blood glucose, total and HDL cholesterol, haemocrome;
- 24-h urine collection.

At the end of the screening, you will receive results of the examinations performed on you so that you can inform your physician. Biological samples will be stored at the biobank of the National Centre for Epidemiology, Surveillance and Promotion of Health of the National Institute of Health.

Your participation is free and will involve no cost to you. You can withdraw from the study at any time and without giving any reasons. Withdrawal from the study will have no consequences on your clinical controls.

Any information about you collected in this study will be used exclusively for scientific purposes.

The examination will be performed on .....

at .....

Fasting from at least 12 hours is required. It is also recommended to bring eyeglasses and the boxes of medicine you regularly take, in order to take note of prescriptions made by your physician.

We hope you can accept to contribute to the collection of relevant epidemiological data on prevention of cardio-cerebrovascular diseases

Thank you for cooperation

Date ..... Dr .....

## **8. QUALITY ASSURANCE**

### **Training programme**

Training is the key element of standardization and quality assurance.

All members of the national survey team, both those working at the ISS and all fieldwork staff members received the training programme. It was essential for the quality of the survey: secretaries and assistants working at the central survey office, those who contacted the selected persons, sent the invitations and scheduled the visits, data managers, statisticians and all fieldwork staff members understood aims of the survey and the whole data collection process.

All survey staff members were recommended to read the manual before training sessions and eventually update it at the end of the screening on the basis of suggestions arisen during fieldwork procedures.

The ISS was responsible for training fieldwork staff members. The training sessions took place during the first week of the screening in each selected centre although its duration varied depending on the previous survey experience of the selected staff members and distribution of tasks between the fieldwork members. To allow substitution of other fieldwork members when needed and rotating tasks, each team member was trained to handle several measurements. If screening lasted more than planned, refresh training sessions were foreseen.

The training included both general issues for all staff members, general fieldwork skills and practices for the fieldwork staff, and specific training for each selected measurement. Practical measurement sessions were needed also for the experienced staff members to ensure that the standards were followed correctly.

The training for all survey members included following topics:

- purpose and aims of the survey: it was important that all staff members had understood the importance of the survey and were able to describe the aims and purpose of the survey to the participants in a standard way;

- ethical issues and confidentiality: what was intended with data confidentiality and how it was assured by all staff members, why an informed consent was needed, what was meant by the informed consent.
- random samples and the importance of high participation rates: how people were selected, and why all selected persons were equally important regardless of their health status or other characteristics, how participation could be encouraged and motivated;
- the importance of standardization and quality assurance: understanding the aims of audit visits and quality assurance, the role of the survey manuals, the importance of consulting supervisors when needed;
- survey organization: roles and responsibilities of each staff member at the central office and in the fieldwork teams;
- working with the local health care professionals e.g. to build and maintain good collaboration, so that they encourage their patients to participate in the survey, and referring participants with abnormal measurement results to their GPs or other local health care professionals;
- how the survey results were reported and published, publicity rules and working with local media during fieldwork;
- data management system and IT skills for data entry, handling and reporting.

The training for the members of the field work teams who were carrying out the measurements included the following topics:

- specific procedures for each interview module or instrument;
- specific measurements: rationale why they were measured, measurement techniques, including practical training;
- how give the feedback to participants concerning measurement results;
- information to physicians and local health care professionals when needed;

- safety of the fieldwork team members (e.g. actions needed in case of needle stick injuries, violently acting and aggressive participants).

For example, the personnel responsible for collecting blood samples were trained following the part of the protocol that pertains to blood collection, the safety instructions for protecting the participant and the nurse or technician during the blood sample collection. Similarly those who carried out the blood pressure measurements needed specific information on why standardized blood pressure measurements were needed, what were the key steps in the measurement protocol, how the results were recorded and how the results were explained to the participants. The practical training included e.g. carrying out adequate number of measurements observed by supervisors and feedback sessions. The double stethoscope is used to check the readings of blood pressure measurement. Differences more than two mmHg between the trainer and the nurses were not allowed.

The trainers participated in the trained seminars organized by the EHES; they were encouraged to read the survey manuals before the training sessions, during and/or after the training. The manuals of operation, continuously updated until 2011, formed the basis for all training.

Open discussions between all field work members and other survey staff members were encouraged during the training sessions. During the fieldwork, meetings with the supervisors, audit visits and feedback sessions supported learning and point out the importance of standardization.

### **Duration and timing of the training**

In the OEC/HES 1998-2002 the training section was centralized in Florence at the ANMCO-HCF offices with a two days course; all the nurses involved in the fieldwork and the local cardiology responsible of the survey participated to the meeting. During the screening a site visit was organized in order to check how the measurements were done and how the data were collected; that visit was also important for quality control.

After that experience we have realized that the course was not helpful for those centres which were not able to start immediately. Planning the OEC/HES 2008-2012 we agreed that the best time for training could be the first week of screening: in this way, trainees had the possibility to observe trainers and progressively substituted to them in performing measurements as they

gained expertise. To allow substitution of fieldwork team members when needed and rotating tasks, each team member was trained to handle several measurements. Retraining during fieldwork should be organized if the fieldwork lasts for more than two or three months to ensure that the standards were kept. Retraining was essential also if observer effects or non-adherence to survey standards were observed during audit visits or by other forms of quality control during the fieldwork.

### **Quality control**

Standardisation of measurements, training of personnel and quality control are essential to assure reliable and comparable data. Only instruments with sensitivity/specificity assessment were used. It was also important to periodically check that instruments were perfectly functioning. They were used for the whole duration of the survey.

Measure variability was recorded:

- season of year;
- time of day (morning/afternoon);
- time of last;
- time from the venipuncture and the centrifugation of blood samples; that was important for the fasting blood glucose test the value of which reduces of 5% each hour.

### **Survey management**

The ISS coordinated the training and testing of survey personnel, standardization of measurements, quality control. The survey project leader availed of the support of a group of experts in order to assure an effective survey organization.

The OEC/HES manual of operation 2011 describes in a detailed manner the procedures to be used in the survey. All recommendations provided by the EHES Manual of Operations were followed and all deviations from them were discussed with the EHES Reference Centre (RC). The OEC/HES manual was developed in Italian and in English to facilitate communication with the EHES RC and other partner countries

([http://www.cuore.iss.it/eng/factors/pdf/National%20manual%20operations-Italy\\_Dec-2011.pdf](http://www.cuore.iss.it/eng/factors/pdf/National%20manual%20operations-Italy_Dec-2011.pdf)).

## **Organizing quality control for the survey procedures**

### ***Internal quality control***

The procedure for examinations were planned carefully and a routine retraining of all personnel doing examinations was performed.

To comply with EHES recommendations, some changes have been introduced with respect to the previous survey OEC 1998-2002. For example, two consecutive measurements of blood pressure were performed in the OEC 1998-2002 survey, three were performed in the OEC/HES 2008-2012 survey although position of participant and methodology were the same. To study temporal trends, the mean value of 1<sup>st</sup> and 2<sup>nd</sup> measurements was considered (as in previous surveys), while the mean value of 2<sup>nd</sup> and 3<sup>rd</sup> measurements was considered to make comparison with EHES partner countries.

Weight was measured with a standard electronic scale in the OEC 1998-2002 survey, with a balanced beam scale in the OEC/HES 2008-2012. During the OEC/HES 2008-2012 survey, blood pressure, weight and height measurements were particularly subjected to quality control measures.

For blood pressure:

- proportion of identical blood pressure values in the two/three measurements performed;
- frequency of the last digit: 0,2,4,6,8 should have the same chance of occurring (20% each).

It was useful to check mean values and standard deviations of single parameters measured after the first day of the survey; if standard deviation was too much different from the value assessed in other team, the procedure for blood pressure, weight/height, circumference and other measurement, should be checked and, if necessary, personnel was trained again.

### ***External quality assessment (EQA)***

Data obtained from the OEC/HES 2008-2012 were assessed by the EHES Reference Centre (RC). The EHES RC was responsible for reviewing the version of the OEC/HES national manual and assessing survey and quality control procedures through the site visit. External laboratory quality performance was also assessed by the RC.

### *Site visit of EHES-RC*

The evaluation of the OEC/HES procedures used, data generated in the surveys and data and information generated through external controls were evaluated by the RC, with the help of the national survey coordinator and organizers. The site visit was held from the EHES –RC at Brescia on May 24-25 2011; the complete report at THL-EHES. Here below the conclusion and recommendations of the Italian site visit report:

*The Italian OEC/HES has been built since the 1990s. It is based on collaboration with regional authorities and the methods have been adapted to what is feasible in each region. A specific characteristic of the Italian survey is that separate local fieldwork teams are recruited for each centre (region). This is challenging for the training. Therefore, particular attention during the site visit was paid to the training procedures and the performance of the fieldwork staff. Our observations were positive, and the team was very dedicated to the work. Future surveys can no doubt be built on this excellent work.*

*The main challenge in Italy is the participation. The impression was that rural areas are easier for recruitment and largest cities most challenging, but not much can be done to motivate people to show up. Perhaps one solution could be to increase the awareness of the population about the survey, so they will recognize it when they receive the invitation. So far the participation rates have varied in the Centres from 40 to 78 %.*

*As always, it is possible to further enhance standardization and quality control. Specific issues on this are summarized below:*

- *More attention should be given to the position of the measuring tape for waist circumference measurements;*
- *Attention should be paid to sufficient resting time before and between blood pressure measurements.*
- *Quietness of the room during blood pressure measurement should be ensured. During the site visit the window was open and noise from the traffic could disturb the auscultation.*
- *We did not get a good record of the tubes used in blood drawing; for the centralised blood measures and preservation in the CNESPS-ISS biobank 1 blood collection tube of serum of 10cc and 2 blood collection tubes of 4.5 cc with EDTA are recommended; these quantity allow to preserve 6 paillettes of serum (2 sent to Campobasso for the centralised tests) 6 of plasma, 2 of buffy coat and 2 of red cells; for local blood analysis (to be given*

*to the participant) 1 tube of serum and 1 tube of 4.5cc with EDTA. All the blood collection kit is available at local level and differs from centre to centre.*

- *It is strongly recommended that a routine back-up of the data entry system is taken several times each day.*
- *The relative centrifugal force should be calculated using the formula in the EHES manual. If it is less than 2000g, the centrifuging speed should be increased.*

## **9. DATA MANAGEMENT**

Planning of the data management was part of the general planning of the survey from the beginning. Well-planned data management facilitates good quality and availability of the data for analysis. Data management ensured that the data recorded during the fieldwork were available for analyses, and that the available data were complete (no data collected from the participants were lost), correct and verifiable. In this way the data analyses was done using the correct data, without errors and documented properly, so the whole analysis could be repeated later. Data management included also the security of the data storage.

