ERNDIM - Quantitative Schemes
Cystine in White Blood Cells

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Annual Report ERNDIM-EQAS 2013

1. Purpose

The purpose of the ERNDIM External Quality Assurance Scheme for Cystine in White Blood Cells is the monitoring of the analytical quality of the quantitative assay of cystine in white blood cells in the management and diagnosis of patients with cystinosis. For details see www.erndimqa.nl

2. Participants

36 Sample sets have been shipped to laboratories from 18 countries.

3. Design

The Scheme has been designed, planned and co-ordinated by Dr. Mick Henderson as scientific advisor and Dr. Cas Weykamp as scheme organizer (subcontractor on behalf of SKML), both appointed by and according to the procedure of the ERNDIM Board. The design includes special attention to sample composition and to the layout of the reports.

Samples

The scheme consisted of 2 series of lyophilised samples: one series containing protein pellets and the other supernatants of lysed white blood cells spiked with cystine. As can be seen from table 1 the weighed amounts of protein and cystine were identical in pairs of samples. The nature, source and added amounts of the analytes are summarised in table 1.

Table 1. Pair identification, source and amount of added analytes.

Analyte	Source	Added Quantities Protein (mg/vial)+Cystine (nmol/vial)			
		Sample Pair 85 - 90	Sample Pair 86 - 89	Sample Pair 87 - 92	Sample Pair 88 - 91
Protein	Instruch. 11930	1.00	1.50	2.00	0.50
Cystine	Sigma C8755	0.00	6.00	2.00	4.00

Reports

All data-transfer, the submission of data as well as request and viewing of reports proceeded via the interactive website www.erndimqa.nl The results of your laboratory are confidential and only accessible to you (with your name and password). The

anonymised mean results of all labs are accessible to all participants. Statistics of the respective reports are explained in the general information section of the website.

An important characteristic of the website is that it supplies short-term and long-term reports.
Short-term reports on the eight individual specimens are available two weeks after the submission deadline and provide up-to-date information on analytical performance. Although technically reports could be immediately available a delay time of 14 days has been introduced to enable the scientific advisor to inspect the results and add his comment to the report.
The **annual long-term report** summarises the results of the whole year.

A second important characteristic of the ERNDIM website is the different levels of detail of results which allows individual laboratories the choice of fully detailed and/or summarised reports.
The “Analyte in Detail” is the most detailed report and shows results of a specific analyte in a specific sample.
A more condensed report is the “Current Report” which summarises the performance of all analytes in a specific sample.
The Annual Report summarizes all results giving an indication of overall performance for all analytes in all 8 samples.
Depending on the responsibilities within the laboratory participants can choose to inspect the annual report (QC managers) or all (or part of) detailed reports (scientific staff).

4. Discussion of Results in the Annual Report 2013

In this part the results as seen in the annual report 2013 will be discussed. Please print out your annual report from the website when you follow the various aspects below and keep in mind that we only discuss the results of “all labs”. It is up to you to inspect and interpret the results of your own laboratory.

4.1 Accuracy

A first approach to evaluating your performance in terms of accuracy is comparison of your mean values in the eight samples with those of all labs. This is shown in the columns "your lab" and "all labs" under the heading "Accuracy”. For example for protein the mean of all labs is 1.13 mg/vial, with which you can compare the mean of your lab.

4.2 Recovery

A second approach to describe accuracy is the percentage recovery of added analyte. In this approach the amounts of weighed quantities added to the samples are the assumed target values after adjustment for blank values. The correlation between weighed amounts (on the x-axis) and your measured quantities (on the y-axis) has been calculated. The slope of the resulting relationship (a in $y = ax + b$) in this formula multiplied by 100% is your recovery of the added amounts. The outcome for your lab in comparison to the median outcome of all labs is shown in the column “Recovery”.
It can be seen that the mean recovery of cystine is 107% and of protein is 86%, which is excellent and very reassuring. We are all measuring the same thing.

4.3 Precision

Reproducibility is an important parameter for the analytical performance of a laboratory and is addressed in the schemes’ design. Samples provided in pairs can

be regarded as duplicates from which CV's can be calculated. The column "Precision" in the annual report shows your CV's in comparison to median values for all labs. The best median CV is observed for protein (6.2%). 8.0% and 14.6% are seen for cystine and cystine (nmol ½ cys/mg protein), respectively.

