

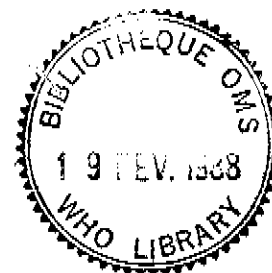


INFORMATION SYSTEMS SUPPORT
FOR INTEGRATED NONCOMMUNICABLE DISEASES PROGRAMMES

Report of a meeting, Helsinki, Finland
29-30 August 1986

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The theoretical basis and action guidelines for integrated noncommunicable diseases programmes have been discussed at several meetings of experts¹⁾²⁾³⁾⁴⁾⁶⁾⁷⁾⁸⁾. Activities have already started in some countries: For instance the WHO Regional Office for Europe has started a Country-wide Integrated Programme for Noncommunicable Disease Intervention (CINDI), in which nine countries are participating. The Regional Office for the Americas is coordinating a similar programme (MORE) in countries of the Region. The Division of Noncommunicable Diseases (NCD) at WHO headquarters has now initiated a global programme, entitled INTERHEALTH, which includes at least two countries in each region. The experiences of these countries will be used for further development of the programme in the regions. The protocol for INTERHEALTH is under preparation and the first training course for the programme managers from participating countries was organized in November 1986 in Finland.

The purpose of the present meeting was to elaborate, on the basis of present knowledge, recommendations for basic information requirements for assessment of health trends related to major noncommunicable diseases. The meeting also examined the possibility of links with and use of MONICA methodology.⁹⁾¹⁰⁾

1. Basic information needed to assess health trends related to major noncommunicable diseases

The core mortality rates for assessing the effects of the NCD programme were considered to be cardiovascular diseases (CVD), cancer, diabetes mellitus (DM) and chronic respiratory infections. It was very important to measure changes in disease rates and then to assess if any change is related to the programme. The group also recommended monitoring of the morbidity of CVD, cancer, DM, chronic respiratory diseases and some other chronic diseases if possible. It was essential that the methodology be as flexible as possible in order to get some sort of picture from the different countries. Also the possibility to pool together the rates of chronic diseases was discussed and recommended in cases where more detailed or well validated information is not available.

-
- 1) NCD/OND/80.1 : Report of a WHO Consultation on an Integrated Noncommunicable Disease Prevention & Control Programme, Geneva, 16-19 June 1980
 - 2) ICP/CVD 020(2) 6634B(EURO) : Prevention and Control of Chronic Noncommunicable Diseases. Report on a WHO Working Group, Zurich, 23-25 October 1980.
 - 3) NCD/82.2 : An Integrated Programme for the Prevention and Control of Noncommunicable Diseases. Report of a meeting (WHO Headquarters, Geneva and Regional Offices for Europe, Copenhagen) Kaunas, Lithuanian SSR, USSR, 16-20 November 1981.
 - 4) NCD/83.1 : The Steering Group Meeting on an Integrated Noncommunicable Diseases Prevention and Control Programme. Report of a meeting held in WHO Headquarters, Geneva, 25-28 October 1982.
 - 6) NCD/84.1 : The Second Steering Group Meeting on an Integrated Noncommunicable Disease Prevention and Control Programme. Report of a meeting held in Malta, 5-8 December 1983.
 - 7) NCD/IP/85/WP/5 : WHO Consultation on an Integrated Programme for Community Health in Noncommunicable Diseases. 16-18 December 1985, WHO Headquarters, Geneva.
 - 8) ICP/NCD/025,8697F : Countrywide Integrated Noncommunicable Diseases Intervention Programme (CINDI). Report on a Meeting of Programme Directors held in Reykjavik, Iceland, 16-21 June 1986.
 - 9) CVD/MNC/Version 1.1: MONICA Manual (except Section 6)
 - 10) CVD/MNC/Version 1.1: MONICA Manual, Section 6, Standardization of Lipid Measurements

1.1 Cardiovascular diseases

It was recommended that all cardiovascular diseases, including myocardial infarction (MI), stroke, hypertension (HT), rheumatic heart disease (RHD) and cardiomyopathy, should be monitored. MI, hypertension and stroke can be validated according to MONICA methodology if available.

For the monitoring of morbidity (incidence) of these diseases, countries were recommended to refer to MONICA methodology and, where it was not available, other methodology such as surveys and hospital discharge data should be selected.

1.2 Cancer

Total cancer mortality should be monitored, with emphasis on sites which could be influenced by the programme, such as lung, colon, breast, stomach and cervical cancers. For collection of incidence data, registers, if possible, are recommended¹⁾ and centres are encouraged to find feasible ways for registering of cancer incidence if established registers do not exist.

1.3 Diabetes

For evaluation, data obtained from population surveys are recommended. Blood glucose measurements and if possible the glucose tolerance test according to WHO recommendations²⁾ are strongly recommended for the population over 45 years.

For the monitoring of incidence, registers are starting in many places. If registers exist, additional information from e.g. surveys should be used for evaluation.

1.4 Chronic respiratory diseases

Chronic respiratory diseases are important in the NCD programme; however for assessment purposes, it would be essential to have a standardized questionnaire for the surveys. As no standardized self-administered questionnaires exist to date, the group was unable to recommend a particular one for the survey. However, if the survey is administered by an interviewer, the respiratory questionnaire from "Cardiovascular Survey Methods" could be used³⁾ (Annex 3).

1.5 Other diseases

Traffic accidents, oral health, and mental health are also important entities in NCD as well as other diseases like musculoskeletal disease. The group recommended that countries select some of these diseases relevant to given situations.

-
- 1) Maclennan R. et al : Cancer registration and its techniques IARC - Scientific publications No. 21; Lyon 1978
 - 2) Diabetes Mellitus, Technical Report Series No. 727, WHO, Geneva, 1985
 - 3) Rose G.A. et al : Cardiovascular Survey Methods. World Health Organization Monograph Series No. 56, Geneva 1982

2. Measurement of risk factors and life style

2.1 Smoking

The questionnaires used in the MONICA project were recommended. Validation (such as measurement of serum thiocyanate) should be considered (see Annex 4).

In addition national consumption figures should be collected whenever possible.

2.2 Alcohol

Each country is recommended to develop its own methodology for measuring the habitual weekly alcohol consumption, since local beverages vary from country to country.

Biological measurements, such as gamma-gt, could also be considered.

2.3 Physical activity

It was recommended that some questions on physical activity should be used in the surveys; those included in CINDI could be adapted for local situations.

2.4 Obesity

Body weight and height measurements according to the MONICA methodology are recommended, and BMI (Body Mass Index, kg/m^2) should be used.

2.5 Stress

There is no standardized methodology for measurement of stress.

2.6 Traffic behaviour

No standardized methodology exists.

2.7 Nutrition

The group emphasized the central role of dietary habits. These should be monitored using a methodology applicable to each country. The group stressed that each country should do its own evaluation in a standardized way.

2.8 Blood pressure

MONICA methodology was recommended (see Annex 5).

2.9 Body weight

MONICA methodology was recommended (see Annex 6).

2.10 Height

MONICA methodology was recommended (see Annex 6).

2.11 Serum cholesterol

MONICA methodology was recommended (see Annex 7).

2.12 Other indicators

The group recommended that countries also consider the following indicators:

- serum creatinin; the test is relatively simple and can be used as an indication for renal diseases (caused by diabetes or other).
- urine glucose, protein and some minerals should also be considered.

3. Links with and use of MONICA methodology

It was considered important that some NCD be monitored in MONICA, such as cancer (lung and colon), respiratory diseases, diabetes and accidents. The MONICA Data Centre could provide guidelines for the monitoring of morbidity of these diseases. MONICA centres are recommended to contact local cancer registers, where these exist, and use the international registration system recommended by WHO.¹⁾ MONICA centres are recommended to monitor the prevalence of diabetes using blood glucose assessment and glucose tolerance tests obtained from population surveys. Questions still to be solved include definition of sample size and age groups to be surveyed. Guidelines for surveying diabetes within the framework of MONICA could be provided by the MONICA Data Centre.

The assessment of alcohol consumption and respiratory diseases by questionnaires is recommended. The basis for evaluation of these variables will be further elaborated by WHO and they will also be presented to the MONICA Steering Committee.

Concerning the monitoring of intervention, the group was informed about the present status of work on evaluation of health care in MONICA. Proposals will be ready for consideration by the MONICA Steering Committee in August 1987. In this connection, the group realized the need for guidelines for broader monitoring of intervention, e.g. health education, training etc.

4. Steps needed to develop new components for monitoring

4.1 Protocol

The draft protocol for INTERHEALTH was reviewed by the group. This will be further elaborated by WHO.

4.2 Operations manuals

The MONICA Data Centre will prepare a manual for monitoring other disease entities and WHO headquarters will prepare a manual for assessment of other risk factors like alcohol consumption. The group stressed the urgent need for a manual for measuring the feasibility, effects and costs (evaluation manual) of noncommunicable disease programmes.

1) MacLennan R. et al : Cancer registration and its techniques. IARC - scientific publications No. 21: Lyon, 1978

4.3 Programme modules

INTERHEALTH includes several modules of which the most essential are: smoking, hypertension, diabetes, nutrition, alcohol, school health, and training programmes. Guidelines for measuring the feasibility, effects and costs (evaluation) of these programmes are needed.

5. Recommendations for plan of action

The group emphasized the need for protocols for the global INTERHEALTH programme and for each of its modules. These would form the basis for further development of the evaluation component.

The MONICA links and the use of its methodology should be further elaborated.

Annex 1

WHO MEETING ON INFORMATION SYSTEMS
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AGENDA

1. Basic information needed to assess health trends related to major noncommunicable diseases
 - 1.1 Lifestyle
 - 1.2 Risk factors
 - 1.3 Diseases entities
2. Links with and use of MONICA methodology
 - 2.1 Monitoring of risk factors
 - 2.2 Monitoring of health interventions
 - 2.3 Monitoring of disease entities other than cardiovascular diseases
3. Steps needed to develop new components for monitoring
 - 3.1 Protocol
 - 3.2 Manual of operations
 - 3.3 Modules for the above
4. Recommendations for plan of action
5. Any other business

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Annex 3

WHO MEETING ON INFORMATION SYSTEMS
SUPPORT FOR INTEGRATED NONCOMMUNICABLE
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RESPIRATORY QUESTIONNAIRE
(for administration by an interviewer)

Use the actual wording of each question. Mark "X" in the appropriate square after each question. When in doubt, record "No".

Cough

1. Do you usually cough first thing in the morning in the winter?
(Count a cough with first smoke or on "first going out of doors".
Exclude clearing throat or a single cough). Yes
No
2. Do you usually cough during the day or at night in the winter?
(Ignore an occasional cough) Yes
No
3. Do you cough like this on most days for as much as three
months each year? Yes
No
Does not apply

Phlegm¹

4. Do you usually bring up any phlegm from your chest first thing
in the morning in the winter? Yes
No
5. Do you usually bring up any phlegm from your chest during the
day, or at night, in the winter? (accept twice or more) Yes
No

If "Yes" to question 4 or 5, ask:

6. Do you bring up phlegm like this on most days for as much as
three months each year? Yes
No
7. In the past three years have you had a period of (increased²)
cough and phlegm lasting for three weeks or more? No
Yes
Yes - two or more periods

-
- 1) Or an alternative word to suit local usage
 - 2) For subjects who usually have phlegm

WHO MEETING ON INFORMATION SYSTEMS
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SMOKING HISTORY (WHO/MNC/82.2)

The smoking questionnaire is part of the general questionnaire. It represents a compromise among different proposals and derives, at least practically, from the WHO Cardiovascular Survey Methods questionnaire. It can be self-administered if it is sent to the home of invited persons together with an invitation to the examination; or it can be administered by a technician or nurse on the screening site.

