A simple, cost-effective quality assurance model for measurement of lipids in a large epidemiological study

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ABSTRACT

Background. Laboratory measurements are an integral part of epidemiological studies in cardiovascular disease. Standardization and quality assurance is of utmost importance in the context of multicentre studies.

Methods. We evaluated a simple and cost-effective method of quality assurance for measurement of total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides in a study involving 10 centres. Three methods for quality assessment were used for the study that involved measurement of cholesterol, triglycerides and HDL cholesterol and included internal quality control, external quality control and 10% repeat analysis in addition to a uniform standardized protocol developed for the 10 centres. External quality control material was prepared and circulated by the coordinating laboratory.

Results. External quality control material was distributed 20 times during the study. The mean variance index suggested a substantial improvement in the performance of participating laboratories over a period of time for cholesterol and triglycerides. This was also evident in the improvement in per cent technical error as a measure of bias and a higher correlation between replicates of samples analysed in the coordinating laboratory and the participating centres for cholesterol, triglycerides and HDL cholesterol.

Conclusion. A cost-effective quality assurance model for laboratory measurement using local capacities was developed and implemented in a multicentre epidemiology study. Such a programme would be useful for developing countries where cost-cutting is important.

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INTRODUCTION

Cardiovascular disease (CVD) is projected to be the leading cause of death in India by 2020 and 50% of these deaths are projected to occur among young and middle-aged individuals.[1,2] To help develop national policies for the control and prevention of CVD, and to demonstrate the feasibility of establishing a surveillance system, baseline data on CVD risk factors were collected from an Indian industrial population.[3] Laboratory measurements formed an integral part of the study. To build local laboratory capacity and to circumvent the need for transportation of samples through a cold chain to a central laboratory, we standardized different laboratories for the measurement of lipid parameters in the study. Since the study involved 10 industrial sites spread across India, a standardized procedure to control for variations arising due to pre-analytical, analytical and post-analytical errors in lipid measurements needed to be devised for laboratories doing the assays. Studies on laboratory standardization report any of the following 3 measures as indicators of bias and precision: internal quality control (IQC) measures, external quality assurance measures and replicate analysis of a small percentage of samples.[4] We report here the standardization procedures developed by us employing the 3 measures for participating laboratories for the epidemiological study.

METHODS

The Department of Cardiac Biochemistry at the All India Institute of Medical Sciences (AIIMS), New Delhi was responsible for biochemical standardization at each centre and coordination of work. Ethical clearance for conduct of the study was obtained from the Ethics Committee, AIIMS, New Delhi. Written consent was taken from all subjects. The laboratory participates in the UK-NEQAS (United Kingdom National External Quality Assessment

Service) for external validation of biochemical assays. The coordinating laboratory organized workshops for training of participating laboratories. To control for pre-analytical variations resulting from problems of phlebotomy, centrifugation, aliquoting of samples, storage and transportation, a workshop detailing these issues was organized for the participating laboratories before the start of the study. A pilot study was conducted after the workshop wherein the participating laboratories were required to collect 25 blood samples each and process them. A workshop was again organized to discuss the problems encountered by the investigating teams. During this workshop a consensus was developed for measures to be taken for control of pre-analytical and analytical variations. For the main study a manual of instruction which carried details of preparation of subjects before sampling, blood sample collection procedures, processing of samples, dispatch of samples and details of analysis was prepared and circulated to all the participating laboratories.

Briefly, the protocol followed by all participating laboratories for the study required the subjects to be fasting, the blood samples to be collected in a 6 ml plain (Red capped, BD) and a 4 ml EDTA tube (Lavender capped, BD) with the subject in the recumbent position. After allowing clot formation (for 30 minutes) the red-capped tubes were centrifuged at 2500 *g* for 15 minutes. After centrifugation, 4 aliquots of serum were to be prepared and stored at –20 °C if the samples were transported within 15 days or in –70 °C deep freezer if the samples were dispatched once in 3 months. The participating laboratories were instructed to use one aliquot of serum for analysis of lipids as per the instructions given in the manual. The other three aliquots were dispatched to the central laboratory on dry ice. All the participating laboratories had at least a –20 °C deep freezer and 8 of 10 had –70 °C freezer. The laboratories were asked to maintain temperature logs and report any breakdown of the freezer. In the event of breakdown the samples were dispatched immediately. The samples were preserved in –70 °C deep freezer in the central laboratory. Blood in the EDTA tube was preserved for DNA extraction.