A detailed plan for the data management including all phases of the survey (sample selection and recruitment; appointment scheduling; survey data collection; recording the laboratory and exams; error checking, corrections, and documentation of the data; data transfer and storage) has been created at the beginning of the OEC/HES.

A central database was developed to store separately personal data and data on measurements; they are maintained in the ISS.

### **Subject identification**

Every participant in the survey was identified using the serial number which included a code identifying the region, a code identifying the centre and a code identifying the subject. The code identifies the region goes from 01 to 20 (in Italy there are 20 regions); for OEC/HES 1998-2002, the code identifying the centre goes from 1 to 87; for OEC/HES 2008-2012, the code identifying the centre goes from 101 to 123 and is consecutive to the time of the survey (in three regions two samples were enrolled); the codes that identifies the subject is consecutive with the presentation of the person at the screening centre and varies from 1 to 880 depending on the number of participants in each region/centre. Participant sensitive information are recorded in a separate file (*Anagrafe*) containing also anagraphical data of non-respondents. Due to the sensitive data (name, surname, data of birth, sex, address) *Anagrafe* file is available only in one computer and accessible to one person with a password. A copy of that file is available at the storage backup device; in that file is recorded the participation status (respondent or non respondent) and eventually the reason for non participation.

### **Sample selection, recruitment and appointment scheduling**

A file with the appointment schedule was sent to the centres at the beginning of the screening: for each day of the screening the list of the participants (name, surname, place and data of birth, address) with the day and time of the appointment for the examination. In the invitation letter was indicates the day and the time of the appointment for the examination; moreover the name, the phone and time to call the nurse was indicated to confirm the appointment. This was important to take note of the number of appointments per day; for example if the subject was unable to participate at the screening in the day scheduled he/she could changes to a more convenient date and time during the screening period. Regarding the future surveys it might be important to keep a record of change appointment times.

### **Survey data**

Recording the survey measurements and getting data from different examination sites to the common database were essential part of the data management.

When the person was welcomed to the screening centre and before signing the informed consent the following information were recorded:

- serial number assigned to every participant: this is unique and was included in all the paper documents, laboratory tests and other exams of the participants (ECG, nutrition questionnaire, ADL-IADL questionnaire, spirometry, bone densitometry);
- anagraphical data (surname, name, married surname), checking that the anagraphical data reported in the appointment list were correct;
- fiscal code;
- sex;
- date and place of birth;
- country of birth (used as a proxy to evaluate immigration)
- address and home or mobile phone number (work, relative's phone number, etc.)
- self-reported weight and height, flu vaccination.

Recording the survey measurements and getting data from different examination sites includes:

- self-administered questionnaires: ADL-IADL was self-administered and input in the software of database later, usually in the afternoon after the screening;
- interview: was a computer assisted data collection; this way reduces the number of manual data transfer and facilitates data checking at early stage;
- recording the values of physical measurements: in values of vital capacity and forced expiratory volume in one second from spirometry; stiffness, t-score and z-score from bone densitometry were input in the database by the result sheet, usually in the afternoon after the screening;
- recording the laboratory test assayed at local level: total and HDL-cholesterol, fasting blood glucose and haemachrome are assayed at local level; these laboratory tests were input in the database software usually in the afternoon after the screening; this tests were not used for the statistical data analysis but they were important as result of clinical examination;
- recording the number of the paillettes of serum, plasma, buffy coat, red cells, prepared in the laboratory and the identification number of the biological samples; this number is different from the serial number of the subject, it correspond to the serial number of the biological specimen bank;
- recording the quantity of 24h urine collection; when the participant come back to the screening centre to give the container with 24h urine, the nurse control the quantity of the urine and input the data in the database and stores 4 tubes in the freezer.

The inclusion in the database software of the biological/physical measurements allowed to prepare the result-sheet for the participant. All the important results (blood pressure, height, weight, hip, waist, body mass index, spirometry, bone densitometry, cardiovascular risk, total and HDL cholesterol, fasting blood glucose and haemachrome) were printed and gave to the participant when he/she come back to the screening centre with the 24h urine collection and food frequency questionnaire.

Usually at the end of 3 month screening, the centralized laboratory sent to the ISS the laboratory results (lipids-total and HDL cholesterol, triglycerides – and fasting blood glucose, the identification number correspond to the serial number of the biological specimen bank. This results were input in the database and were considered in the statistical analysis.

The self-reported diet questionnaire was centralized coded and by optical read getting in electronic format.

Errors and incompleteness of the records were prevented by routine checking of the forms and the data by the staff. For relevant data not obtained by the subject a specific code was used. To prevent loss of records, the subject identification code was recorded at all stages and also laboratory samples were labelled with bar codes with a reference to the subject identification code.

A computer-assisted data collection was used. Each collaborating centre was provided with the 'CuoreOEC/HES' software to collect data from face-to-face questionnaire on health status. The software was segmented into several independent sections, one for each questionnaire topic, and allowed users to insert all information about blood pressure, anthropometric measurements, bone densitometry, spirometry and laboratory analyses on a daily basis. At the end of each section, the software alerted user of missing or incomplete data before saving information, therefore data incompleteness was avoided. The first page of the software was completed during welcome of participants and included name of the region and of the municipality, date of examination, anagraphical data, identification code, address, telephone, fiscal code and self-reported weight and height values. After that, anagraphical data, identification code and date of examination appeared on each page of the software.

After the end of each regional screening, information collected through the computerized questionnaire were stored on an external hard drive. For organizational problem (the person moved from one room to another) some information collected in different rooms (personal data, weight and height measurement, waist and hip measurement, self reported height and weight, blood pressure measurement, room temperature, hours of fasting, level of total, HDL cholesterol, glycaemia) were collected in a paper form and then reported in the database-software.

### **Error checking, correction and documentation of the data**

Mean, standard deviation, minimum and maximum and frequency distribution of every continuous variable were calculated and compared with standard values. Frequency distribution of every categorical variable was calculated and compared with standard values as well.

The paper form was checked if a value was found to be out of standard interval or internal to the standard interval but close to the lower bound or upper bound. If the variable was not included in

the paper form the plausibility of the value was checked evaluating linked variables, e.g., weight was checked evaluating height, waist and hip circumference. When evaluation regarded laboratory determinations the centralized laboratory was contacted.

When the value was recognized as not acceptable is recorded as missing value.

### **Transfer and storage of the data**

Computerized data are stored on an external hard drive from which data were further transferred into the central OEC/HES database. Collected data, protected by two different passwords, can only be accessed by the researcher responsible of the database in order to prevent disclosure of information to unauthorized individuals. The access to information is allowed only through proper identification and authentication of the user. The database does not include personal data but only the personal identification number. Individual records are kept anonymized in a protected file (*Anagrafe*), separate from that which includes names or other information that could be used to identify the participant. The information connecting survey data to personal identification of the subject is available only to persons authorized to have access to such data.

The ‘CuoreOEC’ software offered also data backup, which was usually performed on a daily basis to prevent accidental loss of data.

### **Statistical analyses**

The SAS Software through a syntax saved in a file was used to document data management quality controls and all modification of the database. The distributions of lifestyles, risk factors and prevalence of cardiovascular diseases were published (Eur J Prev Cardiol 2015). Data are reported for the age range of 35–74 years. All data, graphics and tables are age-adjusted by direct methods, considering the European population at 2013 as the standard, and are presented by gender and time trends (1998–2002 versus 2008–2012) of means and proportions (with respective confidence intervals at 95%) and assessed at the two educational levels, low education (primary/middle school) and high education (high school/university). Differences are considered statistically significant when 95% confidence intervals do not overlap.

## 10. SELECTED MEASUREMENTS

Measurements have been divided into core and additional. Core measurements are the EHES minimum set of measurements which should be included. Additional measurements were included in the OEC/HES survey.

### Criteria for selecting the measurements

#### *EHES core measurements*

The EHES core measurements meet the following criteria, but also the additional measurements should be evaluated considering criteria reported for EHES (rational and importance of criteria).

<b>The criteria for selecting the measurements</b>	<b>Rationale and importance of the criteria</b>
Availability of international standards	Internationally standardized measurement protocols are recommended to be used when ever possible; this ensures comparability of the results between countries and in time.
Clear interpretation of the results	The measurements need to be reliable.
Practicality, easy to administrate	The measurements need to be feasible at population level.
Interesting for participants	It is recommended to have at last one measurement that motivates people to take part. This may increase the participation rate. Personal results can also be interpreted and used to estimate needs for care and preventive activities for the individual.
Acceptability to the participant	The selected measurements should not be too time consuming, causing extra burden, pain or discomfort for the participants.
Ethical acceptability	Measurements have to be ethically approved and safe for the participants, as well as accepted by health care professionals. If deviations from normal values are identified, access to care and preventive activities needs to be assured.
Costs	Costs of measurements and available funds need to be in balance. Selecting one expensive measurement may drop out several cheaper ones.

Public health importance	Selected measurements should address key public health problems.
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The core EHES physical and clinical measurements, analyses of blood samples, and questionnaire items, collect data mainly on major chronic diseases (e.g. cardiovascular diseases and diabetes), and their risk factors (e.g. obesity, high blood pressure and high serum cholesterol) which are preventable at both individual and community level.