It is important that the same procedure should be applied throughout the study in the same centre.

In the case of self-administered procedures, the questionnaire should be reviewed by a technician or a nurse for completeness and consistency of answers.

In the case of direct administration, some general rules should be followed:

- use the same wording written on the questionnaire;
- ask the questions a second time and in the same way if on the first occasion the subject does not answer or appears not to have understood;
- ask the questions a third time using different wording but having the same meaning if the subject again does not answer or understand;
- record answers and do not interpret them;
- do not induce certain answers;
- ask all questions and record all answers unless otherwise stated.

Interviewers should be trained and their performance evaluated and tested for precision and accuracy.

The protocol states that the smoking questionnaire should be validated by physical methods, normally serum thiocyanate or carbon monoxide. If such measurements cannot be done on all subjects, do them on a sub-sample of one out of ten subjects, irrespective of their smoking history. Standardization of such measurements is described here.

Serum Thiocyanate Analysis (Section 7 of MONICA Manual)

Smoking habits, e.g., inhalation, may vary locally and with time, all may not be reflected in the information collected by the smoking questionnaire. Moreover, socio-cultural factors may affect the truthfulness of the answers. Among the objective variables for validating smoking questionnaire responses, carboxyhaemoglobin has a half-life of 3-4 hours and serum thiocyanate of 10-14 days. Thiocyanate is thus less subject to short-term variation in smoking.

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Moreover, serum thiocyanate has been validated as a predictor of total and coronary 15-year mortality, being either superior or equal to the smoking questionnaire in two populations, respectively¹.

Among non-smokers, the standard deviation of thiocyanate was found to be approximately 25% of the mean, and 15% among those smoking ten cigarettes or more a day. The "baseline" level of thiocyanate is also affected by the diet.

In MONICA, thiocyanate determination may serve two purposes:

- (a) to validate the smoking questionnaire data for the population and its sub-groups, and
- (b) to provide an aggregate measure of trends in population exposure to smoking.

For (a), a sufficient number of data for each sex, age and smoking category should be collected, while for (b) four groups only at the initial and the terminal surveys, respectively, would be the minimum requirements: male versus female, non-smokers versus present smokers. The non-smokers are necessary for showing trends in the background level dependent on the diet. Only the complete coverage of a MONICA survey sample would provide sufficient accuracy for (a), while for (b) as little as a 20% random sample would be satisfactory.

For the validation of population trends in smoking, thiocyanate obviously should be analyzed at least twice, both at the initial and the terminal surveys. On the other hand, for a stratified study of the validity of the questionnaire - or comparing questionnaires - the mid-point survey would have merit in providing data equally applicable over the entire ten-year period.

Determination of Thiocyanate in Serum

When ferric ions in acid solutions are added to thiocyanate, a brownish-red complex of ferric cyanate with an absorption peak at 455 nm is formed. Based on this reaction, RG Bowler has published a method to determine thiocyanate in serum (Biochem. 38, 385-388, 1944). Later, this method was adapted for AutoAnalyzer II by William C. Butts et al (Clin. Chem. 20, 1344-1348, 1974). Both are given here with the following modifications:

I - In the automated method of Butts:

- the determination is run at 60 samples per hour with a sample-to-wash ratio of 8:2
- better absorbances are obtained when the usual flow-cell of 15 mm light-path is replaced by a 50 mm cell.

II - In the manual method of Bowler, the following alterations are suggested:

- in order to obtain higher absorbances, the amount of sample is increased to 2 ml to give a final dilution of 1:3 in the reaction mixture, and
- the volumes of diluent and TCA are decreased
- the final ferric nitrate concentration is maintained at less than 10 g/l, as at higher concentrations the absorption peak at 445 nm is deformed.

1) Heliövaara, M. et al. Serum thiocyanate concentration and cigarette smoking in relation to overall mortality and to deaths from coronary heart disease and lung cancer. J. Chron.Dis. 1981, 34:305-11.

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(1) Automated method for AutoAnalyzer II

Reagents:

Ferric nitrate, 10 g/l. Dissolve 10 g of $\text{Fe}(\text{NO}_3)_3 \cdot 9 \text{H}_2 \text{O}$ p.a. in 1,5-M nitric acid and dilute to one litre with the same acid. Filter, store at room temperature in a dark bottle. Prepare every two weeks.

Sodium perchlorate, 0,1-M. Dissolve 12,25 g of NaClO_4 p.a. in de-ionized water and dilute to one litre with water. Filter, add 1 ml of Brij-35 (300 g/l), mix, store in a dark bottle at +14°C.

Dialyzer recipient. To one litre of de-ionized water add 1 ml of Brij-35 (300 g/l), mix well.

Standards:

Stock Thiocyanate Standard, 1000 $\mu\text{mol/l}$. Dissolve 97,2 mg of potassium thiocyanate (KSCN , p.a.) in de-ionized water and fill the solution to one litre with water. Stable at room temperature for two to three months.

Working standards of 50, 100, 150, 200 and 250 $\mu\text{mol/l}$ are prepared from the stock standard by diluting with water. Working standards are not stable for more than two weeks at room temperature. Secondary standards, prepared by adding known amounts of stock solution to a serum pool can also be used instead of water standards.

Controls:

It is recommended to prepare a serum pool from the blood of smokers and freeze the control serum in suitable portions. A frozen control stored at -20°C can be used for six months at least.

(2) Manual method

Reagents:

Ferric nitrate, 20 g/l. Dissolve 20 g of $\text{Fe}(\text{NO}_3)_3 \cdot 9 \text{H}_2 \text{O}$ p.a. in 1,5-M nitric acid and dilute to one litre with the same acid. Filter, store at room temperature in a dark bottle. Prepare every two weeks.

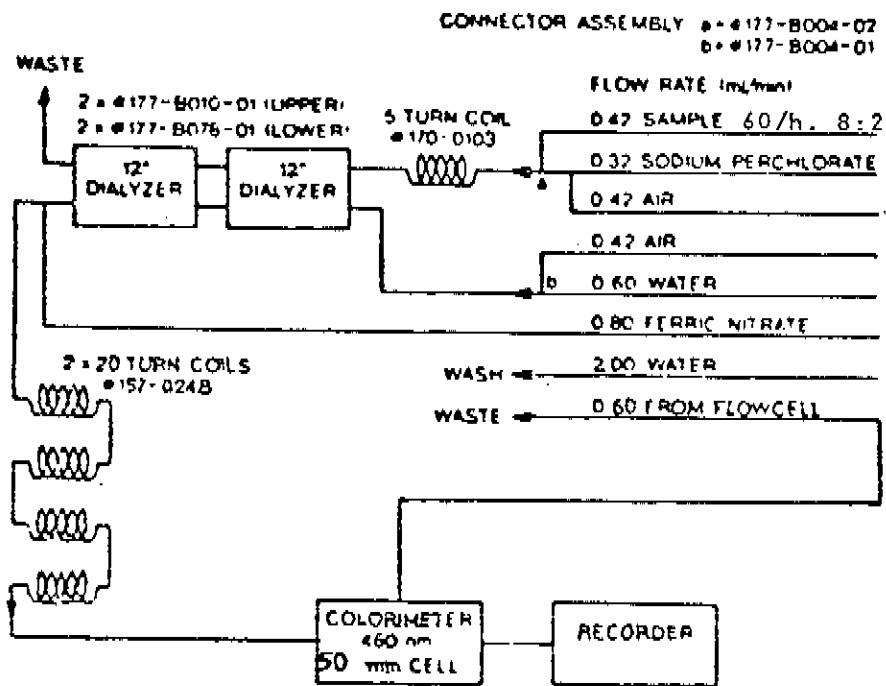
Sodium perchlorate, 0,1-M. Dissolve 12,25 g of NaClO_4 p.a. in de-ionized water and dilute to one litre with water. Filter, and store in a dark bottle at +14°C.

Trichloroacetic acid, 20%. Dissolve 20 g of TCA in 100 ml of de-ionized water, filter. Store at room temperature.

Standards:

Stock Thiocyanate Standard, 1000 $\mu\text{mol/l}$. Dissolve 97,2 mg of potassium thiocyanate (KSCN , p.a.) in de-ionized water and fill the solution to one litre with water. The standard is stable for two to three months at room temperature.

Working standards of 50, 100, 150, 200 and 250 $\mu\text{mol/l}$ are prepared from the stock standard by diluting with water. Working standards are not stable for more than two weeks at room temperature.



From: William C. Butts, Martha Kuehneman, and Graham M. Widdowson:
Automated Method for Determining Serum Thiocyanate, to
Distinguish Smokers from Nonsmokers.
Clin.Chem.20,1344-1348(1974).

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Controls:

It is recommended to prepare a serum pool from the blood of smokers and store it at -20° frozen in portions of 2,5 ml. The control serum is stable for six months at least.

Working scheme:

Into a centrifuge tube pipette (preferably conical) put
 standard or sample 2 ml
 NaClO₄ 1 ml

mix, and add
 TCA 1 ml

Mix vigorously, leave to stand for 20 minutes at room temperature, then centrifuge down the precipitated protein.

Take
 clear supernatant 2 ml
 ferric nitrate 1 ml

mix and immediately measure the absorbance at 455 nm (using a spectrophotometer) or at a wavelength in close vicinity (using a filter photometer) immediately, or within a few minutes after mixing (if a series of samples is measured). The colour begins to fade after 30 minutes in sunlight (and more rapidly from standards).

SCN laboratory performance guidelines

Due to the broad variability of measurement procedures and to the purpose of the SCN data, the guidelines for laboratory performance, relative to quality control sample reference values are less stringent than those for cholesterol. Bias should not exceed 10% of the reference value. The CV(%) as measured by the standard deviation of the individual laboratory determination for a given pool divided by the reference value for that pool multiplied by 100 should be no greater than 5 for any pool.

Carbon monoxide analysis using the Ecolyzer (WHO/MNC/82.2)

The Ecolyzer is an instrument which measures the carbon monoxide concentration of expired air. Carbon monoxide is a gas produced by incomplete combustion, and is commonly present in the environment as a result of smoking, automobile exhaust, gas appliances, etc. When this gas is inhaled, it enters the blood stream and becomes attached to haemoglobin molecules in the same site ordinarily occupied by oxygen. Carbon monoxide is more strongly bound to haemoglobin than is oxygen, and as a result the carboxy-haemoglobin gradually dissociates back to its constituent parts over a period of many hours. As carboxy-haemoglobin dissociates, carbon monoxide is released back into the lungs and is expired in the normal process of breathing out.