4.4 Linearity

Linearity over the whole relevant analytical range is another important parameter for analytical quality and is also examined within the schemes. A comparison of the weighed quantities on the x-axis and your measured quantities on the y-axis allows calculation of the coefficient of regression (**r**). The column "Linearity" in the annual report shows your **r** values in comparison to the median **r** values for all labs. Ideally the **r** value is close to 1.000 and this is indeed observed with values between 0.991 and 0.994 for the respective analytes.

4.5 Interlab CV

For comparison for diagnosis and monitoring of treatment for one patient in different hospitals and for use of shared reference values it is essential to have a high degree of harmonization between results of laboratories. Part of the schemes' design is to monitor this by calculating the Interlaboratory CV. This, along with the number of laboratories who submitted results is shown in the column "Data all labs" in the annual report. We see an interlab CV of 14.1% for protein and of 26.1% for cystine (nmol ½ cys/mg protein).

4.6 Number of participating labs and submitting results

36 labs received specimens, of which 3 did not submit results and 2 did not submit sufficient results to allow calculation of the annual report.

4.7 Interrelationships between results

Cystine (nmol ½ cys/mg protein) is a ratio of the assays of cystine (nmol/aliquot) and protein. The precision will be the cumulated precision of both assays.

4.8 Report in correct numbers

As we have indicated in previous reports it is important to report in the correct units. Although we feel that nearly all labs do that now, some strange results of individual labs might be traced back to "clerical errors". So if you have a deviating result, please check if you reported your result in the correct units.

4.9 Your performance: red and green flags

After some years of discussion and planning a system to judge performance of individual laboratories is implemented starting from January 2009. In the annual report of an individual laboratory red flags indicate poor performance for accuracy, precision, linearity and recovery. Amino acids with satisfactory performance for at least three of the four parameters (thus no or only one red flag or no result) receive a green flag. Thus a green flag indicates satisfactory performance for analysis of that particular amino acid while a red flag indicates that your laboratory has failed to attain satisfactory performance. Criteria for red flags can be found in the general information on the website (general information; interactive website, explanation annual report).

4.10 Poor Performance Policy

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of red flags observed. 68% of the laboratories have no red flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 10% of laboratories with more than 25% red flags.

Following intensive discussion within the ERNDIM board and Scientific Advisory Board (SAB) and taking into account feedback from participants we have been able to agree on a harmonised scoring system for the various branches of the Diagnostic Proficiency schemes and qualitative schemes. We have also tested a scoring system for the quantitative schemes as described in our Newsletter of Spring 2009. In parallel to this the SAB has agreed levels of adequate performance for all the schemes and these will be re-evaluated annually. The scoring systems have been carefully evaluated by members of the SAB and have been applied to assess performance in our schemes from 2007 onwards. The ERNDIM Board has decided that the Scientific Advisor will judge the performance of the individual laboratories based on these levels of satisfactory performance and issue a letter of advice of failure to achieve satisfactory performance to those laboratories which do not achieve satisfactory performance. The letter is intended to instigate dialogue between the EQA scheme organiser and the participating laboratory in order to solve any particular analytical problems in order to improve quality of performance of labs in the pursuit of our overall aim to improve quality of diagnostic services in this field.

Table 2. Percentage Red Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	10%	10%
20 – 25%	10%	20%
15 – 20%	3%	23%
10 – 15%	3%	26%
5 – 10%	6%	32%
0 – 5%	0%	32%
0%	68%	100%

4.10 Certificates

As for other schemes the performance as it is indicated by the red/green flags in the individual laboratories annual report is summarised in the new style of annual participation certificate. The certificate lists the total number of amino acids in the scheme, the number for which results have been submitted and the number for which satisfactory performance has been achieved. It is important to bear in mind that the certificate has to be backed up by the individual annual report in the case of internal or external auditing.

5. *Summary*

We feel that the scheme is well-established now. The mean performance of the labs, especially the recovery of added cystine and protein, is fine. Of course the performance of some individual labs require improvement. The Interlab CV demonstrates lack of standardisation which requires improvement. We would like to emphasise the need for all laboratories to use internal quality control. At its simplest this can be made from pooling surplus supernatants from assayed samples. We think that some of the aberrant results are still caused by simple calculating errors.

6. *Preview of the Scheme in 2014*

The design of the 2014-scheme is the same as in 2013.

7. Questions, Comments and Suggestions

If you have any questions, comments or suggestions please address to the scientific advisor of the Scheme, Dr. Mick Henderson (mick.henderson@leedsth.nhs.uk) and/or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).