For quality assurance, importance of running IQC samples was highlighted and the laboratories were asked to establish intra- and inter-assay co-efficient of variance (CV) for cholesterol and triglycerides using IQC samples. For minimizing analytical variation arising due to the use of different lots of reagents, the purchase of reagents for all the laboratories was made by the coordinating centre. Total cholesterol, triglyceride and high density lipoprotein (HDL) cholesterol estimation kits were purchased from Randox Laboratories Ltd. Care was taken to send the same lot of reagents to all the participating laboratories. Cholesterol estimation was done by the CHOD-PAP (cholesterol oxidase/p-aminophenazone) method, triglycerides by GPO-PAP (glycerolphosphate oxidase–peroxidase aminophenazone) method and HDL cholesterol was estimated by the precipitation method using phosphotungstate/magnesium precipitation of apolipoprotein B containing lipoproteins followed by estimation of cholesterol in the supernatant by the enzymatic method. Analyses at the centres were done using semi-automatic analysers from different manufacturers. All the instruments were compatible with reagents from Randox Laboratories.

After the laboratories were adequately standardized in the estimation of cholesterol, triglycerides and HDL as assessed by inter- and intra-assay CVs, the laboratory performance was assessed for the next 4 months through IQC Levy–Jennings plots and external quality assessment. Three levels of IQC (levels 1, 2 and 3 from Randox Laboratories) were required to be run once a week during the standardization period. This was in addition to running three levels of IQC samples with each batch of 20 samples; the standard protocol followed in clinical chemistry laboratories. The target and range of values for the three levels of IQC samples which were used in the study are given in Table I. The internal controls were purchased by the coordinating labora-tory and sent to all participating laboratories. The same lot of IQC material was sent to all the laboratories. The participating laboratories were advised to establish Levy–Jennings plot of the three IQC samples which were reviewed by the coordinating laboratory periodically.

For external validation, lyophilized quality assessment materials were prepared as per WHO guidelines[5] by the coordinating laboratory and distributed. Homogeneity of the prepared quality control in a particular lot was ensured by randomly reconstituting and analysing 20 vials at the coordinating laboratory. The standards used for calibration of lipids were serum based and had traceability to standards from the National Institute of Standards and Technology, Gaithersburg, MD, USA. One lyophilized quality control material was sent every 15 days during the standardization period.

In the subsequent year the impact of the standardization procedure was assessed through a continuing external quality assurance programme and a 10% repeat analysis of the samples collected by the participating laboratories again by the coordinating laboratory. For internal quality assurance the participating laboratories incorporated three levels of IQC samples with every batch of 20 samples. External quality control material was distributed 8 times during the next 1 year. For 10% repeat analysis, blood samples were collected and processed by the laboratories locally and analysed for cholesterol, triglycerides and HDL cholesterol using Randox kits as described above. An aliquot of every tenth serum sample collected at each of the centres was frozen at –20 °C and dispatched periodically (within 15 days) to the coordinating laboratory for repeat analysis till one year. Cholesterol, triglycerides and HDL cholesterol were estimated at the coordinating laboratory using the same batch of reagents as

TABLE I. Target and range of the three levels of internal quality controls used for the study

Analyte	Level 1	Level 2	Level 3
Cholesterol			
(mmol/L)	4.24 (4.03–4.84)	6.68 (5.67–7.70)	10.14 (8.37–11.65)
(mg/dl)	163.1 (155.0–187.3)	256.9 (218.1–296.1)	390 (321.9–448.1)
High density lipoprotein cholesterol			
(mmol/L)	0.83 (0.65–1.01)	1.53 (1.30–1.77)	1.87 (1.58–2.16)
(mg/dl)	31.9 (25.0–38.9)	58.8 (50.0–68.1)	71.9 (60.8–83.1)
Triglycerides			
(mmol/L)	0.83 (0.65–1.01)	1.53 (1.30–1.77)	1.87 (1.58–2.16)
(mg/dl)	72.2 (56.5–87.8)	133.1 (113.0–153.9)	162.6 (137.4–187.8)

supplied to the participating laboratories and using the same lot of IQCs as internal controls.