Since everybody is exposed to trace amounts of carbon monoxide, as well as producing small amounts by metabolism, it is normal to have a concentration of carbon monoxide in expired air in the range of 2-8 parts per million (ppm). Individuals who have been exposed to relatively high doses of carbon monoxide from inhaling cigarette smoke typically have carbon

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monoxide levels in the range of 9-40 ppm. These levels decline with a half life of about four hours, which works out mathematically to remaining detectable for about 24 hours after smoking cessation. For this reason, smokers will tend to have elevated carbon monoxide levels even if they have not smoked since the previous evening.

Techniques for using the Ecolyzer to determine expired air carbon monoxide levels have been standardized in order to provide measurements which most reliably reflect smoking exposure. These standardized techniques, which were developed in the MRFIT programme, are presented below.

Instructions for measuring expired air carbon monoxide levels of survey participants

In an attempt to fix the time interval between possible smoking and the Ecolyzer measurements, the Ecolyzer procedure should be the first one carried out after the participant has been called from the waiting room. Both the time of day when the participant arrives at the clinic and the time when the ecolyzer test was performed should be recorded.

- (1) Explanation of the procedure to the participant. The instructions to each participant should start with an explanation of the purpose of the measurement, e.g., "This instrument measures the amount of carbon monoxide in your breath. Trace amounts of carbon monoxide are ordinarily present in the air everyone breathes out, and larger amounts may be present in individuals who smoke or have unusual exposure to automobile exhaust". (Incidentally, it is very rare for the carbon monoxide level to be elevated above 10 ppm by automobile exhaust, and/or occupational exposure).
- (2) Instructions to the participant. Before collecting the breath sample, the participant should be told that he/she will be asked to:
 - (a) inhale deeply
 - (b) hold breath for 15 seconds
 - (c) exhale half of the breath into the air
 - (d) exhale remaining half slowly into the collection bag until it is nearly full.

Ask if the participant has any questions.

- (3) Collection of expired air sample and measurement of carbon monoxide level. Follow these steps:
 - (a) before measurement, check to see that the selector switch has been left at zero
 - (b) turn the selector to the "on" position
 - (c) attach the disposable mouthpiece to the collection bag
 - (d) squeeze the collection bag flat on a flat surface so that there is no residual gas remaining
 - (e) direct the participant through the four steps mentioned above [(2), (a)-(d)]
 - (f) pinch the mouth of the bag shut, remove the disposable mouthpiece and plug the sample into the intake port of the Ecolyzer
 - (g) allow the meter to reach a maximum level and record this value, lining up the needle with the mirror as usual. Read to the nearest ppm.
 - (h) repeat the whole series of procedures to obtain a second carbon monoxide reading
 - (i) if the two measurements do not agree within 4 ppm, repeat the procedure until agreement is obtained.

SMOKING QUESTIONNAIRE (WHO/MNC/82.1)

- 1a) Do you smoke cigarettes now?
 Yes, regularly
 No (if "no", go to question 2)
 Occasionally (usually less than one cigarette/day)

- 1b) On the average, about how many cigarettes do you now smoke a day?

- 1c) How old were you when you began to smoke cigarettes? Age:
 (go to question 2)

- 2(a) Did you ever smoke cigarettes?
 Yes, regularly
 No, never
 Occasionally (usually less than one cigarette/day)

- 2b) What is the maximum number of cigarettes you ever smoked
 per day for as long as a year?

- 2c) How old were you when you began to smoke cigarettes? Age:

- 2d) When did you stop smoking cigarettes? Year, 19
 If in the last year, less than one month ago
 1-6 months ago
 6-12 months ago

- 3a) Have you ever smoked cigars/cigarillos?
 No
 If "no" go to question 4(a)
 Used to, but not now
 If "not now", go to question 4(a)
 Now smoke occasionally (less than one per day)
 Now smoke regularly

- 3b) About how many do you smoke per week? Number:

- 4a) Have you ever smoked a pipe?
 No
 Used to, but not now
 Now smoke a pipe occasionally (less than one a day)
 Now smoking regularly

- 4b) About how many grams of tobacco do you smoke per week? Grams:

Annex 5

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BLOOD PRESSURE MEASUREMENT (WHO/MNC/82.2)

It is important that measurement of blood pressure (BP) is as precise as possible. This is essential for valid comparisons. Therefore a strict order of doing BP measurement should be kept as a fixed routine.

1. The subject should be instructed to avoid the following activities for at least one hour before the BP measurement: strenuous exercise, eating, drinking of anything other than water, smoking, drugs that affect the blood pressure; full bladder affects the blood pressure and patients should be advised accordingly.
2. The participant should have removed outer garments, jackets, etc. The sleeve of shirt, blouses, etc., should be rolled up so that the upper right arm is bare for the blood pressure cuff. The shirt should not constrict and the blood pressure cuff should not be over the garment. Garments must be removed if obstructing and a short-sleeved jacket provided.
3. The examination should take place in a quiet room with constant controlled temperature.
4. The equipment used should preferably be the random-zero sphygmomanometer. The cuff (bladder-size) should be 12-12.5 cm wide and sufficiently long to surround at least 2/3 of the upper arm.
5. The BP should be measured after resting with no change of position for at least 5 minutes, in sitting position and using the right arm - unless there is a deformity. When seated the subject's arm should be allowed to rest on a desk so that the antecubital fossa is level with the heart. To achieve this, either the position of the subject in the chair should be adjusted, or the arm may be raised or lowered on a comfortable support. The subject must always feel comfortable.
6. The cuff should be applied firmly enough to prevent slipping. The rubber tubes should lie symmetrically on each side of the cubital fossa (to have the central part of the rubber bladder covering the brachial artery). The lower edge of the cuff should be 2-3 cm above the cubital fossa, to allow sufficient room for the bell of the stethoscope. The top edge of the cuff should not be restricted by clothing.
7. The observer should be in a comfortable position in relation to the examination table. The sphygmomanometer's mercury column should be in a perfectly upright position, the centre of it at the eye level of the examiner. The mercury column should face the observer and should not be in the subject's view.

The cuff should be connected now with the sphygmomanometer.

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8. After the subject has rested five minutes in this position - during which the whole process of BP measurement could be explained to him/her - first the peak inflation level should be established. This is the level to which the pressure should be raised for the first blood pressure measurements.

The procedure is the following:

- (a) feel the subject's radial pulse with your left hand fingers;
 - (b) inflate the cuff and note the level of the top of the meniscus of the mercury column at the point when the radial pulse disappears. Then immediately deflate the cuff by disconnecting the cuff and the sphygmomanometer;
 - (c) write down the level of the mercury column (where the radial pulse disappeared) to the nearest 2 mm reading and add to this number 30: this sum is called the peak inflation level.
9. Reconnect the cuff and the sphygmomanometer and wait for at least 30 seconds, or raise the arm for 5-6 seconds. This is to allow the return of venous blood to the forearm).

Locate the brachial pulse. The point of maximal pulsation, immediately below the cuff where the bell of the stethoscope should be placed. If it is not possible to feel the brachial pulse, the bell of the stethoscope should be placed over the area of the upper arm immediately inside the biceps muscle tendon. The bell should not touch the cuff, rubber or clothing.

Looking at the manometer with the centre of the scale at eye level, and the column perfectly upright, inflate the cuff rapidly to a pressure equal to the peak inflation level. From this point let the column of mercury fall at a rate of 2 mmHg per second.

Continue to reduce the pressure steadily at this rate until recording the systolic and phase 5 diastolic level. Then deflate the cuff rapidly (as above).

Blood pressure values should be recorded to the nearest 2 mmHg (reading from the top of the rounded meniscus. If the top of the meniscus falls half way between two markings, choose the marking immediately above.

10. Reconnect the cuff and the sphygmomanometer, raise the arm for about 5-6 seconds, or wait at least 30 seconds, and then repeat the measurement exactly the same way the first one was carried out.

Whenever having difficulties in hearing the sounds, the cuff must be completely deflated and at least 30 seconds must pass before making the next measurement.

11. Record the values of both measurements.

The above procedures for BP measurement should be applied regardless of the instrument used (i.e. simple mercury, random-zero or School of Hygiene sphygmomanometer).

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For the random-zero machine additional instructions apply. These include:

- (a) connect the cuff tubing to the random zero device;
- (b) ensure that the mercury reservoir valve is in the operating position, i.e. turned fully to the right and extending past the right side of the case; turn the bellows cock on the face of the device to the right, to the position marked OPEN;
- (c) turn the thumb wheel at the right side of the device, by gently stroking it two times with the thumb of the right hand. If the wheel is not free to spin in either direction, the bellows are not completely deflated and the bellows check position should be rechecked;
- (d) inflate rapidly by the same method as for the standard device, to the peak inflation level for this series of random zero readings;
- (e) by closing the bulb thumb valve, hold the pressure at this level for five seconds (count to five slowly), and then turn the control valve to the left, to the position marked CLOSE;
- (f) by carefully controlling the thumb valve, with the bell of the stethoscope over the brachial artery deflate the cuff at 2 mm/second until the mercury level is 4-6 mm below the diastolic reading;
- (g) open the thumb valve fully and disconnect the tubing to the random zero device, allowing the mercury to fall to its zero level for this reading;
- (h) record the systolic and 5th phase diastolic readings, uncorrected;
- (i) read the zero level for this reading and record it on the form in the spaces provided beneath the uncorrected systolic and 5th phase diastolic readings. Subtract the zero level to obtain the correct readings and record on the form.

WHO MEETING ON INFORMATION SYSTEMS
SUPPORT FOR INTEGRATED NONCOMMUNICABLE
DISEASES PROGRAMMES

29-30 August 1986, Helsinki

HEIGHT AND WEIGHT (WHO/MNC/82.2)

HEIGHT

Procedures

1. Height is measured in conjunction with the weight measurement. It may precede or follow this procedure.
2. The height rule must be taped vertically to a hard flat surface, with no moulding, with the base at the floor level. A carpenter's level should be used to assure vertical placement of the rule.
3. The floor surface must be hard (tile, cement, etc.) and cannot be carpeted or have other soft materials. If only a carpeted surface is available, a wood platform should be laid down to serve as the floor. A mat or small carpet should be used between the chair and rule.
4. The participant is asked to remove his/her shoes and heavy outer garments (jackets, coats, etc.).
5. To measure height, the participant should stand with his/her back to the height rule. The back of the head, back, buttocks, calves and heels should be touching the wall, feet together. The top of the external auditory meatus (ear canal) should be level with the inferior margin of the bony orbit (cheek bone). This position is aided by asking participant to hold head in a position where he can look straight at a spot, head high, on the opposite wall.
6. Place the triangle on the height rule and slide down to head so that the hair is pressed flat.
7. Record information on survey form to nearest centimetre. For example, if 187.4, record as 187; If 187.5, record as 188. If 187.6, record as 188.
8. Self-reported heights are not acceptable in ambulatory participants and should not be reported (mark as refusal). Only persons who are not ambulatory (e.g. amputees) may self-report their heights. Be sure to note this on the form.
9. To measure extreme heights, a short rule is used in addition. It is placed at the top of the long rule and the extra height is added.