Physical measurements are needed because self-reported data are not sufficiently reliable to follow population trends or to make comparisons between populations. These selected measurements are also the ones that have been measured in previous national HESs conducted in Europe.

The core physical measurements are: height, weight, waist circumference, blood pressure.

The core biological samples are: non-fasting blood for total and HDL cholesterol and fasting blood sample (8-14 hours) for glucose.

The core questionnaire includes questions on household size, sex, age, marital status, socioeconomic status (education, occupation and household income), height and weight, general health, chronic diseases, use of medication, smoking.

Age and sex enable reporting of the HES results by sex and age group and the age-adjustment of the results for comparison between populations.

#### *Additional measurements*

In addition to the core measurements other physical measurements and questions were included in the OEC/HES. When choosing the additional measurements, the criteria shown in the table were kept in mind.

The additional measurements were: hip circumference, lung function test, carbon monoxide assessment, ECG Minnesota code, bone densitometry, 24h urine collection for sodium and potassium excretion, diet and physical activity questionnaire, family history for cardiovascular diseases, dyslipidemia and diabetes; ADL-IADL score, MMSE, self-reported health rate; they were added with the following rationale:

- assessment of trend of some diseases. On the basis of the rich tradition of epidemiological studies of cardiovascular diseases and the experience in ECG reading through Minnesota code we have decided to include an ECG at rest to assess the prevalence of some conditions, such as left ventricular hypertrophy, atrial fibrillation and ischemic cardiopathy and to validate history of ischemic heart disease;
- assessment of some indicators of respiratory function, such as the Forced Vital Capacity and Forced Expiratory Volume in 1 Second;
- assessment of carbon monoxide exposure (cigarette smoke, chimneys, etc...);
- assessment of calcium in bones through bone densitometry;
- diet in order to evaluate food consumption, daily intake of food items, food groups and nutrients, and validation of sodium/potassium with 24h urine excretion;
- assessment of disability and self perception of health;
- assessment of cognitive function in persons aged 65 years and older.

The EPIC food frequency self-administered questionnaire was used; the same questionnaire is usually administered to the participants enrolled in the cancer longitudinal epidemiological studies. This is a semi-quantitative food frequency questionnaire.

These additional measurements were chosen to assess the health status of the Italian adult population, distribution of health determinants, risk factors and prevalence of major chronic diseases. Moreover the choice of some examinations, such as bone densitometry, was attractive and increased participation.

After the launch of salt reduction campaign of WHO and the agreement with Italian bread makers associations, the 24h urine collection was suggested as indicator for monitoring the sodium consumption in the general population. Potassium excretion is a good indicator of vegetables and fruit consumption.

The ADL-IADL were registered by a self-administered questionnaire similar to the one used in the WHO Eleven Countries Study. The Italian translation of the questionnaire was done by the research group in the FINE study (two independent translators) from the English version and checked by Morosini in 1985 (National Institute of Health, Rome). Each item was coded using 4 categories (1 to 4: not able to perform, some help needed, with difficulty but without help, without any difficulty). For the classification of subjects a hierarchical scheme was used,

concerning limitations in Basic ADL, Mobility, and IADL. For this purpose the items were recorded combining the first two categories (disabled) and the third and fourth category (not disabled).

Global cognitive function in persons 65years or more was examined with the Mini-Mental State Examination (MMSE), taken by a trained nurse. The test includes questions on orientation to time and place, registration, attention and calculation, recall, language and visual construction. The test was originally created for a clinical setting but is extensively used in epidemiological studies. The Italian version was translated by the research group of the FINE study (two independent translators) and checked by Morosini in 1985. The maximum score is 30 points. A higher score indicates a better cognitive performance. In the case of one, two or three missing items (out of a total of 20), the item was given the value zero, since it was assumed that people refuse to answer when they don't know the answer. If four or more items were missing no summary score was calculated. The score should be adjusted by level of education

Self-reported health rate, a scale with 10 steps, step 1 corresponds to bad health and 10 step to optimal.

All procedures and methodologies used in this additional measurement followed international recommendations and quality controls.

Biological samples (serum, plasma, buffy coat and red cells, 24h urine) were stored at the biological bank of the CNESPS of the ISS and will be preserved for a minimum period of 30 years, which is the minimum time period for the development of a consistent number of cerebro and cardiovascular events necessary to study the association between risk factors, cardiovascular disease and other chronic disease and follow disease trend. A copy of informed consent is preserved at the ISS.

## 11. NON-PARTICIPANT INFORMATION

In order to assess the non-participation bias, it was important to collect information on non-participants to evaluate potential biases in estimates. Follow-up of vital status was updated until December 2014 for participants and non-participants. Some key information such as age, sex, and some aspects of social status can be obtained from the sampling frame.

### **Data to be recorded**

For each person invited to participate, number and type of contact attempts was recorded, if refused or dropped out after having agreed to participate, if completed or not completed examinations. Reasons behind refusal were recorded as followed:

- *Refused*: no reason given
- *Refused*: lack of time
- *Refused*: personal principle
- *Refused*: health problem (e.g. disability restricting access to the examination site or is hospitalised)
- *Refused*: feeling healthy (therefore thinks that there is no reason to participate)
- *Not contacted*: not reached (no address/phone number available, outdated information)
- *Not eligible*: moved abroad (not known at the address; the letter was back to the sender)
- *Not eligible*: age out of survey range
- *Temporarily unavailable*: e.g. holiday
- *Language problems*
- *Not eligible*: died
- *Impossible to examine* for other reason (this reason should be specified, if feasible)

## 12. SELECTING THE EXAMINATION SITE

All examination sites have their advantages and disadvantages

	<b>Participant's home</b>	<b>Temporary examination site</b>	<b>Examination site within existing health care premises</b>	<b>Mobile examination site</b>
Access by participants	Easy access	Requires effort	Requires effort	May be easy if mobile examination site can be taken close to the participants
Cost for participants	None	Travel costs	Travel costs	Some travel costs
Environment				
Atmosphere	Relaxed	Some tension	Some or a lot of tension	Some tension
Privacy	Limited privacy if other family members at home	Can be controlled	Can be controlled	Can be controlled
Temperature	Cannot be controlled by the survey team	Can usually be controlled	Can usually be controlled	Can be controlled
Quietness	Cannot be controlled by the survey team	Can usually be controlled	Can usually be controlled	Can be controlled
Safety of the field work staff	Limited and cannot be controlled	Can be controlled	Can be controlled	Can be controlled
Travel cost of field work staff	Expensive	Some	Some	Some
Traveling for field work staff	Lot of traveling	Some	Some	Some
Restriction to measurements	Only measurements for which devices can be transported easily and which do not have specific environmental requirements	Generally none	Generally none	Generally none, sometimes a lack of facilities for specific measurements may come up (e.g. limited space)
Calibration/standardization of the measurements	Difficult	Can be done	Can be done (but if using equipment of health care centre, standardization and	Can be done

			calibration may be difficult)	
Acceptability	Some people are not willing to let the survey team into their home	Generally accepted	In some countries may not be highly valued among some people	Generally accepted
Time and cost for setting up an examination site	Minimal	Time consuming	Takes some time (depends on equipment used, if equipment from the health centre are used, careful calibration before fieldwork is needed, otherwise like temporary examination site)	Time consuming and costly
Cost of the maintenance of the examination site	None	Some costs	Some costs (depends on agreements with the local health care administration)	Costly

In the OEC/HES 2008-2012, one centre (municipality) for each region was selected, only three regions had two centres, due to the large population, for a total of twenty-three centres.

Within each region the centre was selected within existing health care premises only if it meets the general requirements reported in the study protocol and manual of operations and necessary to implement the screening procedures.

Once the centre had been selected, the sample was stratified by age and sex and was randomly selected from the list of residents.

The requirements to be selected for the survey is described in other section.

The ISS provided the instruments necessary to perform measurements. Two complete sets were used in all the centres, in particular: mercury sphygmomanometer with stethoscope; balanced beam scale, statimeter or wall height rule and measuring tape; containers for 24h urine collection and urine storage tubes; paillettes filling machine, paillettes, visotube, glasses; bone densitometer; spirometer; electrocardiographer; carbon monoxide (CO) measuring instrument; chronometer; thermometer to measure ambient temperature. A strict time schedule with dates of the screening for each region was essential.

In selecting the examination site, also the following issues have been considered:

- participants should have easy access to the examination site, which therefore should be near the participants' workplace or residence. Especially in urban areas, transportation is not easy and people are not necessarily willing to travel to another side of the city, but in rural areas longer distances can be considered acceptable. In addition, the OEC/HES did not foresee reimbursement of travel costs afforded by participants. In the city of Rome, to obviate to transportation problems, the sample was extracted in the district of the screening centre (S. Giovanni hospital);
- the availability of public or different way organized transportation to the examination site needs to be assured;
- access of participants with limited functional ability (e.g. those using a wheelchair) needs to be assured;
- the examination site should be near a hospital or a laboratory to enable blood sample storage and analyses.

The only way to be sure that the examination site was suitable for carrying out physical measurements is to visit the place before selecting it. This has required adequate time and personnel resources during survey preparation.

### 13. QUESTIONNAIRE AND ITS ADMINISTRATION AND VALIDATION

#### OEC/HES Questionnaire

Questionnaire adopted in the OEC/HES is standardized and validated, and commonly used in epidemiological studies. The questionnaire included face-to-face ‘core’ questions, data on physical measurements and biological samples.

The core questionnaire included the majority of EHES ‘core questions’. Only some questions (educational level; job; monthly income; diseases diagnosed by a physician; prescribed medications; passive smoking) differ slightly from the EHES ones. During the Italian HES, the screening conducted in Noale (Veneto) and Torino (Piemonte) demonstrated that there were no great differences in answers when administrating both questionnaires.



Questions were closed-ended, except those investigating current or past (for persons in retirement) job, preferred brand of cigarette, medications for hypertension, hypercholesterol, diabetes and other hospitalizations.

