Impact of external haematology proficiency testing programme on quality of laboratories

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Received June 19, 2006

**Background & objectives:** A reliable and reproducible report from a laboratory needs internal quality control within the laboratory and participation in external proficiency testing programmes (EPTP). This study conducted at the Department of Haematology, All India Institute of Medical Sciences (AIIMS), New Delhi, which has been conducting an EPTP since 1992, was undertaken to assess the efficacy of this programme in improving the performance of participating laboratories in reporting test samples sent for Hb, total leucocyte count (TLC), reticulocyte count and assessment of peripheral blood smear (PBS).

**Methods:** The samples were prepared in our laboratory according to the International Standards Organization (ISO) guidelines. The performance of individual laboratories was assessed using robust Z score, which is an indicator of acceptability of the test result.

**Results:** An improvement in the overall percentage of laboratories with acceptable reports was seen during the study period. It has increased from 38, 40, 40 per cent in 1992 to 85, 90, 94.7 per cent in 2006 for Hb, TLC, reticulocyte count, respectively. However, the results for peripheral smear assessment improved only marginally.

**Interpretation & conclusions:** The external haematology proficiency testing programme run by our department for Hb, TLC, reticulocyte count, and peripheral blood smear assessment, has helped in improving the reporting standards of these parameters in Indian laboratories.

**Key words** Haemoglobin - peripheral blood smear - proficiency testing - reticulocyte count - total leucocyte count

Quality control is a systematic method that ensures any laboratory to maintain the standard and reliability in reporting a test result. It aims at achieving internal as well as external quality control. Quality assessment (QA) includes application of objective statistical methods to assess the precision and/or accuracy of a test result. Precision testing constitutes internal quality control and is assessed using control charts, or duplicate testing of patient samples. External quality assessment (EQA) or proficiency testing (PT) programmes assess accuracy of a test result. A proficiency testing programme is conducted by a competent referral laboratory, and involves distribution of test samples to participating laboratories and assessment of their performance on return of the test results by means of inter-laboratory tests at the nodal laboratory.
With accreditation by National Accreditation Board for Laboratories (NABL) becoming mandatory, these quality control test programmes are fast becoming an integral part of the day-to-day laboratory practice. World Health Organization (WHO) initiated an international QC programme in haemogram in 1987 in which many international and national haematological laboratories from both developing and developed countries participated. These programmes need a sustained financial support, technical expertise and dedicated human resource.

The Department of Haematology, All India Institute of Medical Sciences (AIIMS), New Delhi, has been conducting an external proficiency testing programme (EPTP) for parameters like haemoglobin (Hb), total leucocyte count (TLC), reticulocyte count and peripheral blood smear (PBS) examination with nationwide laboratories as participants since 1992. There were 38 participants in 1992, which gradually increased to 401 in 2006. This study was carried out to assess the efficacy of this programme in improving the performance of participant Indian laboratories in reporting parameters like Hb, TLC, reticulocyte count, and peripheral blood smear assessment.

Material & Methods

General awareness was created in practicing government and private laboratories through lectures, pamphlets, hands on Wet workshops were done periodically from 2003-07 and advertisements in various Indian journals. Laboratories that volunteered to participate in the quality assurance programme were considered in this study. The parameters evaluated included haemoglobin, reticulocyte count, total leucocyte count (TLC), and peripheral blood smear (PBS).

Haemolysate for haemoglobin estimation, fixed avian red cells for TLC, PBS and reticulocyte count for manual and mechanical testing were prepared in our laboratory. All the samples prepared and obtained were in accordance with WHO standards and specifications. Preparation of haemolysate: A unit of packed red blood cells was obtained from blood bank. After separation of plasma, RBCs were washed in normal saline. Carbon tetrachloride was added and then shaken for one hour, and stored in refrigerator overnight. It was centrifuged, after which a button of debris was formed at the bottom of the container with an overlying layer of carbon tetrachloride. Haemolysate was carefully pipetted out. Stability of haemolysate was maintained by addition of preservatives and antibiotics. The preparation was transferred into small vials which were sealed to make them air proof before dispatching to participants. The same batch of specimen was kept at the ambient temperature in the laboratory for about 7-8 days after the dispatch and was processed at different intervals to check its stability and homogeneity.

Preparation of sample for WBC counts by fixed avian red cells: Blood from a single chicken was obtained and plasma was discarded. Sample was then centrifuged which separated the cells into discrete layers. Cells were then fixed in glutaraldehyde and dissolved in normal saline. Required amount of preservative and antibiotic was then added. After adjusting the counts the preparation was mixed with the haemolysate.