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BODY WEIGHTProcedure

1. The floor surface on which the scale rests must be hard and cannot be carpeted or have other soft materials. A mat or small carpet with non-skid backing should be used between the chair and the scale.
2. The scale should be balanced with both weights at zero and the balance bar aligned.
3. The participant should have removed his/her shoes and heavy outer garments (jackets, coats, etc.).
4. The participants should stand in the centre of the platform as standing off centre may affect measurement.
5. The weights are moved until the beam balances (the arrows are aligned).
6. The weight is read and recorded on form. Record weights to the nearest 200 g.
7. Under no circumstances is the participant to self report his/her own weight or do the reading of the scales.
8. Self-reported weights are not acceptable in ambulatory persons. Refusals to be weighed should be recorded as refusals. Only participants who are not ambulatory (e.g. amputees) may self report their weights. Be sure to note this on the form.



WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTE

DISTR.: RESTRICTED
DISTR.: RESTREINTEWHO MONICA PROJECT: MEETING OF PRINCIPAL INVESTIGATORS
Berlin, German Democratic Republic, 9-11 April 1987

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ENGLISH ONLY

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Section 6 Standardization of Lipid Measurements

This section provides details on the methods to be used for measurement of Lipids in the MONICA Project and their standardization.

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CVD Unit
2.2.87

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LIST OF ABBREVIATIONS

Apo-B	Apoprotein B
CDC	Centers for Disease Control, Atlanta, Georgia 30333, USA
EDTA	Ethylene diamine tetra acetic acid
EQA	External quality assessment
G	Gravity
HDL	High density lipoproteins
HDL-C	High density lipoprotein cholesterol
IKEM	Institute for Clinical and Experimental Medicine, Prague, Czechoslovakia
IQC	Internal quality control
LDL	Low density lipoproteins
LRC	Lipid Research Clinics
MMC	MONICA Management Centre
MQC	MONICA Quality Control Centre
OD	Optical Density
QA	Quality Assurance
R.p.m.	Rotations per minute
RV	Reference value
SCN	Serum Thiocyanate
St	Total standard deviation
TC	Total cholesterol
TG	Triglycerides (Triacylglycerols)
VLDL	Very low density lipoproteins
WHO-HQ	WHO Headquarters
WHO-KRIS	WHO-Kanaus-Rotterdam Intervention Study
WHO-LW	WHO Lipid Workshop (Prague, 26-28 January 1982)
WHO-RLRC	World Health Organization Regional Lipid Reference Centre (WHO Collaborating Centre for Blood Lipid Research In Atherosclerosis and Ischaemic Heart Disease at the Institute for Clinical and Experimental Medicine (IKEM), 14000 Prague - 4, Czechoslovakia P.O. Box 10)

6.1 Obligatory and Voluntary Biochemical Measurements*

6.1.1 Total cholesterol (TC) and serum thiocyanate (SCN) (for SCN see Section 7) are the minimum obligatory parameter to be measured in blood (serum, plasma) samples of the MONICA study subjects in all cooperating centres. However, wherever possible, samples should also be analysed for HDL-C and further parameters on a voluntary basis. Laboratory directors who attended WHO/LW agreed upon HDL-C measurements in MONICA study centres.

6.1.2 HDL-cholesterol (HDL-C) is strongly recommended and will be measured by many centres (who must standardize their technique on the Reference Centre). Other voluntary but complementary measurements might include, e.g.: triacylglycerols (triglycerides), lipoprotein typing and composition (electrophoresis, ultracentrifugation, ultrafiltration and nephelometry, chromatography of lipoproteins, etc.), apoprotein (12) concentrations (immuno-electrophoretic, radial-diffusion and/or nephelometric methods), glucose, uric acid, urea, electrolytes, creatinine, thyroxine, haemoglobin, carboxyhaemoglobin, and in certain situations, trace elements. If myocardial infarction is suspected, the aspartate amino transferase, alanine amino transferase, lactate dehydrogenase may be measured.

6.2. Sample Collection and Initial Processing

6.2.1 Centres should collect MONICA blood samples in comparable seasonal periods (i.e. during the same months in subsequent years) to prevent effects of seasonal variation on the levels of measured serum/plasma parameters. Centres collecting MONICA blood samples in the course of the whole year should randomize blood taking for sex and age groups to obtain each year approximately the same number of subjects of different categories in comparable time periods (months). Medical staff should keep detailed records on circumstances which may affect lipid levels and/or methods (certain drugs, alcohol consumption, pregnancy, etc.). Whenever possible, lipid affecting medication should be interrupted before blood taking. The interruption period should be sufficient to cancel the lipid influencing effect of the drug(s), including a rebound effect.

Venous blood samples should ideally be taken after a 12-16 hour overnight fast (water, tea or coffee without milk is allowed) particularly if TG are also measured.

It is recognized that it is not feasible to obtain fasting samples in all cooperating Centres and subjects. Since circulating levels of serum/plasma TC and HDL-C are relatively stable with respect to immediate dietary history, non-fasting samples can be admitted in this study for measurement of TC and HDL-C [34]. Centres must standardize their procedures so that they are consistent as to time of day and fasting status from one survey to the next. The time of venipuncture should be recorded.

*Abbreviations are listed on page 2. (certain drugs, alcohol consumption, pregnancy, etc.). Because the MONICA survey is concerned with risk factor levels in the community, however they have been modified, lipid affecting medication should not be interrupted before blood taking.

Subjects should be asked before venipuncture about their food intake during the previous 12-16 hours and adequate records made if a non-fasting sample is taken and analysed.

6.2.1.1 Blood should be drawn from the subject or patient in a sitting position by venipuncture. Prolonged venous occlusion can cause changes in the apparent concentrations of blood constituents (3). Use of tourniquet should therefore be avoided. If a good flow cannot be obtained in some subjects and the tourniquet has to be used, it is released prior to withdrawal of blood.

Standardization of the position (sitting position is recommended) is necessary since plasma volume changes occur when a standing subject assumes a recumbent position [20,3].

6.2.2 Serum should be used in preference to plasma. It is realised that the group of laboratories standardized with CDC may have to continue using these methods for MONICA. It follows that these laboratories may continue using EDTA plasma prepared according to the LRC prescriptions [20].

6.2.2.1 Either 10ml vacuum tubes or syringes and glass tubes may be used to collect blood. Glass tubes should be equipped with stoppers made from material inert to the blood components. When vacuum tubes are used, the type with stoppers not lubricated with glycerol should be selected (glycerol causes interference with TG assays in enzymatic methods).

6.2.2.2 The use of vacuum tubes containing EDTA is recommended if plasma is used (group of laboratories following LRC methods).

6.2.3 For serum preparation, blood samples are allowed to clot at not more than 20°C usually for up to one hour before centrifugation. There is evidence (personal communication from the Helsinki Centre) that this period could be prolonged by up to three hours.

Blood specimens should be centrifuged at a temperature of not more than 20°C (warning: prolonged running of a non-refrigerated centrifuge may result in considerable warming of the inner centrifuge compartment and centrifuged samples) at a minimum of 1500 G for at least 10 minutes to separate serum from the clot. If a refrigerated centrifuge is not available, it may be necessary to cool blood samples before centrifugation (for instance, in a refrigerator or on wet ice). With a refrigerated centrifuge, centrifugation should be done preferably at 4°C. Whole blood samples must not be frozen during processing (this would cause haemolysis).

6.2.4 For plasma preparation the tube(s) filled with blood must be immediately stoppered (if vacuum tubes are not used) and inverted about 10 times gently to ensure prompt and thorough mixing of blood sample(s) with EDTA. Mixing should not be vigorous. According to LRC recommendations the blood samples are then cooled on wet ice. Within 3 hours (and preferably

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within one hour) the tubes should be centrifuged at 4°C in a refrigerated centrifuge at 1500 G for 30 minutes. If a refrigerated centrifuge is not available within 3 hours of collection, the samples may be centrifuged at room temperature within 1 hour of collection, and the plasma stored at 4°C.

6.2.5 After centrifugation, the serum/plasma should be promptly separated from clot or cells (transfer to a clean tube). The white cell layer (buffy coat) is not transferred with plasma.

6.2.6 Haemolytic serum/plasma samples should be discarded and new samples should be retaken from the subjects and analysed.

6.3. Storage of Serum/Plasma Samples

6.3.1 It is recommended that the TC and HDL-C levels should be estimated on the day of sample collection.

6.3.2 Samples can be stored for up to four days at 4°C [1,3,5]. If analyses of TC cannot be performed within 4 days the serum or plasma samples should be immediately stored at -20°C or lower in tightly closed glass vials [1].

6.3.3 Precipitation of Apo-B containing (LDL + VLDL) lipoproteins (see 6.4.5) should preferably be done on fresh (non-frozen) serum aliquots on the day of blood collection. Should it be impossible to perform precipitation on fresh samples, the serum or plasma for HDL-C determination should be immediately frozen at -20°C or lower [1,3]. Precipitation should then be performed within 14 days. The sample should be thawed only once and well mixed. It should be recognized, however, that freezing may introduce a systematic error of up to 4% in HDL-C measurements [3].

6.3.4 Storage of serum/plasma samples for other purposes than immediate analysis of TC, HDL-C and SCN: if samples are to be stored for voluntary analysis, laboratories must select adequate storage conditions at which the measured parameters' concentrations would be stable.

6.4 Recommended Analytical Procedures for TC and HDL-C Measurement

6.4.1 It is recommended that an enzymatic cholesterol method with practically 100% cholesterol ester hydrolysis be used. Advantages include non-corrosive reagents, easy and fast operational steps, specificity, possibility of using automated methods as well as manual methods with inexpensive instruments, unification of methodology in the project, etc. However, it is realised that a group of laboratories standardized with CDC using LRC methods based on extraction into isopropanol, may continue using their methods for the MONICA if the purpose of their own research would require such continuity.

6.4.2 The use of a number of commercial brands of enzymatic reagents for cholesterol analysis might diminish comparability of results between laboratories due for example to non-homogeneity of production lots or due to loss of kit enzyme activities on storage. Information collected at the WHO-LW showed that most of the participating laboratories have worked or are going to work with the same commercial brand which is widely used in Europe. This is fortunate for the project. Suitability of selected enzymatic methods will be checked by means of the EQA.

6.4.3 It is possible that a few laboratories taking part in the MONICA may be unable to comply, for local reasons, with the recommendation to use an enzymatic method. These laboratories probably will continue to use the so-called "direct", "one-step", "Liebermann-Burchard" method(s) in manual or automated version(s), etc. The WHO-RLRC will circulate a questionnaire concerning analytical and associated procedures to be used in the different centres, with the aim of obtaining full information on the use of methods other than the LRC or enzymatic methods. It is the policy of MONICA to accept, in exceptional cases, participants not able to use the recommended methods. In such cases, particular attention to methods for ensuring comparability will be necessary if performance within allowable EQA limits is unattainable with use of primary pure standards. The laboratories concerned may have to calibrate with serum calibrators provided by the WHO-RLRC (MQC).

6.4.4 Every participating laboratory should provide a full detailed description of their entire procedure of sample collection and analysis to MMC, (WHO - Cardiovascular Diseases unit) and to Dr D. Grafnetter (WHO-RLRC) at the latest six months before analysis of MONICA samples start in the centre concerned. An example of a recommended sequence of quality control standardization and participant samples for total cholesterol and HDL cholesterol determination are shown in Figure 1.