The questionnaire was available in electronic version and included different independent sections. A specific software to collect data from questionnaire was available. The software was segmented into several independent sections, one for each questionnaire topic, and allowed users to insert all information about blood pressure, anthropometric measures, bone densitometry, spirometry and laboratory analyses on a daily basis. The software was designed to check data

quality, therefore data inconsistencies were not allowed. At the end of each section, the software alerted user of missing or incomplete data before saving information. Anagraphical data, identification code and date of examination appeared on each page of the software. After the inclusion of all data and examinations results, the software permitted to print the cardiovascular risk score (“Print the risk score”) or, alternatively, the CUORE Project risk chart (“Print the CUORE chart”), depending on the availability of the HDL cholesterol value. The risk score could be printed only for participants aged 35-69 years free from cardiovascular events. All participants were provided with a printed copy of results of all instrumental and laboratory examinations (“Print patient data”).

The software offered also data backup, which should be performed on a daily basis.

A PDF version of the questionnaire is reported in the website ([www.cuore.iss.it](http://www.cuore.iss.it)).

The EPIC food frequency questionnaire was used to collect information on dietary habits. It is a validate semi quantitative instrument self administered and checked by trained personal after compilation. The quantity of the food consumed was assessed from the respondent’s selection of an image of a food portion. A computer programme, Nutrition Analysis of Food Frequency Questionnaire, was developed by the Epidemiologic Unit the Istituto Nazionale Tumori of Milan, to convert questionnaire dietary data into frequencies of consumption and average daily quantities of food, energy and nutrients consumed.

The EPIC questionnaire was automatically read by an optical scanner, therefore the participant was instructed to fill in fully the circle even if the answer to the question on the consumption of a particular food item was ‘never’.

## **Checking questionnaires and interviewing**

### **Interviewing**

The questionnaire were administered by properly trained personnel. Fieldwork staff were trained about how to read questions (tone of voice, time necessary to read questions and to wait for answers).

The interviewer was recommended to read the questions and all possible answers as they were written, trying not to influence the answer through intonation of the voice or facial expression. If the participant did not seem to understand the question, in the training section it was suggested to repeat the question three times. If, after three attempts, the participant was still unable to answer, the order of the words or the words themselves could be changed provided that the meaning of the question was not altered and the answer was not suggested.

It was recommended to give positive feedback at the end of the interview so that the participant felt that his/her responses and time spent with the interview were valued.

At the end of interview all problems encountered (problems in communication skills, cognitive ability, hearing or reading, proxy use, other disturbing factors, etc.) were recorded.

### **Checking the self administered questionnaires**

Self-administered questionnaire includes: ADL-IADL and food frequency. The food frequency questionnaire, used to collect information about dietary habits, was self-administered and usually filled out at home and then returned together with 24-hour urine sample. At the end of examination visit or when the participant came back, the fieldworker checked if the respondent has filled all items in the right way.

Checking the self-administered questionnaires included the following steps in front of participant:

- trying to fill in missing items: the fieldworker gave some further explanations.
- correcting items where the respondent had selected several options in questions where only one option was allowed;
- correcting if jump rules have not been followed: the fieldworker helped the respondent to clarify the situation.

## 14. MEASUREMENT PROCEDURES IN THE EXAMINATIONS

International procedures and methods were followed; they are well described in the EHES manual and here reported:

### Blood pressure

In Italy, blood pressure has been, and still is, measured with the mercury sphygmomanometer. Recently, due to toxicity of mercury, the European Union has banned the use of mercury devices and the use of automated devices has increased. Therefore, for the future, it is recommended to maintain the same instrument or at least perform a validation study to see how comparable readings with the mercury sphygmomanometer and the automated devices are.

The standardization of the blood pressure measurement procedures is important to obtain as valid readings as possible. Blood pressure levels are affected by the following issues:

Activity	Systolic blood pressure (mmHg)	Diastolic blood pressure
Full bladder	Increases 10 to 15 mmHg	
Not resting 3 to 5 minutes before measurement	Increases 10 to 20 mmHg	Increases 14 mmHg
Back/feet unsupported	Increases 5 to 15 mmHg	Increases 6 mmHg
Supine posture instead of sitting posture	Increases 3 to 10 mmHg	Decreases 1 to 5 mmHg
Legs crossed	Increases 5 to 8 mmHg	Increases 3 to 5 mmHg
Participant talking during the measurement	Increases 10 to 15 mmHg	Increases 6 to 10 (refs.)
Arm below heart level	Increases up to 10 mmHg	Increases up to 11 mmHg
Arm above heart level	Decreases	Decreases
Physical exercise	Increases up to 22 mmHg	Increases 7 to 8 mmHg
Left arm instead of right arm	Decreases 1 to 3 mmHg	Decreases 1 mmHg
Diaphragm of the stethoscope instead of bell	Decreases 2 mmHg	Decreases 0 to 2 mmHg
Cuff too small	Increases 10 to 40 mmHg	Increases 5 to 12 mmHg
Cuff too large	Decreases 10 to 30 mmHg	
Cuff over clothing	Unreable	Unreable

## Equipment used in OEC/HES

### Measurement by the mercury sphygmomanometer

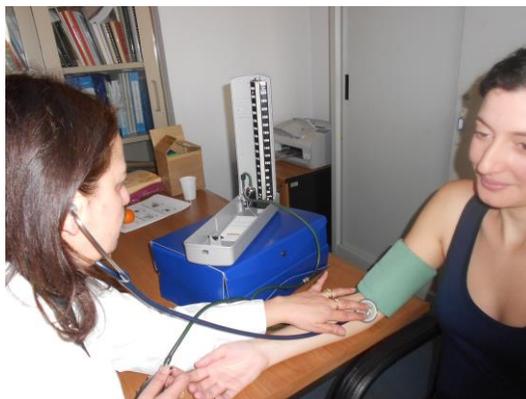
- Mercury sphygmomanometer
- Stethoscope
- 2 cuffs of different size (medium and large):

Cuff	Arm circumference	Bladder size
Medium (13cm)	$\leq 34$ cm	11.5 cm x 27 cm
Large (14.5cm)	$>34$ cm	12 cm x 35 cm

- non-elastic measurement tape, for measuring arm circumference
- thermometer, for recording room temperature
- stop watch (or a watch with a second hand), for recording the time of measurement

Blood pressure was taken before blood drawing, on the right arm, with the subject in a sitting position after 5 minutes rest, using the double-cuff (normal adult and obese adult) Riva-Rocci mercury sphygmomanometer and the bell of the stethoscope.

### Measurement procedures



### Setting up the measurement site

Blood pressure should be measured in a quiet room with a comfortable temperature. The room temperature should be recorded for each participant. The measurer should be in a sitting position in front of the subject. The sphygmomanometer should be placed on a platform 10-15 cm higher

so that the measurer has his/her eyes at the level of the mercury column; mercury column should be at 0 level. If this is not so, the sphygmomanometer should be slightly bent toward the side of the mercury column bulb.

The chair of the participant should be adjusted to the proper height and should have a backrest so that he/she can feel relaxed and comfortable.

### **Preparation for the measurement**

Before coming to the examination, participants should be instructed to abstain from doing heavy physical activity, eating, smoking and avoid exposure to cold temperature for one hour before measurement. The participant should be also instructed to empty his/her bladder before the measurement and to remain relaxed and avoid talking during measurement.

The participant should remove outer garments and all other tight clothing. The sleeve of shirt, blouses, etc. should be rolled up without being constrictive so that the upper right arm is bare for the cuff; the remaining garments should not be constrictive and the cuff should not be placed over any garment.

The participant should be in a sitting position so that the arm and back are supported. The participant's feet should be resting firmly on the floor and legs should be uncrossed.

### **Position of the arm**

The measurement should be made on the right arm. If not possible, e.g. the arm is deformed, amputated, or has rashes, adhesive dressing, casts, open sores, hematomas, wounds, arterovenous shunt or any other intravenous access device, the left arm should be used. The use of left arm and reason for this was recorded on the questionnaire.

The arm should be resting on the desk so that the antecubital fossa (the triangular cavity of the elbow joint that contains a tendon of the biceps, the medial nerve, and the brachial artery) is at the level of the heart and the palm is facing up. The arm forms a 45 degrees angle with the trunk.

### **Selection of the cuff**

A set of 2 cuffs of different sizes are usually available and special attention should be paid to using the proper cuff width in relation to arm size, measured using a non-elastic measurement

tape (medium if arm circumference is  $\leq 33$  cm, large if arm circumference is  $\geq 34$  cm) placed at the mid-point between the acromion process of the scapula and the olecranon. The measurement should be read to the nearest centimetre and recorded.

The length of the bladder of the cuff should be at least 80% of arm circumference and be placed so that its lower edge is about 2 cm above the antecubital fossa; the tubes from the cuff should not be under the arm or the arm should not rest on the tubes.

The subject should sit with the cuff around the arm for at least 5 minutes before the measurement can be taken. During this time, data on blood pressure measurement (time of measurement, room temperature, arm circumference and type of cuff) are recorded on the questionnaire.

### **Number of measurements**

Three consecutive blood pressure measurements are recommended, a few minutes apart (the time usually necessary for blood pressure values registration). One-minute pulse rate was measured at the wrist between the first and the second measurements.

### **Measurement protocol**

1. Participant is asked not to talk and not to look at the mercury column during the measurements
2. Measure the arm circumference and select the correct cuff size.
3. Place the cuff on the right arm so that its bottom edge is 2-3 cm above the antecubital fossa. The top edge of the cuff should not be restricted by clothing. Make sure that the tubes from the cuff are not under the arm or otherwise tied up.
4. Palpate the radial pulse with left hand fingers.
5. Inflate slowly the cuff closing the valve of the inflation bulb and watch the mercury column: the level of the top of the meniscus of the mercury column is noted at the point when the radial pulse disappears; deflate the cuff quickly and disconnect the cuff tube from the sphygmomanometer.
6. Record the value and add 30 mmHg to this value to obtain the peak inflation level.
7. Wait for 30 seconds or raise the arm for 5-6 seconds and connect again the cuff tube to the sphygmomanometer. Locate the brachial pulse and place the bell of the stethoscope at

this point (it is advisable to use the bell as it eliminates background noises). Earpieces of the stethoscope should be placed into the external auditory meatus (from back to front facing outward). If it is not possible to feel the brachial pulse, the bell of the stethoscope should be placed inside the bicep muscle tendon and never below the cuff.