Preparation of peripheral smear: Three ml of blood was drawn in EDTA vial and required number of peripheral smears were made and fixed in methanol immediately. Slides were dispatched as either unstained after methanol fixation or as stained with Jenner’s Giemsa.

Preparation of smear for reticulocyte counts: Three ml of blood was mixed with equal volume of solution made of brilliant cresyl blue in normal saline and incubated at 37°C for 15 min and smears were made.

Testing for stability and homogeneity: In a proficiency testing (PT) programme, the test material distributed to various laboratories must be homogenous and stable; otherwise any differences observed between laboratories may not be real or genuine. It is important that these aspects are taken care of at the time of selection, procurement, preparation and distribution of material. In our programme, randomly picked up vials from the dispatch lot were kept at 4°C, and subjected to manual and automated counter tests for eight consecutive days to check for stability. Analysis of variance technique (ANOVA) was used for examining homogeneity of test material and stability was tested using F ratio as statistical methods.

Dispatch of samples: Samples were transported by courier service in cardboard boxes with cool packs along with instructions to keep the specimens at 4°C immediately after receiving them and process them as early as possible and to send the results within ten days.

Frequency of distributions/year: The number of distributions of sample per year was four at three-month intervals.
Methods of evaluation:

Robust Z score for assessment of results between laboratories - Pairs of results were obtained from the participants laboratories and the robust inter-laboratory Z scores were calculated by the formula $Z = \frac{S(A+B) - \text{Median}(S)}{\text{NIQR}}$. Where $A$ is the 1st value and $B$ is the second value thus $A+B$, is the sum of paired results of a laboratory, Median $(S)$ is the central value of the respective parametric results of all the participants. NIQR is normalized interquartile range calculated by the formula, $0.7 \times \text{interquartile range (IQR)}^3$.

Interpretation of Z scores - The calculated Z scores were tabulated in the report along with the corresponding results and the results were assessed based on their Z score. The results of Z score was interpreted, by standard method\(^3\).

- $Z < 2 = \text{Satisfactory}$
- $Z > 3 = \text{Unsatisfactory}$

An outlier was defined as any result/pair of results with an absolute Z score greater than 3 \(i.e.,~Z>3 \text{ or } Z<-3\). The outlier criterion $|Z| > 3$ had a confidence level of about 99.7 per cent indicating that there was less than 1 per cent chance that the result was a true member of the population and it was far more likely that there was a problem with this/pair of results. Laboratories which had a Z score in between 2 and 3 were encouraged to take a close look at their results. The sign of the values of Z score is also important. The $Z>3$ indicated that both results for pair were too high. Similarly a negative value ($-3$) indicated that the results were too low.

The evaluation of the performance of each participant laboratory was communicated to the participants along with suggestions regarding corrective measures to those participants whose performance scores were not satisfactory and encouraged persistent outliers to attend the workshops organized by our department.

A feedback on the suitability of test samples at the time of receipt was obtained through feedback proformae sent along with the samples.

Evaluation of peripheral smear assessment - As the peripheral smear examination is based on morphology, it is subjective. The result was therefore considered satisfactory as long as it did not grossly deviate from the true diagnosis required for clinical management.

Results

A significant increase in the number of participant laboratories was observed from 38 in 1992 to 401 in 2006. Number of participant laboratories in 1992 was 38. Nine more participants joined in 1995 \(i.e.,\) there was an increase of 23.7 per cent from 1992 to 1995 in EQAPT programme at the AIIMS. There were 17 more participants in 2001 \(i.e.,\) 44.7 per cent increase and more than 10-fold increase was observed in 2006 as compared to 1992 (Table I).

The change of PT programme over the period of time region-wise was analyzed by using chi square trend. There was an increasing linear trend in south (0.7816), no significant difference in central part (0.37), whereas a significant linear trend (decreasing) was observed in north-east (<0.001) and west region (<0.001). The numbers of participants southern, northern and western region in 2006 were significantly different ($P = 0.04$).