6.4.5 Isolation of the HDL-fraction:

6.4.5.1 Laboratories are recommended to use the "Phosphotungstate-Mg²⁺" (PT) precipitation method [2, 10, 11, 15, 31, 32, 33] for isolation of HDL from LDL + VLDL. In this method final concentrations after mixing serum (or plasma) with precipitation reagent(s) are: 3.6 g/litre for phosphotungstic acid and 0.045 M for Mg²⁺ [21, 24]. According to these papers, precipitation of LDL + VLDL is complete if pH of the final mixture (serum/plasma + PT reagents) remains below pH 7.6. This method (see 6.4.5.2 - 6.4.5.7.2) was in general use at the time this Manual was initiated. By 1986, many centres had already switched to the new PT method.

6.4.5.1.1 Some laboratories, e.g. those with the LRC methodology, may need to continue with other precipitation techniques (Mg²⁺-dextran, Mn²⁺-heparin). Acceptance of these methods is based on the same policy as mentioned under section 6.4.3.

6.4.5.2 Preparation of PT method [31, 32] reagents for precipitation at pH below 7.6 (for alternative concentrations and use of PT reagents see sections 6.4.5.7 - 6.4.5.9).

6.4.5.2.1 Sodium phosphotungstate, 40.0 g/l, pH 7.4. Dissolve four grams of phosphotungstic acid (reagent grade) in about 60 ml distilled water. Add gradually under mixing and using pH meter as much of 1 mol/l NaOH as to reach pH 7.4 (usually somewhat less than 16 ml). Then make the volume up to 100 ml.

6.4.5.2.2 Magnesium chloride, 2.0 mol/l: Dissolve 40.6 grams of $MgCl_2 \cdot 6H_2O$ (analytical grade) in about 80 ml of distilled water and make the volume up to 100 ml.

6.4.5.2.3 Reagents should be kept at 4°C between use and can be used as long as 6 months, provided they show no bacterial growth.

6.4.5.3 Use of PT reagents described under 6.4.5.2.1 and 6.4.5.2.2:

6.4.5.3.1 Add 100 μ l of the phosphotungstate solution (6.4.5.2.1) to 1.0 ml of sample and mix (preferably by vortexing). Add 25 μ l of magnesium chloride solution (4.5.2.2) and again mix well. Let each precipitated sample stand for ten minutes at room temperature and then centrifuge. For centrifugation conditions see Section 6.4.5.4.

6.4.5.3.2 Volumes of sample and reagents could be reduced (e.g. halved) for the precipitation step.

6.4.5.3.3 It is also permissible to use pre-mixed reagent. This is prepared and used as follows: phosphotungstate and magnesium chloride solutions are mixed on the day of use in the ratio of 100:25 (e.g. 4 ml + 1 ml). then 125 μ l of the pre-mixed reagent are added to 1.0 ml of sample and the whole is mixed well.

6.4.5.4 Centrifugation after precipitation: Since centrifugation at +4°C on one hand, and +20°C on the other hand can lead to certain differences in HDL-C levels in some reconstituted lyophilized quality control materials (observations made in the WHO-RLRC) but not in fresh sera, it is recommended (for all but the LRC methods laboratories) to centrifuge "HDL samples" after PT precipitation of Apo-B containing lipoproteins at room temperature (not below +15C or above +25C) at 2000 G for 30 minutes.

6.4.5.5 After centrifugation the supernatant should be immediately transferred to a clean and dry tube.

6.4.5.6 With study subject samples, only clear (non-turbid) supernatants should be used for subsequent cholesterol analysis. With the PT method (if TG are elevated) the supernatant may occasionally show some turbidity. In that case the precipitation step should be repeated with the serum/plasma sample diluted 1:1 with 0.9% sodium chloride solution in distilled water (saline, physiological solution). This usually results in a clear supernatant (but precision of the cholesterol assay is decreased; remember that the result must be multiplied by two if the 1:1 diluted sample is used - see also section 6.4.6.4). Should dilution of serum/plasma sample not give a clear supernatant after precipitation and centrifugation, do not proceed. Any such irregularity and/or difficulty in obtaining clear supernatant should be recorded on the form for recording results.

6.4.5.6.1 In some lyophilized quality control materials slight turbidity of supernatants may be unavoidable. The WHO-RLRC will distribute instructions for use of pools with individual shipments of EQA sets.

6.4.5.7 Alternative concentrations and use of PT method reagents:

Special (adjustable or constriction) pipettes are necessary to deliver the unusual volumes (e.g. 25 μ l or 125 μ l) of the above "classical" PT method reagents (see 6.4.5.1 - 6.4.5.6.1.) The following modified reagent concentration (33) and their use (see sections 6.4.5.7.1, 6.4.5.7.2 and 6.4.5.7.3) yield the same result, but enable the use of normal precision pipettes.

6.4.5.7.1 Sodium phosphotungstate, 48.0 g/l, pH 7.4: dissolve 4.8 grams of phosphotungstic acid (reagent grade) in about 50 ml distilled water. Add gradually under mixing, and using a pH meter, as much of 1 mol/l NaOH as necessary to reach pH 7.4. Then make the volume up to 100 ml.

6.4.5.7.2 Magnesium chloride, 3.0 mol/l: dissolve 60.9 grams of $MgCl_2 \cdot 6H_2O$ (analytical grade) in about 80 ml of distilled water and make the volume up to 100 ml.

6.4.5.7.3 Use of the modified PT method reagents:

Precipitation reagent is prepared by mixing 5 parts of sodium phosphotungstate (6.4.5.7.1) with one part of magnesium chloride (6.4.5.7.2) solution (e.g. 5 ml + 1 ml) on the day of use. Then 100 μ l of the premixed reagent are added to 1.0 ml of serum/plasma sample and the whole is mixed well. Further steps (centrifugation, etc.) are the same as those starting at section 6.4.5.4. However, multiplication (dilution) factor (6.4.6.3) will be 1.10 in this case (not 1.125 as before).

6.4.5.8 Some laboratories have used a commercial PT precipitation kit and followed its working instructions, and they may wish to continue doing so.

This is possible if results obtained with the use of such a kit are found compatible with the EQA criteria.

6.4.5.9 A modified precipitation method for determination of HDL-cholesterol using PT was developed and has been commercially available for several years (kit "HDL-Cholesterol. Precipitant;" Cat.No. 543004; Boehringer Mannheim GmbH). This technique is described in detail in the operation sheet inserts with each kit. The method has been shown to compare well with the reference ultracentrifugation method. It uses a precipitant solution with decreased Phosphotungstic acid and Magnesium chloride concentrations; only 200 μ l of sample are required for one determination on a sample using the semimicro version of the method. Samples become more diluted than in the conventional PT method(s), thus, turbid supernatants occur only very exceptionally. In short the procedure is as follows:

500 μ l (or 200 μ l in the semimicroversion) of sample are mixed with 1000 μ l (500 μ l) of the reagent. In the semimicro version the Precipitant solution must be diluted with water (4 + 1 volumes) to obtain the precipitation reagent. After mixing, the mixture is left to stand 10 minutes at room temperature and then centrifuged for 1 minute at 4000 r.p.m., or 2 minutes at 12,000 r.p.m. Cholesterol is determined in the supernatant using the enzymatic method (e.g., Monotest Cholesterol or Peridochrom, Boehringer Mannheim GmbH). 200 μ l of sample (or blank, or standard) supernatant are mixed with 2000 μ l of the Monotest or Peridochrom reagent in the conventional manual method (one can also use proportionally less of sample and reagent in the automated methods, or where allowed by the cuvette volume of the photometric instrument). After mixing and 10 minute incubation at 20^o - 25^o C (or 5 minutes at 37^o C) the developed colour is measured at Hg 546nm or at 500nm, using a 1 cm. Cuvette concentration is calculated on the basis of "water soluble" standard(s) (e.g., Preciset Cholesterol; Boehringer Mannheim GmbH), or using the manufacturer's factor if the kit procedure is followed exactly as described in the insert.

The Precipitant solution used in this method contains Phosphotungstic acid (0.55 mmol/l) and Magnesiumchloride (25 mmol/l in distilled water). For use in the semimicro version of the method (200 μ l sample + 500 μ l reagent) it should be diluted as mentioned above, i.e. 4 + 1 (4 volumes Precipitant + 1 volume distilled water).

6.4.6.0 Determination of cholesterol in the HDL containing supernatant

6.4.6.1 The determination should preferably be done shortly after precipitation of LDL+VLDL and the centrifugation step (on the same day). If it is necessary to store supernatant(s) analysis is recommended within 4 days if stored at +4^o C. Prolonged storage requires at least -20^o C. Storage is recommended in small volume glass tubes (vials) with leak-proof stoppers to prevent volume and concentration changes (evaporation, freezing out). After storage and before analyses the samples brought to room temperature should be gently mixed.

6.4.6.2 Cholesterol should be determined in the HDL-containing supernatants by the same method as used in the laboratory concerned for TC estimation. Should the method sensitivity be low (optical density readings in unreliably low range) it might be necessary to use a photometric cuvette with greater optical length and/or to increase the supernatant sample volume. Reliability of the method must be checked carefully in such cases [30].

6.4.6.3 When serum/plasma sample is diluted 1:1 before use (see section 6.4.5.6) one must multiply the result by two to obtain final serum/plasma HDL-C concentration.

6.4.6.4 Duplicate or single TC and HDL-C measurements: If possible, all MONICA serum/plasma samples should be analyzed in duplicate. For analysis of cholesterol by extraction chemical methods two separate extracts would be required for duplication (do not perform two analyses on one extract). For HDL-C analysis duplicate LDL+VLDL precipitations would be required. Mean results are reported if analyses are performed in duplicate. (MDC must be notified as to whether single analysis or mean values are reported, the "power" of the means is greater in statistical evaluations.)

6.4.6.5 For IQC and EQA samples all analyses are performed in duplicate (but mean values are not reported to the MQC, both values are recorded and reported).

6.4.6.6 The IQC rules shown below (see section 6.7.7) give criteria for each single measurement result on control samples, from which the maximal allowable difference between side-by-side duplicates is in fact also defined. Daily use of IQC information is of prime importance for maintaining good precision, since EQA information is only retrospective and primarily devoted to following interlaboratory comparability.

It is recommended in addition that laboratories analyzing not only control but also MONICA project samples in duplicate should reanalyse the samples with duplicates differing more than 0.40 mmol/l for TC (15.0 mg/dl), or 0.20 mmol/l (8.0 mg/dl) for HDL-C. Should duplicate results frequently differ that much, the method should be revised and every effort be made to improve precision.

6.5 Calibration of Methods

Recommendation: Primary standards and/or secondary serum/plasma calibrators are used at least in duplicate for calibration.

6.5.1.0 Each participating laboratory is responsible for its own analytical primary standards and/or secondary serum calibrators.

6.5.1.1 It is assumed that all participating laboratories will use the best and most appropriate pure substances and method reagents. The cholesterol substance used for preparation of standards should be of more than 99% purity.

6.5.1.2 Secondary (serum) calibrators should preferably be prepared and/or labelled with correct TC and/or HDL-C concentrations in the WHO-RLRC and/or CDC. It is understood, however, that staffing and budgetary restraints may be a limiting factor in such an undertaking.