For assessing the external quality assurance programme, the values obtained from the participating laboratories were compared and analysed for variance (variance indicated the result of a particular laboratory compared against the mean of all participating laboratories). The calculation of variance was by the method described by Whitehead.[6] Briefly, for each distribution the mean and standard deviation (SD) of all laboratories was calculated for the analytes. Results outside two SD limits were excluded and the mean, SD and CV were recalculated. Variance was estimated by subtracting the calculated mean of all laboratories from the actual result of the participating laboratory and dividing this by the CV. If the variance was <1, a score of '0' was assigned, if it was between 1 and 2 a score of '1' was assigned with a further corresponding increase in the score depending on the recalculated variance. The variance index was arrived at by dividing the cumulative score (total score accumulated) with the number of participating laboratories. A lower variance index indicated a good performance by the participating laboratory for the given analyte.

Statistics

Statistical analysis was done using SPSS software (version 11.5, Chicago Inc.). For the 10% samples, re-analysis frequency distribution was calculated and overlapping plots were prepared to compare values of the coordinating centre and the participating laboratories. As the SD of values was high the data were log transformed and the geometric mean was calculated. The mean differences between the two laboratories were compared by calculating intra-class coefficient. Technical error (TE) was computed as ($\Sigma d^2/2N$) where d is the within pair difference and N is the number of split pair samples. For calculation of per cent TE, the TE was multiplied by 100 and the result was divided by mean of all samples. Within pair median CV and overall bias was also computed for replicate analysis for the three analytes.

RESULTS

Internal quality control

The intra- and inter-assay CV which was initially established by each laboratory using three levels of IQCs before the start of the study for the three analytes was <2% and <3%, respectively for cholesterol and <2.5% and 3.5% for triglycerides and HDL cholesterol. The performance of the participating laboratories in the internal quality control as assessed by Levy–Jennings plots were satisfactory with (i) at least 2 levels falling within 2 SD limits 95% of times and (ii) 3 levels falling within 2 SD limits 90% of times (data not shown). Westgard rules were followed for rejection of runs.

External quality assessment

External quality control samples sent during the entire duration of the study had a range of values: 2.75–6.99 mmol/L for cholesterol and 0.95–3.45 mmol/L for triglycerides. Figure 1 shows the mean variance index of all laboratories for total cholesterol and triglycerides for the 12 distributions during the standardization period (6 months). The mean index score was computed from the fifth distribution onwards as the participation of laboratories was low in the first 4 distributions. The mean variance index score was 0.86 in the fifth distribution which dropped to 0.73 by the twelfth distribution for total cholesterol and from 0.73 to 0.53 for triglycerides. For HDL cholesterol, the stability of external quality control material was a concern and therefore was not analysed.

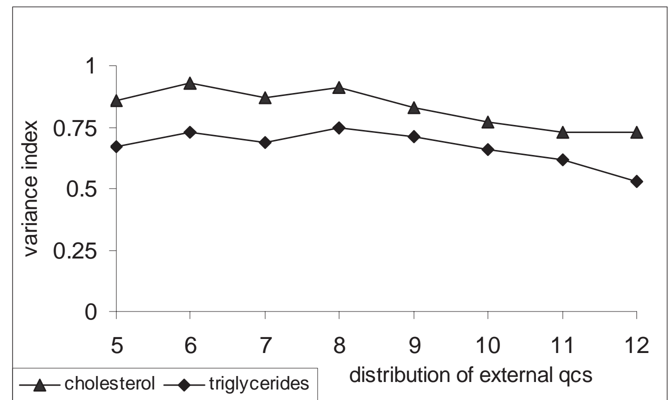

FIG 1. Mean variance index during the first 6 months of the study
qcs quality control sample

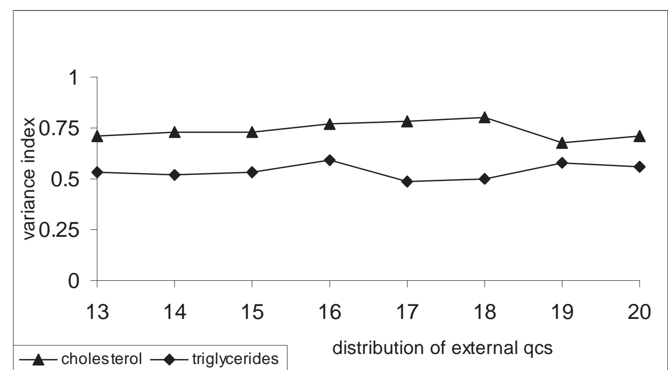

FIG 2. Mean variance index during the following year
qcs quality control sample

Figure 2 shows the mean variance index from the thirteenth to twentieth distribution. The mean variance index remained around 0.75 and 0.55 for cholesterol and triglycerides, respectively during the subsequent 1 year showing a consistency in performance.