8. Inflate rapidly the cuff to the peak inflation level and then deflate the cuff at a rate of 2mmHg per second. Read the value corresponding to the top of the meniscus of the mercury column. Blood pressure measurements should be recorded to the nearest millimetre. If the top of the meniscus falls halfway between two markings, choose the marking immediately below. Record systolic and diastolic blood pressure: the systolic level corresponds to the first appearance of a clear sound followed by an equal one (Korotkoff Phase I); the diastolic level (Korotkoff Phase V) corresponds to the last sound heard. Slowly deflate the cuff until at least 10 mmHg after the complete disappearance of heart rate. Then rapidly deflate the cuff by fully opening the valve of the inflation bulb. Disconnect the cuff tube from the sphygmomanometer.

9. After the first blood pressure measurement, heart rate should be measured for one minute. For the second and third measurements, repeat steps 7 and 8.

### **Pulse rate**

Pulse rate should be measured once at the end of the first measurement of blood pressure.

It should be measured at the right wrist by placing the middle fingers of the left hand on it to locate the radial artery; when a pulse is found, the number of beats felt within a one minute period should be counted using a stop watch (or a watch with a second hand).

### **Feedback to participants**

The participant was informed about his/her blood pressure levels. The mean of the three measurements was communicated to the participant and reported on the results' form.

### **Quality assurance**

Quality assurance of the blood pressure measurement includes following components: training of the personnel; checking the equipment and regular calibration of the devices; audit visits and

evaluation of the measurement data during the fieldwork; assess the proportion of identical blood pressure values in the three consecutive measurements; assess the chance of the digit occurrence; use the double stethoscope.

### **Training of the measurers**

Training of the blood pressure measurers should include:

- Theory about why it is important to measure blood pressure in the general population; why blood pressure measurement has to be standardized and the implications of the changes in the procedure.
- Practical training ideally with people with different blood pressure levels [Training manual, R. Prineas]

In our experience, we have observed that training of local staff during the first week is very useful: it allows to check the positions, better blood pressure hearing, choose the right time necessary for measurement. The main aim of the HES is to describe the characteristics of the general population, usually healthy; therefore it is important to perform measurements and examinations according to standardized procedures and respecting the required time. Performing measurements in the right way may be useful also in clinical practice.



The use of the double stethoscope allows to check the level of attention of the measurer, the velocity the cuff is deflated and the reliability of measurement. After measurement, the trainer and the measurer should record blood pressure values; comparison is made after at least 10

measurements. The measurement made by the measurer should not be more than 2 mmHg different from that made by the trainer. Double stethoscope should be used until differences between measurements reduces.

### **Checking the equipment and regular calibration of the devices**

All equipment should be checked regularly. If any problems are observed during the checks, the device in question should be replaced and this is recorded.

### **Checks or when new examination place is set up**

When the examination site is set up in new centre, the following issues from the equipment should be checked:

#### *From the mercury sphygmomanometer*

The shape of the meniscus (top of the column of mercury) is a smooth, well-defined curve; check that the mercury rises easily in the tubing and the mercury does not bounce noticeably when inflated; disconnect the inflation system from the cuff and confirm that the meniscus of the mercury in the glass manometer tube is zero, check for cracks in the glass tube; check the screw at the top of the calibrated glass tube to make sure it is securely in place; check the coiled air tube for cracks, tears.

Moreover, test inflation system for air leaks; connect each cuff size to the inflation system and wrap it around the corresponding calibration cylinder; inflate to 250 mmHg; open valve and deflate to 200 mmHg and close valve; and wait for 10 seconds; if mercury column drops more than 10 mmHg, there is an air leak in the system; and if a leak is detected, change the cuff, check the coiled tubing and repeat the test.

#### *From the cuffs*

Cuff material is clean, intact; rubber tubing and inflation bulb (in mercury sphygmomanometer) is smooth, has no cracks or tears; pressure control valve opens and closes smoothly without sticking (mercury sphygmomanometer).

### *From the stethoscopes*

Stethoscope has no cracks in the tubing; earpieces of the stethoscope are securely attached; head of the stethoscope is securely attached to tubing; diaphragm is secure, no cracks.

### **Quality control by coordinating office during the field work**

The coordinating office organizes audit visits to the field to monitor the measurements. At regular intervals, the following indicators should be checked from the data:

- distribution of terminal digits for systolic and diastolic measurements: frequency of the last digit 0,2,4,6,8 should have the same chance of occurring (20% each);
- mean and standard deviation of the systolic and diastolic blood pressure measurement should be checked, to determine if the measurers are producing readings that are systematically lower or higher than the average;
- the proportion of identical measurements for the same participants to determine if the measurers are really doing all 3 measurements for each subject.

### **Anthropometric measurements**

#### **Height**

To obtain valid and reliable values, it is important to perform anthropometric measurements following standardized methodologies. Following the recommendations provided by the EHES manual takes a few minutes. It is recommended to perform the anthropometric measurements in one room with the participant undressed, with only underwear on: this facilitates measurement and reduces variability due to weight of different outer garments. In some cultures undressing may not be acceptable. This should be respected; in that case the waist circumference can then be measured over a thin shirt.

### **Measurement protocol**

#### **Equipment**

- Height rule (stadiometer)
- Steps

- Carpenter's level

### **Setting up the measurement site**



The height rule is attached vertically to the hard flat wall surface with the base at floor level. A carpenter's level is used to check the vertical and horizontal (triangle) placement of the rule. The height rule does not have a fixed rod, it is recommended to mark with a tape the straight line for the rule down to the floor. The floor surface must be hard and not covered by elastic or soft material (moquette).

### **Exclusion criteria**

Height should be measured to all participants except if a person is immobile or in a wheelchair; if has difficulties in standing straight; prevents the proper use of the equipment.

### **Measuring height**

The participant is asked to remove his/her shoes, heavy outer garments and is asked to stand with his/her back to the wall, feet together and back of the head, shoulder blades, buttocks and heels touching the wall. The participant is asked to look straight ahead so that the top of the external auditory meatus (ear canal) is level with the inferior margin of the bony orbit (cheek bone).

Obese persons with difficulties keeping feet together may be allowed to stand with toes looking slightly outward but heels close to each other.

If the participant is taller than the measurer, the measurer should stand on steps so that he/she can properly read the height rule.

Once the participant has reached a correct position, the triangle placed on the height rule should be slid down to the head until the hair is pressed flat. If the last significant digit is followed by 5, round down if last significant digit is even, round up if odd (e.g 187.5 becomes 188; 186.5 becomes 186).

### **Recording**

Height is recorded on the questionnaire after the participant has moved away.

### **Feedback to the participant**

The participant is informed about his/her height. The height is reported in the results' form. Also BMI is calculated.

### **Quality assurance**

Quality assurance of height measurement includes following components: training of the personnel; checking the equipment and calibration of the devices when a new examination place is set up; audit visits and evaluation of the measurement data during the fieldwork

### **Training of the measurers**

Training of local staff is performed during the first week of screening. If survey prolongs, a refresher session about the measurement should be organized at three months interval.

### **Calibration and checking the equipment**

When setting up examination site the instructions of the device need to be followed carefully. The horizontal and vertical placement of the height rule must be checked by using the Carpenter's level.

### **Quality control on the field**

Regular monitoring of the performance of the measurers.

### **Quality control by coordinating office**

Surprise audit visits to the examination sites to observe the measurers.

Regular monitoring of the measurement results by measurer (digit preference, mean and standard deviation).

### **Weight**

#### **Measurement protocol**

#### **Equipment**

- Balanced beam scale
- Calibrated weights (e.g. 10 kg up to 100kg)
- A carpenter's level

#### **Setting up the measurement site**

Weight is measured with a balanced beam scale. The scale must be placed on a hard floor surface.

The floor should not be carpeted or otherwise covered with soft material. A carpenter's level (portable or in scale) should be used to verify that the scale is in horizontal position.

#### **Exclusion criteria**

Weight should be measured in all participants, except immobile or wheelchair bound individuals, or persons who have difficulty standing steady.

#### **Measuring weight**

The participant is asked to undress to his/hers underwear. If participant refuses to undress, he/she should be asked to take off the shoes, remove heavy outer garments, and at least he/she should empty his/her pockets (mobile phone, keys, wallet, belt) before getting on the scale. If the participant is currently wearing a prosthesis, this needs to be recorded.

The interviewer should stand in front of the participant and never behind.

### **Balanced beam scale**

The participant is asked to stand in the centre of the platform about 10 cm gap between the heels.

The weight should be distributed on both legs and the participant is asked to stand still.

The weights are moved until the beam balances, meaning the arrows are aligned.

### **Recording**

Weight is expressed in kilograms and hectograms and recorded to the nearest 200 gr on the questionnaire. If the value in hectograms is odd, it must be rounded down.

### **Feedback to the participant**

The participant is informed about his/her weight. The weight is reported in the results' form. Also BMI is calculated.

### **Quality assurance**

Quality assurance of the weight measurement includes following components: training of the personnel; checking the equipment and calibration of the devices when a new examination centre is set up; audit visits and evaluation of the measurement data during the fieldwork

### **Training of the measurers**

Training of local staff is performed during the first week of screening. If survey prolongs, a refresher session about the measurement should be organized at three months interval.

### **Calibration and checking the equipment**

All the calibrations and checking the equipment, and possible actions, should be recorded.

### **When setting up new examination site**

Standardized weights should be used to check the scale when setting up and breaking down the examination site. 10 kilos weights are placed one by one on the scale up to 100 kilos and checked that the scale works properly. If the error is greater than 0.2 kg it should be corrected.