6.5.1.3 The WHO-RLRC will distribute during the pre-standardization period a set of at least three cholesterol standards for use in testing linearity over the working range. After preliminary agreements are made a shipment of a commercial cholesterol standards pack (6 standards with concentrations of 50-400 mg%) will be sent to each laboratory. Such sets should enable control of linearity and at the same time calibration of several enzymatic and/or extraction cholesterol methods. Some cholesterol methods (the so called "direct" Libermann-Burchard chemical methods) can not be calibrated by these "water soluble" standards (falsely high results might be obtained without special arrangements), but the linearity response of these methods can be judged on the basis of these standards.

6.5.1.4 Each standard (calibrator) should be run at least in duplicate.

6.5.1.5 Each laboratory should perform tests on linearity over the usual working range (0.5 - 10.0 mmol/l of TC), even if the WHO-RLRC or other body were not able to supply them free of charge with appropriate control material for this purpose.

6.5.1.6 It is essential that linearity should be checked repeatedly during the prestandardization period. During the study linearity should be checked regularly, and particularly if greater than allowable imprecision and/or inaccuracy (allowable limits on accuracy and precision - see 6.7.8.5 and 6.8.3.8) is detected. Ideally, linearity should be checked with at least three standards in each run in which MONICA samples are analysed. It is understood, however, that this will not always be possible in all laboratories, and that it may not be necessary to use standards to judge linearity where adequate data can be obtained from IQC and EQA samples (see 6.7.3.2). Laboratories are free to check on linearity by any reasonable method guaranteeing acceptable results within the limits of accuracy and precision.

It is understood that linearity enabling use of a mean calculation factor (which asks for calibration line passing through the X,Y intercept after subtraction of reagent blank) may not be attainable in some participating laboratories (e.g. because of limitations in the photometric equipment available for the assay). Non-linearity need not necessarily mean wrong results. Linearity must be checked, and every effort should be made to

attain it. If unattainable the laboratory should derive results mathematically from regression equation or graphically from standard curves covering the working range of the method. (Enzymatic cholesterol methods can usually provide linearity up to 11.5 mmol/l. When higher serum/plasma cholesterol concentration is expected analysis should be repeated after appropriate dilution of the sample according to the instructions of the manufacturer of the enzymatic kit).

6.5.1.7 More detailed information on checking on linearity, calibration, and QA can be found in the literature [4, 14, 19, 20].

6.6 Units of Reporting

Recommendation: All results should ideally be reported to 2 decimals in the International SI Units [23].

However, the older units (e.g. mg/dl) are still used in some laboratories because continuity of methods is desirable until certain related projects are finished. In the MONICA project results for TC and HDL-C will therefore be accepted both in mmol/l and mg/dl since units can easily be converted by computer.

6.6.1 Results obtained in mmol/l should be calculated and reported to two decimals.

6.6.2 Results obtained in mg/dl should be calculated to one decimal, but TC concentrations should then be reported rounded off to whole numbers and HDL-C concentrations as calculated, i.e. to one decimal.

6.6.3 The conversion factor for cholesterol from mg/dl to mmol/l is: 0.025864. The conversion factor for triglyceride from mg/dl to mmol/l is: 0.0114

Example: (250mg/dl) x (0.025864) = 6.466 = 6.47 mmol/l for TC
(250mg/dl) x (0.0114) = 2.85 mmol/l for TG.

6.7. Internal (Intralaboratory) Quality Control (IQC)

Recommendation: at least two serum control pools are used for IQC. The preparation, checking, and use of these pools are described under 6.7.3 and 6.7.7.

6.7.1 Performance of methods within IQC limits is a prerequisite for successful participation in EQA programme and for reliable analysis of MONICA samples.

6.7.2 The following recommendations on the preparation of IQC pools and how to proceed with IQC represent the views of the WHO-LW on the desirable

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methods of quality control. It is understood, however, that some laboratories will not be in a position to comply with all of these recommendations, and others will already have instituted adequate but different quality control procedures, which they are unable to change. In these cases each participating laboratory should inform the WHO-RLRC centre of their quality control and standardization methods. In other words, systems other than the recommended internal quality control system are permissible as long as there is adequate indication that analytical results, as judged by IOC and EQA, are within the IOC and EQA control limits described below. It follows that use of self-made and/or commercial frozen or lyophilized internal quality control materials is allowed. If lyophilized material is used for HDL-C, it should be material specifically designed for this purpose.

6.7.3 Establishment of pools: Each laboratory should establish at least two control serum pools for internal quality control. Each pool should last through the whole of one phase of MONICA and in any case should be sufficient to last for one year of normal operations. The pools under 6.7.3.1 and 6.7.3.2 are the only two pools that are strictly required for MONICA.

6.7.3.1 TC + HDL-C pool: This pool should be prepared from non-turbid human serum containing "normal" TC and TG concentrations. The pool should be distributed in suitable aliquots into tightly closed glass vials and ideally kept frozen at -60°C or below.* Should it be impossible to use a self-made or commercial frozen serum pool, a lyophilized pool could be used. In the latter cases, there should be a guarantee that the pool was prepared only from human sera, without use of additives or enrichments which could affect TC and HDL-C determinations, and it should be suitable for the HDL-C determination. The TC + HDL-C pool would be used both for TC and HDL-C method control, including the precipitation step in the latter case (the quality control specimen should be treated in exactly the same way as the test samples). Lyophilized pools do not require -60°C for storage. Even $+4$ to $+6^{\circ}\text{C}$ may be satisfactory for a defined expiration period.

6.7.3.2 Low total cholesterol pool (LTC pool): This pool should contain 1.30-1.60 mmol/l cholesterol and it should be used for control in the low TC range. It could be prepared from a human serum pool diluted to the appropriate cholesterol concentration with 0.15 M NaCl. Alternatively, animal (bovine, horse) serum pool, eventually slightly diluted to the desired CH concentration level, could be used.

*CDC laboratories found -20°C unsatisfactory for long term storage (personal communication). However, a number of MONICA laboratories may have to store at only -20°C . In such cases the TC + HDL-C pool should be periodically checked for HDL-C concentration stability in runs containing EQA samples.

6.7.3.2.1 Ideally, the appropriately (4-5 times) diluted TC + HDL-C pool (under 7.3.1) should be used as a LTC pool, with the advantage that both pools could be used also for linearity checking (plot of absorbance against concentrations or dilution factors should result in a line passing through the X, Y intercept). This checking will work only with enzymatic and/or extraction cholesterol methods. In the "direct chemical cholesterol methods the concomitant dilution of interference (by bilirubin, tryptophane, proteins, etc.), as well as changed viscosity in automated versions, may result in non-proportionality of absorbances to dilution.

6.7.4 Some laboratories may elect to establish a further (or several more) pool(s) for TC and/or HDL-C measurements, e.g. one with the cholesterol concentration in the upper part of the working range. The HDL-C pool(s) should have a low TG concentration (under 1.5 mmol/l).

6.7.5 Useful information concerning the preparation of self-made pools can be found in the literature [13, 14, 16, 17, 18, 22, 26, 27, 28, 29].

6.7.6 Use of IQC material to ensure analytical precision for TC and HDL-C analysis:

6.7.6.1 Once opened, an ampoule (vial) of quality control materials should not be used for more than one day's operation. Accurate reconstitution of lyophilized materials must be ensured (to prevent introduction of errors not related to the method itself). If a lyophilized pool is used for HDL-C control (precipitation included) each laboratory concerned should standardize the time period between reconstitution and analysis.

6.7.6.2 Each analytical run should begin with calibration standard(s). This could be the primary standard(s) and/or secondary serum calibrator(s) (see example in Appendix I)

6.7.6.3 IQC samples should follow. All quality control samples should be analysed in side-by-side duplicates. The results obtained from quality control samples following the standard(s) are used to indicate whether the run is in control and whether the analysis of study samples can be begun (for criteria see 6.7.8.1).

6.7.6.4 When a new pool is introduced both the next and the old pool should be in use together (overlap period) for at least the number of runs (analyses) required to establish the starting mean (see 6.7.6.6), to ensure adequate calibration of the new pool and method control.

6.7.6.5 Control of drift in a run:

In each analytical run (or part of analytical run) containing MONCIA

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samples, quality control material should be placed on every tenth position among the samples (to monitor within run stability). This can be other than the LTC+HDL-C or LTC pool material and need not necessarily be of human origin nor accurately calibrated because it serves only for monitoring within-run reproducibility (see Figure 1).

6.7.6.6 Before a pool is introduced, its starting concentration mean must be established. This is done as follows: at least 40 values are collected on at least 20 pool vials randomly distributed in independent analytical runs over at least 20 days (do not use 40 times in one run). Finally, the whole set of values should be examined for potential outliers [19]. If any value differs from the overall mean (\bar{X}) by a number greater than $3.3s_t$ (i.e. if any $X_i > \bar{X} + 3.3s_t$), the pair of results in which it occurs is discarded and \bar{X} recalculated. Only one such outlier is permissible. Should there be more outliers, the method should be revised and complemented with further runs and checks on outliers as above.

(s_t is calculated only for the purpose of checking on outliers; it is not needed for IQC in MONICA, common control limits are stated below.)

Total (overall) standard deviation (s_t) is calculated on the basis of individual repeated measurements as follows:

$$s_t = \sqrt{\frac{\sum (X_i - \bar{X})^2}{n - 1}} = \sqrt{\frac{\sum X_i^2 - (\sum X_i)^2/n}{n - 1}}$$

where X_i = individual measurements results,

\bar{X} = the mean of all measurements $(\frac{\sum X_i}{n})$,

n = the number of measurements,

$\sum X_i$ = the sum of the measurements results.

Relative standard deviation (coefficient of variation-C.V.) is calculated:

$$C.V. = \frac{s_t}{\bar{X}} \times 100$$

For an example of mean and s_t calculation see Appendix I.

6.7.7 IQC criteria for acceptability of the run: Once the acceptable pool mean (\bar{X}) is obtained, the precision control limits are defined as follows (for an example of the quality control chart for visual follow up of method performance see Figure 2):

Warning limits for TC (levels above 2.60 mmol/l or 100 mg/dl) analysis: +3% from the mean ($+0.03 \bar{X}$).