At the start of the programme in 1992, a majority of laboratories employed manual (74%) and semi-automated methods (21%). Of the 38 laboratories joined for EQAPT in 1992, 20 were found to be the same (53%) and have shown improvement in quality of reporting of Hb and TLC. In 2006, nearly 90 per cent laboratories were doing the haemogram parameters (Hb, TLC) by automated methods (Table II). Most commonly used automated counters were Sysmex (151, 42%), Beckman Coulter (43, 12%) and Cell dyne (14, 3.8%). No information regarding the type of automated counter used by the rest of the participants was available. Of the 401 participants, 380 were private laboratories and 21 were government institutes. There were 83 NABL accredited participants in 2006. The outliers were maximum in the manual group and least in the automated group (Table III). Moreover, the percentage

<table>
<thead>
<tr>
<th>Region</th>
<th>1992 (n=38)</th>
<th>1995 (n=47)</th>
<th>2001 (n=55)</th>
<th>2006 (n=401)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South</td>
<td>9(23)</td>
<td>14(29.7)</td>
<td>17(30.9)</td>
<td>132(33)</td>
</tr>
<tr>
<td>North</td>
<td>21(55)</td>
<td>22(46.8)</td>
<td>26(47.2)</td>
<td>125(31)</td>
</tr>
<tr>
<td>East</td>
<td>4(11)</td>
<td>4(8.5)</td>
<td>5(9.0)</td>
<td>29(7)</td>
</tr>
<tr>
<td>West</td>
<td>4(11)</td>
<td>6(12.7)</td>
<td>6(10.9)</td>
<td>105(26)</td>
</tr>
<tr>
<td>Central</td>
<td>4(11)</td>
<td>1(2.1)</td>
<td>1(1.8)</td>
<td>10(2.5)</td>
</tr>
</tbody>
</table>

Figures in parentheses are percentages

Overall chi square =21.87, $P =0.04$
of laboratories reporting acceptable results over this period. This can be attributed to an increased awareness of participants achieved through conducting annual/biannual workshops organized by our department where special emphasis was given to explain the advantages of usage of cost-effective automated counters, importance of effective and optimal training of the personnel, distribution of state-of-art standard operating procedure manuals to the participants and also by encouraging new participants. Also, the laboratories became more conscious of producing reliable results to ensure an accreditation from NABL, a statutory authority entrusted with maintaining the quality of laboratory results in the country. A majority of our participants exercised internal quality control methods that suited their laboratory.

Parameter-wise performance of participating laboratories was also assessed by acceptable Z score of 0-2 over four time points. A consistent increase in proportion of participants with acceptable performance was observed for all the four parameters. While the improvement in reporting of Hb, TLC, and reticulocyte counts was substantial, only a marginal improvement was observed in peripheral blood smear assessment (Fig.).

**Discussion**

The successful run of this programme, the first of its kind in India over 14 yr has been reflected by the consistent increase in proportion of participation by laboratories from all regions of the country and also a consistently increasing proportion of laboratories reporting acceptable results over this period. This can be attributed to an increased awareness of participants achieved through conducting annual/biannual workshops organized by our department where special emphasis was given to explain the advantages of usage of cost-effective automated counters, importance of effective and optimal training of the personnel, distribution of state-of-art standard operating procedure manuals to the participants and also by encouraging new participants. Also, the laboratories became more conscious of producing reliable results to ensure an accreditation from NABL, a statutory authority entrusted with maintaining the quality of laboratory results in the country. A majority of our participants exercised internal quality control methods that suited their laboratory.

At the start of the programme in 1992, there were a higher proportion of outliers who used manual methods for testing. With a large number of laboratories using automated counters in 2006, the proportions of outliers have decreased emphasizing the efficacy of automated counters. There were nine (2%) persistent outliers defined as those participants who had poor robust Z scores >3 for six consecutive distributions whose scores when added and multiplied by 6 was more than 1004.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1992 (n=38)</th>
<th>2006 (n=401)</th>
<th>1992 n(%)</th>
<th>2006 n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb/TLC</td>
<td>28(74)</td>
<td>34(8.5)</td>
<td>8(29)/8(29)</td>
<td>11(32)/13(38)</td>
</tr>
<tr>
<td>Semi-automated</td>
<td>8(21)</td>
<td>6(2)</td>
<td>5(62.5)/6(75)</td>
<td>5 (83)/6(100)</td>
</tr>
<tr>
<td>Automated</td>
<td>2(5)</td>
<td>36(90)</td>
<td>2(100)/2(100)</td>
<td>325(90)/342(95)</td>
</tr>
</tbody>
</table>

Fig. Parameter-wise performance status of participant laboratories.
participation was little in this programme. As is evident from this study, the improvement in acceptability of peripheral smear assessment was marginal. With the increasing use of high-end counters in some high-volume laboratories, these problems can also be partially taken care of.

In conclusion, the PT programme for Hb, TLC, reticulocyte counts and peripheral blood smear assessment run by the AIIMS, New Delhi, has been successful in generating awareness about haematology proficiency testing and improving the quality of reporting of these parameters in Indian laboratories.

References


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