### **During the fieldwork**

Balancing of beam balance scale to zero level should be done at the beginning and end of each examination day. The balanced beam scale is balanced with both sliding weights at zero and the balance bar aligned.

### **Quality control on the field**

Regular monitoring the performance of the measurers.

### **Quality control by coordinating office**

Surprise audit visits to the examination sites to observe the measurers.

Regular monitoring of the measurement results by measurer (digit preference, mean and standard deviation).

### **Waist circumference**

#### **Measurement protocol**

#### **Equipment**

- non-elastic measuring tape
- full body length mirror with 10 cm x 10 cm grid lines (if mirror is used, Carpenter's level is needed)

### **Setting up the measurement site**

Waist is measured with a non-elastic measuring tape, making sure that the finger of the measurer is not between the tape and the participant's body. This measuring tape is graduated on one side only.

If mirror is used it is placed against the wall or if the mirror stands its own feet next too the measurement place. By carpenter's level it should be verified that the grid lines on the mirror are horizontal.

### **Exclusion criteria**

Waist circumference should be measured in all participants, except if the person is immobile or in a wheelchair; has difficulty standing straight; is pregnant (over 20 pregnancy weeks); has a umbilical hernia.

### **Measuring waist circumference**

The participant is asked to reveal the waist, by loosing the belt, lowering the pants/skirt and lifting the shirt. The participant is also asked to stand still, with feet fairly close together (10cm) and weight equally distributed on both legs. The hands are hanging loosely beside the body. Waist circumference is measured at a level midway between the lower rib margin and the iliac crest, with the measurer sitting on a chair in front of the participant; The measurer, in sitting position, checks that the measuring tape is in horizontal position by asking the participant to turn over or checking from the mirror. The measuring tape is held firmly, ensuring the horizontal position. The tape should not be too tight or too loose. The participant is asked to breath normally; the reading is taken at the end of light exhale.

### **Recording**

Waist circumference is recorded on the questionnaire.

### **Feedback to the participant**

The participant is informed about his/her waist circumference. The waist is reported on the results' form.

### **Quality assurance**

Quality assurance of waist circumference includes following components: training of the personnel; checking the equipment and regular calibration of the devices; audit visits and evaluation of the measurement data during the fieldwork

### **Training of the measurers**

Training of local staff is performed during the first week of screening. If survey prolongs, a refresher session about the measurement should be organized at three months interval.

### **Calibration and checking the equipment**

The length of the measurement tape is measured by using calibrated length rods at least one a month.

If the measurement tape is stretched it should be replaced.

### **Quality control on the field**

Regular monitoring the performance of the measurers.

### **Quality control by coordinating office**

Surprise audit visits to the examination sites to observe the measurers.

Regular monitoring of the measurement results by measurer (digit preference, mean and standard deviation).

### **Additional measurements**

Suggested additional measurements performed in the OEC/HES 2008-2012 are listed in the following Table:

<b>Carbon monoxide assessment</b>	assessed using the Micro Smokerlyzer and measured in parts per million (ppmCO); two measurements are performed and the higher value is recorded
<b>Bone densitometry</b>	Portable real-time ultrasonic bone densitometer is used; measurable

	parameters at the right heel are: STIFFNESS, T score (% of variation compared to healthy young adults) and Z score (% of variation compared to a population of the same age)
<b>Electrocardiogram</b>	6-lead ECG at rest is performed and is coded by a certified reader according to the Minnesota code (9 items)
<b>Spirometry</b>	portable spirometer, SpiroPro, is used. Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 Second (FEV1) are assessed; two measurements are performed and the best of the two is recorded
<b>24-h urine collection</b>	for determination of urinary sodium, potassium, creatinine, microalbumin, and iodine concentrations

### **Feedback to participants**

The feedback to the participants was an important part of the fieldwork procedures. For many participants, this was one of the main reasons to participate to the HES. The feedback on anthropometric measurements and blood pressure could be provided to the participant right after the measurements. Results of the blood analysis, usually needed more time and could not be provided during the same visit when blood samples were collected.

Examination results were collected in a folder and given to the participant at the end of examination visit (or mailed to the participant later on). The folder also contained explanations of the examinations and lifestyle recommendations, including healthy diet and physical activity suggestions. For those participants who have both total cholesterol and HDL assessments, the individual risk score for persons aged 35-69 years free from previous cardiovascular event was printed; for those participants who have total cholesterol only, risk chart for persons aged 40-69 years was printed. For all participants it was possible to print results of measurements, examinations and blood tests.

The folder also contained laboratory blood results, ECG, spirometry and bone densitometry printouts, and a copy of the signed informed consent.

Results are provided when the participant come back to the centre to give the 24-hour urine collection.

## **15. ANALYTIC LABORATORY, BLOOD SAMPLE COLLECTION AND STORAGE OF THE SAMPLE**

The purpose of the OEC/HES was to collect data and biological samples from a random population.

One laboratory technician was involved in each region. The training in advance was the key factor according to the OEC/HES Manual.

Laboratory work comprised two phases: drawing of blood and the processing of blood samples. Sample processing also included transferring storage tubes into the storage boxes and their freezing.

### **Selection of analytic laboratory**

Analyses were performed both at a local and at the central reference laboratory. Those performed at a local laboratory included total cholesterol, HDL-cholesterol, fasting plasma glucose, and complete haemochrome; determinations were given to the examined persons as result. Criteria for the selection of the local laboratory included: availability of room and materials necessary for blood collection, close to the screening centre, reasonable cost of determinations, availability of a freezer for biological samples storage. For quality control, laboratories were under control following regional standards.

Successively all analyses pertaining to the core measurements (total cholesterol, HDL-cholesterol, glycaemia, triglycerides, creatinine, albuminuria) and the specific analyses (urinary creatinine, urinary albumin, calcium, phosphorus and parathormone) for the CARHES study (CARDIOVASCULAR risk in Renal patients of the Italian Health Examination Survey) were performed at the central Laboratory of Genetic and Ambiental Epidemiology of the Catholic University of Campobasso. A laboratory technician (Amalia De Curtis) worked full time over the duration of the screening and assayed the biological tests of all the population examined. She participated in the training seminar held in Helsinki and was tested with biological specimens from the R.C. of EHES.

Twenty-four hours urine tests for sodium/potassium and creatinine were assayed at Federico II University of Naples Medical Department of Clinical and Experimental Medicine, ESH

Excellence Centre of Hypertension, School from the part-time technician (Ornella Russo) for all the population examined.

### **Assay methods for the blood and urine examinations performed at the central Laboratory of Genetic and Ambiental Epidemiology of the Catholic University of Campobasso**

- Evaluation of serum for:
  - Total cholesterol using the enzymatic colorimetric method;
  - HDL- cholesterol using the direct immuno-enzymatic colorimetric method;
  - Glycaemia using the Trinder-enzymatic colorimetric method;
  - Triglycerides using the enzymatic colorimetric method.
  - Creatinine (in all individuals) to estimate glomerular filtration rate (GFR). The enzymatic method calibrated against the reference method (isotope dilution mass spectrometry - IDMS) is used to measure creatinine.
  - Calcium, Phosphorus and Parathormone (only in the subgroup affected by chronic renal disease).
- Measurement on 24-hour urine collection:
  - Urinary creatinine and urinary albumin (in all individuals) quantified through assessment of urine volume and urine concentration. Urinary creatinine concentration is measured by using the methodology followed for creatinine assessment; urinary albumin concentration was measured using the immunoturbidimetric method.

### **Blood sample collection**

During the entire survey, the blood withdrawal, processing, storage and transport were maintained as uniform as possible by adhering to the manual of operations.

The blood sampling tubes were evacuated and provided by the same national supplier.

Participants were invited to show up at the screening centre between 8 and 9 am as blood extraction required a fasting period of at least 12 hours (specified in the invitation letter). The length of time from the last meal in full hours was reported on the questionnaire.

All blood samples were drawn with the subject in a sitting position. If required by the participant, blood drawing could be performed with the subject in supine position. The subject should also

abstain from intense physical activity and from smoking for at least 30 minutes before blood collection. Blood preferably collected from the vein in the antecubital fossa or from any other vein of the arm and blood stasis should last no more than one minute.

The following safety rules were followed:

- it was prohibited to eat or drink in the laboratory area;
- wearing protective clothing was not allowed outside the laboratory area;
- table surfaces were disinfected at the end of the day; any surface or object which was contaminated with blood or other human specimen was disinfected immediately;
- during blood withdrawal protective gloves should be used; new gloves were worn for each new subject;
- disposable gloves were used throughout during handling of blood samples;
- the sampling needle was removed directly into the needlebox from the adapter/holder without touching the needle;
- waste, generated during blood withdrawal and blood processing, were disposed of in the biological waste container;
- the containers were disposed of according to the local facility regulations.

The blood collection kit included:

- 2 blood collection tubes of 4,5 cc with EDTA;
- 1 blood collection tube of 10 cc without preserving;
- 1 thermal bag;
- tourniquet, skin cleaner, pipettes, skin tape, etc.

Tubes were labelled and have different cap colours (usually, tubes with EDTA have violet caps, those without EDTA have yellow or light blue caps) .

Other equipment includes:

- special boxes for tube transfer and storage;
- a centrifuge, a vortex and a big container for special waste collection;
- 30°C freezer for blood samples storage;

- set of labels with identification code or name, surname, birth date of the subject to mark the tubes.

Most of the above equipment is provided at local level.

### **Sample logistics**

The biological material (serum, plasma, buffy coat, red cells, urine) was collected, stored and sent according to standard methods used in several international studies.

Biological samples were processed using materials resistant to low temperature and simple, highly standardised methods allowing for multiple potential uses of the material.

The procedure foresees that 20 cc more blood than that necessary was drawn for immediate determinations by the local laboratory, to be distributed in 3 tubes: 2 with EDTA and 1 without preserving.

The tubes (2 with EDTA and 1 without EDTA for each person) were placed into the centrifuge, making sure they are properly balanced, and spin at 3,200 rpm for 10 minutes.