Example: at a 5.17 mmol/l (200 mg/dl) level:

- the lower warning limit = 5.02 mmol/l (194 mg/dl)
- the upper warning limit = 5.33 mmol/l (206 mg/dl)

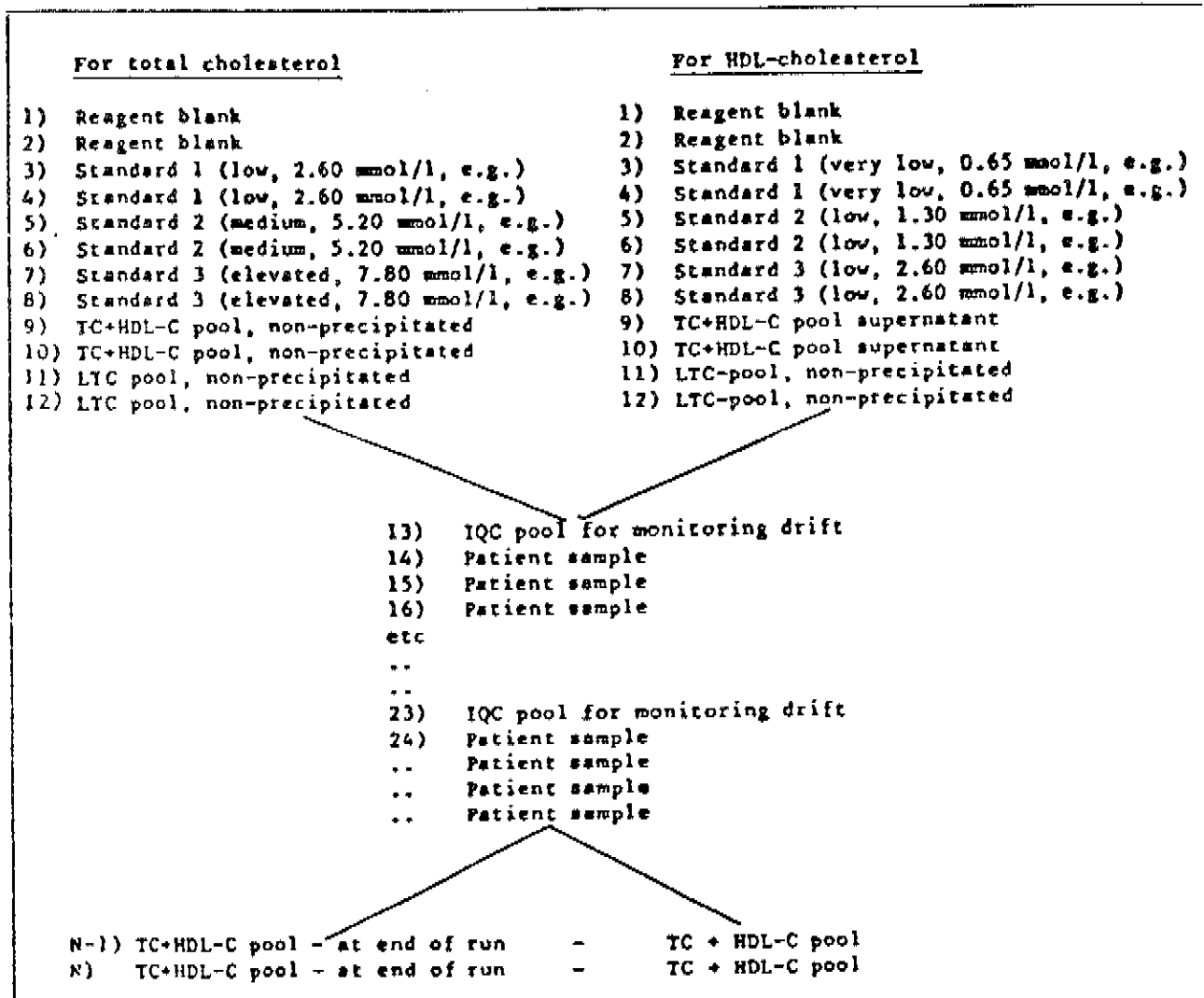
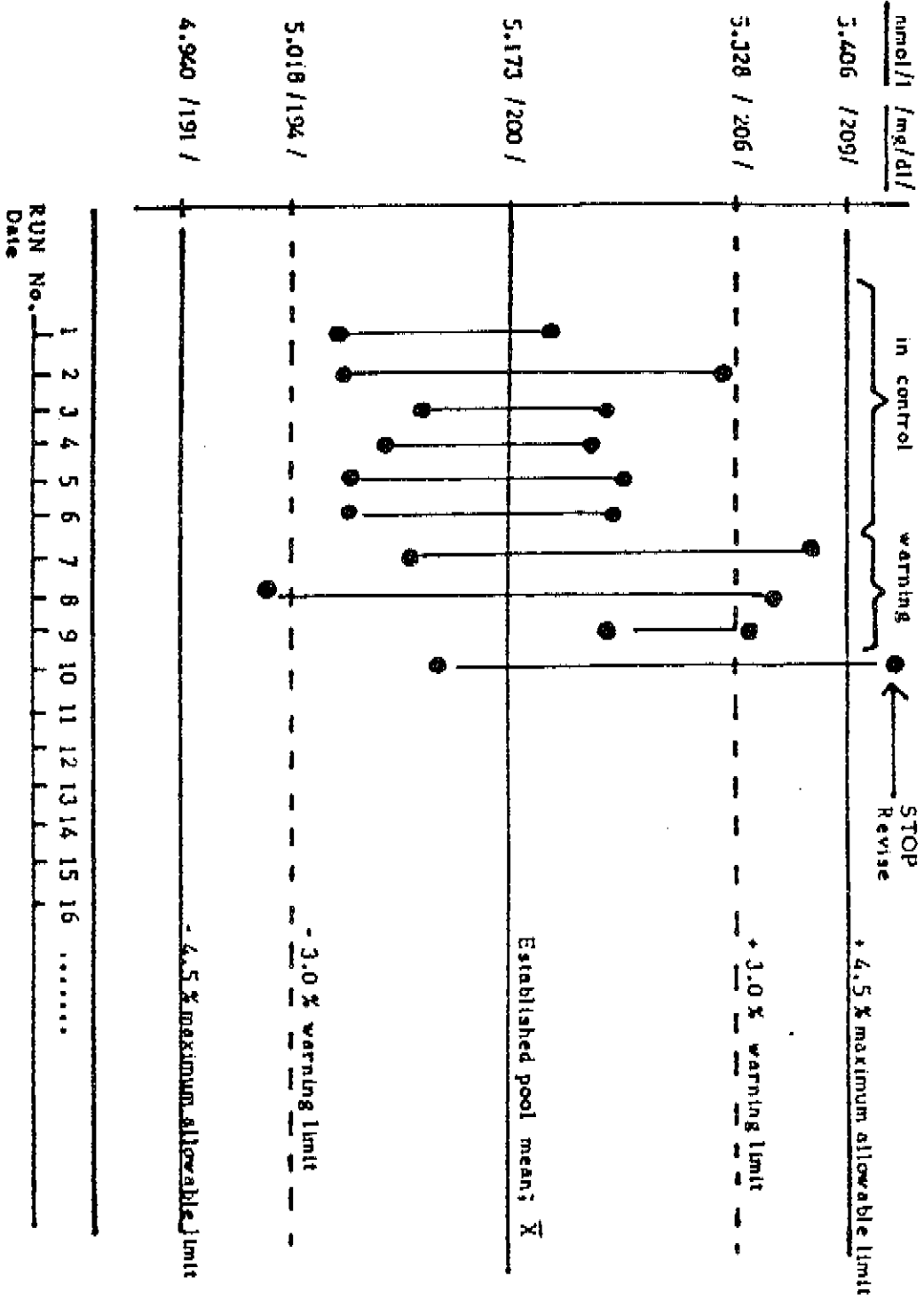


Figure 1. Recommended sequence of N specimens in a run

Note:

- HDL-cholesterols should be preferably analysed in separate runs (or parts of runs) to avoid between sample transfer contamination(s) depending on the instrumentation ("carry over").
- Whenever there could be expected significant carry-over (with older photometers, e.g.) it will be necessary to wash out the photometer cuvette first (notably between "high" standard(s) and first IQC samples) with a part of the next sample/reagent mixture and take the reading on the rest of it.

QUALITY CONTROL CHART - TOTAL CHOLESTEROL



● single values, each control is to be run in duplicate

Figure 2. Example of a Quality Control Chart for Total Cholesterol

Maximum allowable limits for TC (levels above 2.6 mmol/l or 100 mg/dl) measurement: $\pm 4.5\%$ from the mean ($\pm 0.045 \bar{X}$)

Example: at a 5.17 mmol/l (200 mg/dl) level:

- the lower allowable limit = 4.94 mmol/l (191 mg/dl)
- the upper allowable limit = 5.41 mmol/l (209 mg/dl)

Warning limits for HDL-C: $\pm 6\%$ from the mean ($\pm 0.06 \bar{X}$)

Maximum allowable limits for HDL-C: $\pm 9\%$ from the mean ($\pm 0.09 \bar{X}$).

6.7.7.1 Ideally, in runs containing MONICA samples, no single quality control pool value (not the mean of a duplicate) should surpass the warning limits. Should this occur, and the result still be within maximum allowable limits, the run results can be used but it is a strong signal that method(s) should be checked (see standards, calibrator(s), reagents, OD reading instrument(s), pipettes, dispensers, quality control pool expiration dates (see 6.7.3.) eventual microbial growth, possible calculation errors, etc.). The best quality volumetric glass, dispensers, and measurement instruments are recommended.

6.7.7.2 In no case should single control value(s) fall beyond the maximum allowable limits. If this occurs, the run must be stopped, results cannot be used, the cause of trouble must be eliminated, and the method must be brought under control before MONICA samples are started.

6.8 External Quality Assessment (EQA)

Recommendation: the WHO-RLRC is responsible for sending the EQA samples.

6.8.1 EQA is complementary to the IQC and its main purpose will be to check on accuracy (assessment of bias), although it will also supply laboratories with evaluations concerning overall, between-day (between-run) and within-run variability. (Regular use of IQC is a prerequisite for successful participation in the EQA programme).

6.8.2 EQA will be carried out from the WHO-RLRC (Chief: Dr D. Grafnetter, Institute for Clinical and Experimental Medicine (IKEM) Videnska 800, P.O. Box 10, 1400 Prague 4, Czechoslovakia).

6.8.3 EQA assistance to participating laboratories:

6.8.3.1 1982 is considered as a pre-checking (pre-calibration, pre-standardization) period. Each laboratory assigned to analyse MONICA samples (all addresses should be made available to the WHO-RLRC by WHO/HQ, see also 6.4.4) should utilize, before the start of the proper EQA, with "blind" samples (concentration unknown to the laboratories) one or two self-evaluation sets (concentrations known) provided by the WHO-RLRC. Self-evaluation sets contain vials with control sera selected from

lyophilized pools. They are provided with instructions for use and with the list of reference TC, HDL-C, TG (and eventually also Apoprotein AI, AII and B) concentrations. Self-evaluation sets enable a daily check on methods and/or their calibration. They should be used during the pre-checking period together with methods definitively selected for MONICA.

After the "open" period, a "blind" EQA system will start to evaluate laboratory performances. Its principles and methods were explained in detail to the laboratory directors who attended the WHO-LW. Others who are interested or who join the project later may obtain information from the literature [6,7,8,9]. It is necessary to mention here only that the system has been based on repeated analysis of control sera shipped at regular intervals as lyophilizate to laboratories ("sets" composed of 14-21 samples). This system of "blind" EQA sets will be used throughout the MONICA study.

6.8.3.2 Sets should be sent periodically to participating laboratories with clear instructions for use, as well as with relevant information, reporting forms, and eventually also questionnaires. Samples contained in the shipments may originate from commercial pools, as well as from pools prepared in the WHO-RLRC or elsewhere. It will be the responsibility of the WHO-RLRC to check, eventually in cooperation with CDC or other bodies, on the suitability of pools.