Replicate analysis

Table II gives the results of replicate analysis of 295 samples in the coordinating laboratory and the participating centres (includes samples from all centres) collected in 1 year after standardization of the laboratories. The ICC (intra-class correlation coefficient) between the coordinating laboratory and the participating laboratories were >0.90 for all 3 parameters suggesting a good homogeneity of results. The per cent TE in the replicate pair analysis was 6.01 for cholesterol. Similarly for triglycerides and HDL cholesterol the per cent TE were 10.77 and 6.15, respectively. The median TE was comparable to the per cent TE. The overall inter-laboratory bias was 2.94%, 7.95% and 3.77% for cholesterol, triglycerides and HDL cholesterol, respectively. The range of bias was: 0.4%–6.9% for cholesterol values, 2.7%–13% for triglycerides and 0.95%–4.9% for HDL cholesterol.

Figure 3 shows the frequency distribution of 295 serum samples analysed in the coordinating laboratory and the participating laboratories. There was a good correlation between values from the participating laboratories and the coordinating laboratory as is evident from the overlap for total cholesterol, triglycerides and HDL cholesterol.

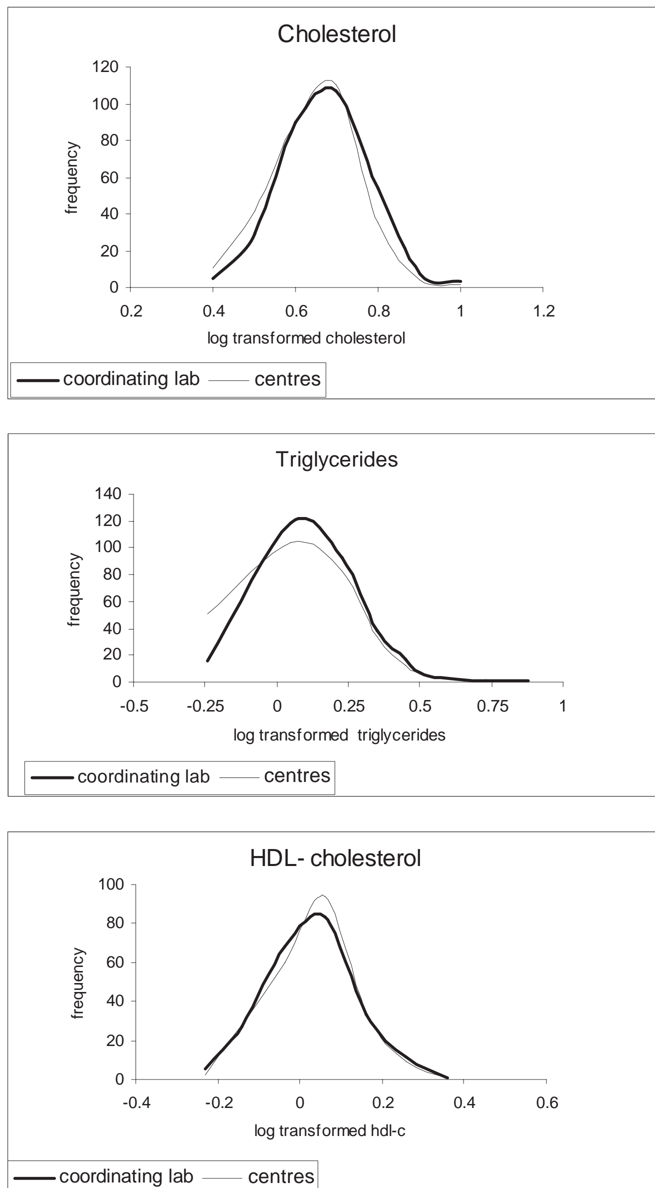

FIG 3. Frequency distribution curves of the centres and coordinating laboratory for the three analytes (10% replicate analysis, *n*=295)

DISCUSSION

Most multicentre epidemiological studies involving laboratory analysis have a single central laboratory doing the analyses.[7,8] Phlebotomy and blood processing is generally done at field centres and training is provided to ensure centre to centre comparability. We attempted to standardize 10 different laboratories to develop a model for the ongoing study as well as for future multicentre studies.