#### *Tubes without conservants*

After centrifugation 4ml of serum was aspirated and poured in a clean plastic tube

#### *Tubes with EDTA*

After centrifugation 4ml of plasma was aspirated and poured in a clean plastic tube; 1ml of buffy coat (1mm above and 1 mm below the grey level between the clotted blood cells and the liquid portion of blood are aspirated) was aspirated and poured into a plastic tube containing 0.25 ml of physiological solution; 1ml of red blood cells was aspirated and poured into a plastic tube containing 0.25 ml of physiological solution.

At this stage, 4 tubes are available, each containing:

- 4ml of serum
- 4ml of plasma
- 1ml of buffy coat + physiological solution

- 1ml of red blood cells + physiological solution

To avoid any waste of biological material after unfreezing, the different samples were introduced into thin plastic cylinders, paillettes, which can be used for different laboratory analyses or at different times. Paillettes, each with a bar code label and a cap (or cotton stopper) of different colour depending on the biological sample contained, were filled and sealed through a special machine (see the manual of operations 2011).

#### Machine functioning



The following set of paillettes was used in the filling process:

- 4 paillettes with **yellow sheath or cotton stopper** (for serum storage) + 2 with white cotton stopper (to be sent to Campobasso)
- 6 paillettes with **red sheath or cotton stopper** (for plasma storage)
- 2 paillettes with **green sheath or cotton stopper** (for red blood cells storage)
- 2 paillettes with **blue sheath or cotton stopper** (for buffy coat storage)

For each person, 16 paillettes were obtained which were stored into the visotubes.

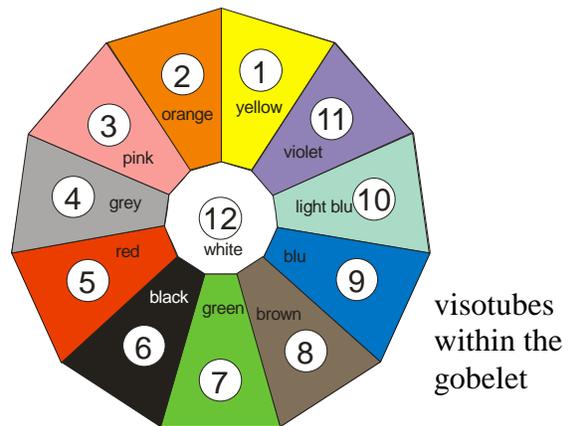


Goblets, plastic-coloured cylinders containing 12 visotubes of different colour (11 triangle-shaped and 1 cylindrical in the middle of each goblet) were used for paillettes storage.

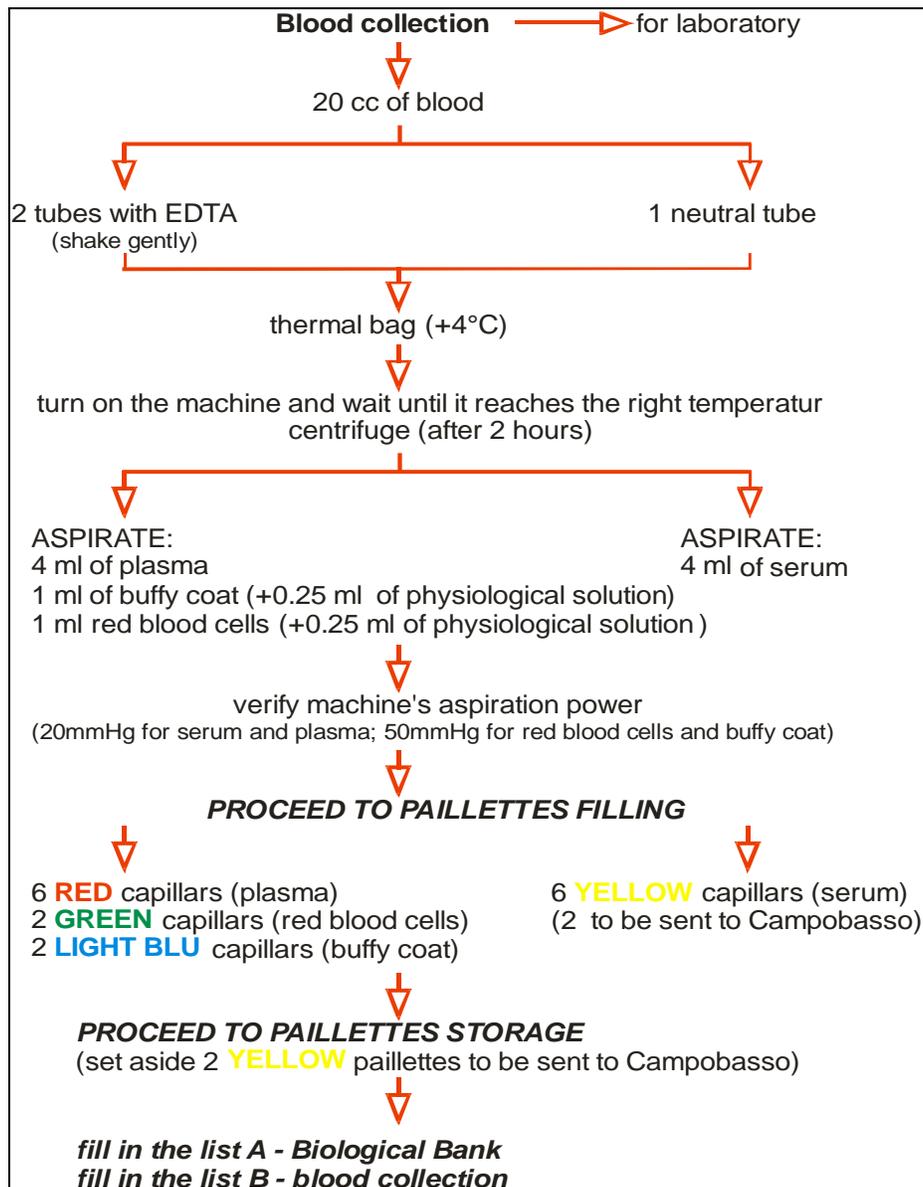
The sequence of colours of visotubes should remain always the same (see the figure). Each visotube corresponds to one person. Goblets were placed horizontally in the freezer at  $-30^{\circ}\text{C}$  and were transferred to the ISS under dry ice. Successively they were placed for one or more days into the freezer at  $-80^{\circ}\text{C}$  and then transferred into liquid nitrogen tanks at  $-196^{\circ}\text{C}$ .

A specific software which keeps track of all the stored samples and their location allows to match the biological bank information with the data bank information.

The cryogenic freezers need to be filled up at regular intervals, depending on the liquid nitrogen containers capacity and the amount of material stored.



## Flow chart of the blood collection procession and storage



### Long term storage of the samples

The biological samples were packaged on dry ice for transfer to the biobank of the National Centre for Epidemiology, Surveillance and Health Promotion (CNESPS). For long term storage reserved for additional measurements and future use, the samples are frozen at  $-80^{\circ}\text{C}$  or in liquid nitrogen at  $-196^{\circ}\text{C}$ .

After performing required determinations, each centre is then required to send data, in electronic format, to Simona Giampaoli of the ISS, responsible of the project.

### **Guidelines on laboratory performance**

The measurement of sodium, potassium and creatinine was performed by the central laboratory in Naples. Urinary sodium and potassium concentrations were measured by ion selective electrode potentiometry and urinary creatinine by a kinetic Jaffe´ reaction using an ABX Pentra 400 apparatus (HORIBA ABX, Rome, Italy). Quality control was effected using urine specific reference samples from UrichemGol BIO-DEV (Milan, Italy). The inter-assay technical error was 0.73% for sodium, 1.16% for potassium and 1.12% for creatinine. A sample was excluded from the analysis if the 24h urine volume was below 500 ml or its creatinine content referred to body weight was found to be lower than the mean minus 2 S.D. from the population mean.

## 16. FIELDWORK STAFF

The OEC/HES was coordinated by the team of the Unit of Epidemiology of Cardiovascular Disease of the CNESPS ISS; the national survey coordinator organised and coordinated the overall fieldwork of the survey:

- coordinating and supervising all fieldwork activities;
- training the local personnel, monitoring quality control and fieldwork;
- extracting the population sample;
- sending survey invitations;
- selecting and agreeing on examination centres (municipality) in the region;
- receiving and processing survey data from the fieldwork teams;
- receiving, storing and analyzing blood and other samples from the fieldwork teams;
- keeping in contact with relevant regional/local administration and health services of the fieldwork sites;
- disseminating results of the survey at population level.

Each local team had a named fieldwork team supervisor. The supervisor worked in close collaboration with the national fieldwork coordinator. The tasks of the field team supervisor included:

- coordinating the work of the fieldwork team, consulting, solving problems and specifying guidelines when needed;
- organizing substitutes for the fieldwork team members in case of sick leaves and other absences;
- keeping a regular contact with the ISS;
- checking daily appointment schedules, checking questionnaires (if needed and not built in computer programmes used in data collection);
- organizing and taking care of transfer of data and instruments.

The fieldwork team supervisors were in contact regularly with the ISS to share up to date information from the centre, brought also information from the ISS back to their teams, e.g. feedback from quality control. They were responsible for monitoring that team members carried

out their tasks well in daily basis. If there were problems, that could not be solved within the team, the supervisors contacted the ISS and unclear issues were sorted out as soon as possible. It was important that the fieldwork team supervisor received support from the ISS and did not deal problems alone.

The satisfaction of the staff was an important issue for the work quality. The national survey coordinator and fieldwork team supervisor have had an important role in creating a positive work environment since it affects the staff satisfaction.

The OEC/HES represents a system of continuous data collection and its objective is to build a permanent survey staff with great expertise able to carry out fieldwork activities, train local fieldwork staff and continue the collection of epidemiological data in the future.