6.8.3.3 Control samples should be used in the laboratories according to attached instructions (reconstitution, sequence of samples, etc.). Each sample should be analysed in side-by-side duplicate on the day of reconstitution. If more measurements are performed, only the first two should be reported to the WHO-RLRC. The rest may serve for internal purposes, e.g. analysis of drift.

6.8.3.4 The decision on how many samples (or sets) should be distributed to individual laboratories will be made on an individual basis, depending on the questionnaires completed and results reported by the laboratories. In most cases 4-5 EQA sets per year will be distributed to each laboratory. Each set should contain about 20 samples, sufficient for about 2 months.

6.8.3.5 Some laboratories may wish to analyse MONICA survey samples (each survey covers approximately 1600 samples) in batches separated by intervals of days or weeks, while others may spread their analyses over year(s). Frequency of EQA sets should be adjusted to their needs. Only sets falling into the survey periods of sample analyses (not collection and/or storage) will be used for evaluation of MONICA results.

Laboratories will be requested to complete analysis of individual sets and report results, at the latest, within 2 months. Failure to do so may mean that a further set will not be received, since only receipt of results will be a signal to the WHO-RLRC to indicate that a further set should be sent. Failure of a laboratory to undertake a sufficient EQA control may result in elimination of its TC and HDL-C results from Monica study evaluations.

6.8.3.6. Based upon the EQA results received, the WHO-RLRC will supply the participating laboratory (and WHO-HQ if needed) as soon as possible with information on the analysis of the EQA set, i.e. on the calculated overall, between-run and within-run standard deviation, and on bias, from which acceptability of results for the different pools and methods can be judged. Performance acceptability criteria are shown in Table 1 and discussed under 6.8.3.8.

6.8.3.7. Reference values (RV) will be obtained in the WHO-RLRC as before by the modified Abell et al. method [18], in cooperation with CDC. CDC will be requested to analyse the EQA pools and make results available to the WHO-RLRC, to ensure comparability between WHO-CDC and WHO-RLRC evaluations. As a secondary check in obtaining the RVs a CHOD-PAP enzymatic cholesterol method with practically 100% cholesterol-ester hydrolysis may be used by WHO-RLRC. For LDL+VLDL precipitation in the HDL-C reference value estimation the described PT reagents and procedures will be used (see sections 6.4.5, 6.4.6).

6.8.3.8 Performance acceptability criteria in EQA (limits on maximum allowable inaccuracy and imprecision) (see Table 1):

Total cholesterol: for any of the control pools, calculated bias (based on the EQA set mean and on RV) should not be greater than 5%. At the same time s_t should be smaller than that shown for individual reference values in Table 1. Table 2 shows allowable limits for CV% according to increasing levels of cholesterol concentration.

HDL-cholesterol: for any control pool, calculated bias (based on the EQA set mean and RV) should not be greater than 7.5%. At the same time s_t should be smaller than that shown for individual reference values in Table 2 (i.e., $CV < 6.5\%$).

6.8.4. Laboratories should not begin analysis of MONICA samples before they are able to show that their analyse of at least two successive blind EQA sets are within the limits of acceptability (see 6.8.3.8) for every control pool.

6.8.4.1 If EQA results suggest trouble with a method a decision will have to be made on the admissibility of data from the laboratory. It is strongly recommended that assistance be provided to help solve such problems with the laboratory concerned, using QA information including IQC data. Laboratories should keep good records on their IQC data to be able to present them upon request.

6.8.4.2. However, a situation might arise when a laboratory demonstrates greater than allowable but fairly stable analytical bias with acceptable precision (small s_t). In such a case the WHO MONICA appointed advisor who receives evaluation copies from WHO-RLRC through WHO-HQ) should consider the case and advise the laboratory and WHO-RLRC immediately of the course to be followed. (Results might perhaps be corrected mathematically provided

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TABLE 1

Performance acceptability criteria in EQA (limits on maximum allowable inaccuracy and imprecision)

Individual standard deviations can be obtained for any RV concentration by interpolation.

	Reference value (RV)		Maximum allowable overall standard deviation (s_t)		Maximum allowable deviation of set pool mean from RV (bias)
	mmol/l	mg/dl	mmol/l	mg/dl	
TOTAL CHOLESTEROL	2.5864	100	0.1681	6.5	+5%
	5.1728	200	0.1940	7.5	+5%
	7.7592	300	0.2198	8.5	+5%
	10.3456	400	0.2457	9.5	+5%
HDL- CHOLESTEROL	0.5173	20	0.0336	1.3	+7.5%
	1.0346	40	0.0672	2.6	+7.5%
	1.5518	60	0.1009	3.9	+7.5%
	2.0691	80	0.1345	5.2	+7.5%

*Calculated by comparing the average of all determination for a given pool in a set to the reference value for that pool i.e. with 0.05 x RV (Total cholesterol) or 0.075 RV (HDL cholesterol).

TABLE 2

MONICA - Total Cholesterol

Limits on maximal allowable imprecision

Reference Value mmol/l	Overall Standard Deviation mmol/l	Coefficient of Variation %	Reference Value mmol/l	Overall Standard Deviation mmol/l	Coefficient of Variation %
2.6	0.1682	6.5	7.0	0.2122	3.0
2.7	0.1692	6.3	7.1	0.2132	3.0
2.8	0.1702	6.1	7.2	0.2142	3.0
2.9	0.1712	5.9	7.3	0.2152	2.9
3.0	0.1722	5.7	7.4	0.2162	2.9
3.1	0.1732	5.6	7.5	0.2172	2.9
3.2	0.1742	5.4	7.6	0.2182	2.9
3.3	0.1752	5.3	7.7	0.2192	2.8
3.4	0.1762	5.2	7.8	0.2202	2.8
3.5	0.1772	5.1	7.9	0.2212	2.8
3.6	0.1782	5.0	8.0	0.2222	2.8
3.7	0.1792	4.8	8.1	0.2232	2.8
3.8	0.1802	4.7	8.2	0.2242	2.7
3.9	0.1812	4.6	8.3	0.2252	2.7
4.0	0.1822	4.6	8.4	0.2262	2.7
4.1	0.1832	4.5	8.5	0.2272	2.7
4.2	0.1842	4.4	8.6	0.2282	2.7
4.3	0.1852	4.3	8.7	0.2292	2.6
4.4	0.1862	4.2	8.8	0.2302	2.6
4.5	0.1872	4.2	8.9	0.2312	2.6
4.6	0.1882	4.1	9.0	0.2322	2.6
4.7	0.1892	4.0	9.1	0.2332	2.6
4.8	0.1902	4.0	9.2	0.2342	2.5
4.9	0.1912	3.9	9.3	0.2352	2.5
5.0	0.1922	3.8	9.4	0.2362	2.5
5.1	0.1932	3.8	9.5	0.2372	2.5
5.2	0.1942	3.7	9.6	0.2382	2.5
5.3	0.1952	3.7	9.7	0.2392	2.5
5.4	0.1962	3.6	9.8	0.2402	2.5
5.5	0.1972	3.6	9.9	0.2412	2.4
5.6	0.1982	3.5	10.0	0.2422	2.4
5.7	0.1992	3.5			
5.8	0.2002	3.5			
5.9	0.2012	3.4			
6.0	0.2022	3.4			
6.1	0.2032	3.3			
6.2	0.2042	3.3			
6.3	0.2052	3.3			
6.4	0.2062	3.2			
6.5	0.2072	3.2			
6.6	0.2082	3.2			
6.7	0.2092	3.1			
6.8	0.2102	3.1			
6.9	0.2112	3.1			

that the bias was practically the same at different concentration levels in successive tests, and that s_t did not surpass allowable limits.) Once s_t is above the allowable limit no allowance for bias consideration can be made, and the laboratory will be requested to revise and correct the method before proceeding with the MONICA study samples.

6.8.5 To help overcome problems of retrospectivity in EQA, the WHO-RLC will include in sets, if possible, a pool with nominated RVs to be made known to participating laboratories for continual self-evaluation.

6.9 Recording and Reporting MONICA and Quality Control Results

6.9.1 MONICA results reported to WHO/HQ and/or an advisor elsewhere will be analyzed under the auspices of WHO. WHO staff will design forms for data reporting suitable for computer analysis.

6.9.2 Laboratories should at all times indicate whether they report single measurements results or means.

6.9.3 WHO-RLC will provide laboratories with reporting forms prepared for EQA sets.

6.9.3.1 Laboratories will be identified in the EQA system by code numbers which will not be made publicly available without written consent from the respective laboratories. However, the code will be open for WHO/HQ and for MONICA appointed advisors (who are also bound to keep it confidential) and WHO MONICA meetings.

6.10 Actions if performance with required accuracy and/or precision cannot be achieved in a laboratory.

Initial action should be to make every effort to bring the method(s) into required performance limits, with the use of information obtained and with special samples for method calibration. WHO-RLRC or CDC should be advised about the problem.

If all efforts fail, WHO may assist in nominating and supporting a visit from a consultant to the Laboratory concerned.

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Example of
Calculation of the pool mean (\bar{X}) and standard deviation (s_c).
Cholesterol in mmol/l

Date	Pool vial number	Determination 1		Determination 2	
		x	x ²	x	x ²
	1	6.31	39.8161	6.41	41.0881
	2	6.47	41.8609	6.21	38.5641
	3	6.26	39.1876	6.21	38.5641
	4	6.21	38.5641	6.41	41.0881
	5	6.41	41.0881	6.31	39.8161
	6	6.36	40.4496	6.36	40.4496
	7	6.21	38.5641	6.26	39.1876
	8	6.26	39.1876	6.36	40.4496
	9	6.21	38.5641	6.26	39.1876
	10	6.23	38.8129	6.44	41.4736
	11	6.44	41.4736	6.23	38.8129
	12	6.26	39.1876	6.36	40.4496
	13	6.21	38.5641	6.26	39.1876
	14	6.26	39.1876	6.21	38.5641
	15	6.21	38.5641	6.36	40.4496
	16	6.31	39.8161	6.41	41.0881
	17	6.41	41.0881	6.21	38.5641
	18	6.21	38.5641	6.26	39.1876
	19	6.21	38.5641	6.47	41.8609
	20	6.41	41.0881	6.31	39.8161
	Σx	125.86	-	126.31	-
	Σx^2		792.1926		797.8491

continued

(continued)

Calculation of the mean: $\bar{x} = \frac{\sum x}{n} = \frac{252.17}{40} = 6.30$

Calculation of the standard deviation:

$$\begin{aligned} \sum x &= (125.86 + 126.31) = 252.17 \\ (\sum x)^2 &= 63589.7089 \\ \sum x^2 &= (792.1926 + 797.8491) = 1590.0417 \\ n &= 40 \\ n - 1 &= 39 \end{aligned}$$

$$\begin{aligned} s_c &= \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n - 1}} = \sqrt{\frac{1590.0417 - \frac{63589.7089}{40}}{39}} \\ &= \sqrt{\frac{1590.0417 - 1589.74273}{39}} = \sqrt{\frac{0.2989775}{39}} = \underline{0.087556} \end{aligned}$$

Calculation of the coefficient of variation:

$$C.V. = \frac{s_c \times 100}{\bar{x}} = 1.389 = \underline{1.4 (\%)}$$

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