MONICA;
Blood sample collection and initial processing

Centres should collect MONICA blood samples in each survey over the same seasons to prevent seasonal variations in the levels of serum/plasma parameters. MCC collecting MONICA blood samples over the course of the entire year should randomize blood taking to ensure equal representation of age and sex subgroups over time.

Lipid laboratories may want to measure lipoprotein fractions and triglycerides and so requires a 16-hour fast. For epidemiological purposes such as in MONICA, where the primary lipid of interest is serum total cholesterol and secondarily high density lipoprotein cholesterol (HDL-C), blood can be taken at any time of day with the subject non- fasting; this facilitates the scheduling appointments at the survey clinic. For comparability with other MONICA centres it is desirable that blood pressure measurements be spread throughout the day and made in non-fasting subjects: for this reason any MCC that wishes to measure fasting lipid specimens should consider doing this separately from the routine survey clinic where blood pressure is measured.

For the purposes of longitudinal comparisons, the methods of measurement of lipids should be consistent between one MONICA survey and another. Any proposed changes such as of time of day and fasting/non-fasting status should be avoided or tested in pilot studies to ensure that they do not introduce bias.

Venipunctures should be carried out with the subject or patient in a sitting position. Prolonged venous occlusion can cause changes in the apparent concentrations of blood constituents Use of a tourniquet should therefore be avoided. If a good flow cannot be obtained in some subjects and the tourniquet has to be used, it must be released before the 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

Serum should be used in preference to plasma. It is realized 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 instructions.

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

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

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 can 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 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 unavailable, it may be necessary to cool blood samples before centrifugation (for instance, in a refrigerator or on melting ice). With a refrigerated centrifuge, centrifugation should be

preferably done at 4°C. Whole blood samples must not be frozen during processing (this will cause haemolysis).

For plasma preparation the tube(s) filled with blood must be stoppered immediately (if vacuum tubes are not used) and inverted gently about 10 times 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 melting ice. Within 3 hours (and preferably 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.

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

Haemolytic serum/plasma samples should be discarded and fresh samples should be taken from the subjects and analysed