A close cooperation between local and ISS personnel was absolutely necessary for the success of the survey. When selecting local staff, usually the preference went to young people, who were more interested in training and could, in turn, train other people to carry out fieldwork activities in order to increase knowledge and expertise in the field of epidemiological data collection. However, this was not always possible as local young staff had already a work contract and could not receive extra funds, therefore retired personnel was also engaged. At the time of the survey, the personnel of the Unit of Epidemiology of cerebro and cardiovascular disease of the ISS was composed of the survey project leader (Simona Giampaoli) who was also actively involved in fieldwork organization and has supported local staff and a technician with expertise in measurements, data collection, IT systems analysis, sample storage and fieldwork procedures (Cinzia Lo Noce); usually they were supported in the training by one other technician of the ISS (Francesco Dima). They were responsible for training local survey personnel during the first week of the screening, verifying that all procedures were performed according to the international quality standard and that equipment was properly functioning. If the survey lasted longer than foreseen, an additional visit at the screening centre was usually necessary, also to collect biological samples.

Interviewers and others in contact with the community were capable, personable, interested, good manners and friendliness towards participants. An additional prerequisite for the ISS personnel was the willingness to travel around the country.

The professional group needed for most measurements consisted of physicians, nurses and other health care professionals. More specifically, with the local supervisor, the minimum personnel required in the OEC/HES survey in each centre (municipality) were:

- a person to perform secretarial procedures, welcome participants, update the participant list on the basis of refusals and eventually select substitute participants; this person also assured that all screening phases were well developed and completed, including delivery of urine box for 24-hour collection and results printing, and check if self-administered food questionnaires have been filled out and data from instrumental and laboratory examinations entered and printed the results for the participant;
- a professional nurse for blood pressure measurement, blood collection and questionnaire administration;
- a professional nurse for performing anthropometric measurements, electrocardiogram (ECG) at rest, bone densitometry, spirometry and CO measurement;
- a laboratory technician for separation and storage of blood samples and for preparation of urine samples.

The above personnel, except for the laboratory technician, were required to work full time over the duration of the screening procedures.

Fieldwork staff was recruited specifically for the survey. An alternative was to use personnel from the local health care organizations (e.g. primary care units or health centres or hospitals) in the selected survey sites. It was usually easier to ensure standardization of measurements, if fieldwork staff was recruited specifically for the survey. When permanent personnel of the local health services were trained to carry out the survey fieldwork they were tempted to follow their regular practices instead of the survey protocols. This happened especially if they also had their regular tasks during the survey, and were carrying out the survey fieldwork only part time. In any case the use of the local personnel in each survey site increased substantially the time and efforts needed for training. The use of regular health service personnel may have affected survey results by the differences in willingness of the survey participants to disclose their personal issues to the practitioners they were familiar with. This familiarity may have both enhanced and restricted open communication

The personnel were motivated to strictly follow the survey protocols to ensure reliability and accuracy of the survey results.

## **17. DISSEMINATION, PUBLICITY AND REPORTING**

Dissemination, communication and publicity were all needed and important aspects of the OEC/HES. With these, survey organizers kept all stakeholders informed about the survey plans, progress and outcomes.

Before starting recruitment of study population, personal contacts and an educational campaign were implemented to make individuals motivated to join the study and community leaders supportive and have pride in their association with the OEC/HES project.

Local authorities of each centre involved in the survey (Director-General, Director of the Health Unit, Regional authorities, Mayor) received the letter of presentation of the project, and the information note of the project explaining that the survey aims at giving a complete picture of the Italian population's health status and facilitates the planning and evaluation of future preventive programmes. The information note and the letter of presentation of the project were also sent to the local general practitioners (GPs) to encourage patients enrolled to participate in the study. In small centres, the parish priest, local pharmacies, elderly clubs, as well as local press and TV were also informed.

A section on the OEC/HES project is available in both English and Italian language on the website of the CUORE Project ([www.cuore.iss.it](http://www.cuore.iss.it)) and gives basic information on the study. Information are kept up to date with the project progress and data about risk factors and health conditions of the population are published as soon as available. This mean of communication serves as an easy solution to communicate to different target audiences. A communication expert helps survey staff in ensuring simplicity but efficacy of web pages.

The OEC/HES survey was also promoted by several local newspapers that highlight the importance of the national HES in providing information on behaviours and health determinants of the general population through direct examination.

Local press conference and releases were also usually organized prior to the start of the screening to raise the awareness of local population about the importance of identifying future health

threats and health care challenges and thus convince stakeholders and population about the importance of national HES.

The key messages for instance were:

- health surveys are vital for understanding the health situation and the behaviours of the population, and they provide an evidence-base for health policies;
- identifying health differences between population groups is a prerequisite in the work to narrow down health inequalities;
- to support healthy aging we need to know the current state of health of adults and children;
- the national OEC/HES is conducted by a reliable public health authority, the methods are secure and science based, and the results do not serve any other interests than the public benefit;
- participating in the survey give participants a free-of-charge opportunity to receive up-to-date information on their own health;
- information about people's health is vital to building an efficient health care system geared to our health needs and that of our families. Each individual's contribution is important in making the study representative;
- the physical health examination survey will verify and complement data collected through other health questionnaires and registries.

## 18. BUDGET AND FUNDING

**Two complete sets of equipment** were available in order to conduct the survey in two different regions contemporary. Here the cost of **one** complete set:

ECG	2.000 Euro
Spirometry	3.000
Bone densitometry	8.000
Carbone monoxide exposure	400
2 mecury sphygmomanometers and 2 stethoscopes	460
Height rute (stadiometer) and two measuring tape	120
Balance beam scale	650
Machine for paillettes (biological specimen bank*)	30.000
Portable computer and printer	1.000

ECG, spirometry, bone densitometry and machine for paillettes were checked every years with a mean cost of 10.000 Euro

\*Paillettes (they are used for storage of biological specimen, have a printed number for the identification of the person; they are very expensive); paillettes and urine containers were provided by the ISS.

For the **centralized laboratory** the cost for **each test** was:

Total cholesterol	0.55 Euro
HDL	3.00
Creatinine	0.95
Fasting blood glucose	0.44
Triglycerides	0.78
Insulinemia	4.5
Glicate haemoglobin (not done for the high cost)	10.0

The cost of the Laboratory Technician was 63.000 Euro per year (for 4 years).

**Two-three persons** of the ISS went to each region **for one week (5 days)** for the training and testing of regional personnel (one research director, plus two technicians), every month of survey:

Research director            400.00 Euro per day

Technicians                    210.00 Euro each per day

Every centre (23) had received a forfait budget of 13.000 Euro every 220 persons examined to cover personal costs, laboratory equipment, laboratory tests at local level and freezer storage.

**Other costs**

ECG, spirometry and bone densitometry papers, biological samples in dry ice were personally transfer to the ISS and the Central Laboratory by the research director and technicians.

## REFERENCES

Giampaoli S, Vanuzzo D, Palmieri L, Lo Noce C, Dima F, De Sanctis Caiola P, Donfrancesco C, Ciccarelli P, Toccaceli V. Progetto CUORE. Epidemiologia e prevenzione delle malattie cardio-cerebrovascolari. Protocollo e manuale delle operazioni dell'Osservatorio Epidemiologico Cardiovascolare/Health Examination Survey 2008-2011. Rapporto Istisan 10/33 Istituto Superiore di Sanità; 2010, v, 80 p.

Primatesta P, Allender S, Ciccarelli P, Doring A, Graff-Iversen S, Holub J, et al. Cardiovascular surveys: manual of operations. *Eur J Cardiovasc Prev Rehabil.* 2007 Dec; 14 Suppl 3:S43-61.

Lupker R, Evans A, McKeigue P, Reddy KS. Cardiovascular Survey methods. World Health Organization, Geneva 2002

Giampaoli S, Vanuzzo D. Atlante italiano delle malattie cardiovascolari. *Italian Heart Journal* 2003, 4(4) suppl.: 1-121.

Osservatorio Epidemiologico Cardiovascolare Italiano. Atlante Italiano delle malattie cardiovascolari 2004, II Edizione. Vol. 5(suppl. 3): 49-93.

MONICA Monograph and Multimedia Sourcebook. World's largest study of heart disease, stroke, risk factors, and population trends 1979-2002 Ed. Tunstall-Pedoe for the WHO MONICA Project. World Health Organization. Geneva 2003

Prineas R. Blood pressure sounds: their measurement and meaning. A training manual. Gamma Medical Products Corporation. Pp 1-80 1978

Giampaoli S, Vanuzzo D, and the Research Group of the Osservatorio Epidemiologico Cardiovascolare/Health Examination Survey. La salute cardiovascolare degli italiani, 3rd Italian Atlas of Cardiovascular Diseases, Edition 2014; *Giornale Italiano di Cardiologia* 2014; 15 (4 Suppl 1): 7S-31S.

Giampaoli S, Palmieri L, Donfrancesco C, Lo Noce C, Pilotto L, Vanuzzo D; Osservatorio Epidemiologico Cardiovascolare/Health Examination Survey Research Group. Cardiovascular health in Italy. Ten-year surveillance of cardiovascular diseases and risk factors: Osservatorio Epidemiologico Cardiovascolare/Health Examination Survey 1998-2012. *Eur J Prev Cardiol.* 2015 Sep;22(2 Suppl):9-37

Giampaoli S, Krogh V, Grioni S, Palmieri L, Gulizia MM, Stamler J, Vanuzzo D; Gruppo di ricerca dell'Osservatorio epidemiologico cardiovascolare/Health Examination Survey. Eating behaviours of italian adults: results of the Osservatorio epidemiologico cardiovascolare/Health Examination Survey.

Pala V, Sieri S, Palli D, Salvini S, Berino F, Bellegotti M, Frasca G., Tumino R, Sacerdote C, Fiorini L, Celentano E, Galasso R and Krogh V. Diet in Italian EPIC cohorts: presentation of data and methodological issue. Tumori, 89: 594-607, 2003.

**[www.cuore.iss.it](http://www.cuore.iss.it)**

**[www.ehes.info](http://www.ehes.info)**