The quality assurance evaluation consisted of a periodic review of Levy–Jennings plot of internal control, trend analysis of external quality assessment materials and replicate studies to calculate correlation coefficient and TE. IQC is a reliable measure of day-to-day consistency whereas external quality control assesses bias and is especially useful in the evaluation of between laboratory comparability. Both types of controls are complimentary and need to be included in any epidemiology study.[9] Validation of individual laboratories by international agencies such as UK-NEQAS would have necessitated considerable expenditure and was not a viable proposition for us in India. In addition, using external validation also impedes local capacity enhancement. An improvement in the mean variance index of all participating centres for cholesterol and triglyceride analysis in the external quality control samples during the first 6 months shows that a validated laboratory can coordinate a cost-effective external quality assessment programme in large multicentre studies being done in developing countries such as India. For the third measure of quality assurance, the replicate analysis, unlike other studies where blind duplicate samples are analysed in the same laboratory, we did repeat analysis in two different laboratories. Estimates of precision based on replicates of samples analysed in two different laboratories are expected to be higher than internal and external controls as the assessment includes in addition to precision of individual laboratories, within pair differences which could arise due to specimen handling such as mislabelling in the field and problems of transportation.

The per cent TE, mean and median CV have been used as measures of precision of replicate analysis in various studies. In the INTERMAP study[10] where precision of microalbumin estimation was evaluated by both TE and CV, the authors suggested that median CV may provide a more accurate assessment of analytical precision. In our study median CV was comparable with per cent TE. The cardiovascular health study[11] employed replicate sample analysis as a quality assurance measure and the

TABLE II. Results of replicate analysis of cholesterol, triglycerides and high density lipoprotein cholesterol

Analyte	Laboratory median (Interquartile range)		Median difference within pair	Intra-class correlation coefficient	Technical error (%)	Median CV (%)	Bias (%)
	Coordinating	Participating					
Cholesterol (mmol/L)	4.47 [171.9] (3.79–5.17) [145.8–198.8]	4.39 [168.8] (3.74–5.02) [143.8–193.1]	0.078 [3.1]	0.904	6.01	7.01	2.94
Triglycerides (mmol/L)	1.33 [115.7] (0.97–1.78) [84.3–154.8]	1.17 [101.7] (0.81–1.72) [70.4–149.6]	0.136 [14.0]	0.955	10.77	12.66	7.95
High density lipoprotein cholesterol (mmol/L)	0.98 [37.7] (0.83–1.16) [31.9–44.61]	1.04 [40.0] (0.89–1.19) [34.2–45.8]	–0.052 [–2.3]	0.907	6.15	8.00	3.77

Values in square brackets are in mg/dl

per cent TE reported for cholesterol, triglycerides and HDL cholesterol in the study was similar to that observed by us in the latter 1 year of the study. A similar improvement in analytical performance of two participating laboratories measuring total cholesterol, triglycerides and HDL cholesterol in quality control samples was shown in the DAIS study.[12] In the KRIS study[13] where two laboratories were responsible for lipid analysis an inter-laboratory bias of 7% was reported initially which later dropped to 2.1%. Our results demonstrate a similar inter-laboratory bias in the latter 1 year of the study with respect to all the three analytes. We adopted similar measures of accuracy and precision as other studies but our study is different in that the lipid analyses were done at 10 geographically distant laboratories. Our purpose was to build local capacities and standardize measurements with inputs from the coordinating laboratory, which we achieved at all the participating laboratories.

Our study has limitations; first, we do not have details on the delay in processing, errors in labelling at respective centres and the delay in transportation. These could have contributed to bias in the replicate analysis. Although a detailed protocol for blood sampling, processing, storage and transportation was prepared and circulated before the study and also training workshops were organized, such errors are likely to occur in large multicentre studies especially involving 10 centres for sample collection and analysis. However, this could have resulted in a larger bias and does not negate the conclusion drawn that replicate analysis and external quality control programme contributed towards improvement of the analytical performance of the participating laboratories. Another limitation of our study was the use of lyophilized material as an external quality assessment because of which we could not stabilize HDL cholesterol measurement in the external quality assessment samples.

In spite of these lacunae, we have demonstrated the feasibility of establishing quality assurance at 10 different laboratories with varying expertise and infrastructure through simple and low cost measures. In conclusion, running IQC and external quality assessment helped in addressing pre-analytical, analytical and post-analytical variations in cholesterol, triglyceride and HDL cholesterol measurement and gave optimal quality assurance of laboratory measurement in the context of a multicentre epidemiological study. Sustainability of the laboratories in terms of quality has been retained by the laboratories as is evident from their mean variance index as well as 10% repeat analysis during the subsequent year of the study. This model, we believe, could be replicated to ensure quality assurance of biochemical laboratories in developing country settings where cost is an important consideration and also in large multicentre studies.

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