ERNDIM
Quantitative Schemes
Cystine in White Blood Cells

ANNUAL REPORT 2021

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
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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*

A total of 36 datasets have been submitted and 2 laboratories did not submit any data at all

3. *Design*

The Scheme has been designed, planned and co-ordinated by Daniel Herrera as scientific advisor and Dr. Eline van der Hagen as scheme organizer (on behalf of the MCA Laboratory), all 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. As a subcontractor of ERNDIM, the MCA Laboratory prepares and dispatches EQA samples to the scheme participants and provides a website for on-line submission of results and access to scheme reports.

Samples

The scheme consisted of two sets of lyophilised samples: one set containing 8 samples protein pellets and the other 8 samples supernatants of lysed white blood cells spiked with cystine. As can be seen from table 1, the weighed amounts of

[1] If this Annual Report is not Version 1 for this scheme year, go to APPENDIX 1 for details of the changes made since the last version of this document

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 2021. 01 - 05	Sample Pair 2021. 02 - 08	Sample Pair 2021. 03 - 07	Sample Pair 2021. 04 - 06
Protein	Sigma P8119	0.8	1.8	1.2	0.40
Cystine	Sigma 49603	2.00	0.125	1.00	1.50

Reports

All data-transfer, the submission of data as well as request and viewing of reports take place 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 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 can 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** summarizes 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 2021

In this part the results as seen in the annual report 2021 will be discussed. Please keep at hand 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 0.956 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 (nmol/aliquot) is 98% and of protein is 89%. The lower recovery in the protein assay may reflect the lack of standardization in the protein assays.

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 the mean value for all labs. The mean CV for protein is 7.5% and for cystine (nmol/aliquot) is 13.3%.

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 a value of 0.986 for Cystine (nmol/aliquot) and 0.991 for Protein.

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 18.6% for protein, 15.4% for cystine (nmol/aliquot) and of 26.3% for cystine (nmol ½ cys/mg protein).

4.6 Interrelationships between results

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

4.7 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.

4.8 Your performance: Flags

In order to easily judge performance of individual laboratories the annual report of an individual laboratory may include flags (in different colours) in case of poor performance for accuracy, precision, linearity and recovery. Analytes with satisfactory performance for at least three of the four parameters (thus no or only one flag) receive a green flag. Thus a green flag indicates satisfactory performance for analysis

of that particular analyte. Criteria for flags can be found in the general information on the website (on this website under general information; interactive website, explanation annual report).

4.9 Poor Performance Policy

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of flags observed. 61% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 5% of laboratories with more than 25% 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 and 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 Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	5%	6%
25%	3%	8%
20 – 25%	0%	8%
15 – 20%	14%	22%
10 – 15%	0%	22%
5 – 10%	17%	39%
0 – 5%	0%	39%
0%	61%	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 annual participation certificate. The certificate lists the total number of analytes 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.

4.11 Additional Specific Remarks of the Scientific Advisor

This year the scheme has piloted again the introduction of clinical information and interpretation of the results. A summary of the results of the interpretative part of the scheme for 2021 is presented below:

Distribution 2021.01. Clinical information: 3 months old presenting with polidipsia, failure to thrive

The median cystine concentration (all laboratories) for this distribution was 5.66 nmol ½ cystine / mg protein and clearly consistent with nephropathic cystinosis. All laboraries agreed that this result was consistent with nephropatic cystionosis. One laboratory had to set methods, measured the concentration correctly but selected the wrong option for one of the method sets.

The laboratories are using different reference ranges and cut-off values for nephropatic cystinosis, ranging from greater than 0.2 nmol ½ cystine / mg protein to greater than 5.0 nmol ½ cystine / mg protein. Laboratories measuring cystine in granulocytes use greater cut-off values than the laboratories using mixed-leucocytes. Ilya et al, Clinical Chemistry 62:5, 766–772 (2016) provides details of the relationship between cystine in granulocytes and mixed-leucocytes and it is an informative publication to read.

The majority of laboratories use cut-off values between 1.0 - 2.0 nmol ½ cystine / mg protein as consistent with nephropatic cystinosis but there are a few laboratories that use much higher cut-off values between 3.0-5.0. Those laboratories should review their cut-off concentrations especially if cystine is measured in mixed-leucocytes

Distribution 2021.02. Clinical information: 45 years old, bilateral crystaline keratopathy, no evidence of renal disease and/or proteinuria.

The median cystine concentration (all laboratories) for this distribution was 0.17 nmol ½ cystine / mg protein. 94 % of the participants agreed that the concentration for this distribution was not consistent with ocular cystinosis or it was consistent with carrier status. Two laboratories selected the option consistent with ocular cystinosis, one of them measured correctly the cystine concentration and made a selection error while the other laboratory measured the cystine concentration at 0.72 nmol ½ cystine / mg protein This laboratory was discussed in detail at the SAB meeting and it was agreed not to be classified as critical error as this initial result would be followed-up in a real setting by a subsequent repeat sample and DNA analysis of the CTNS gene.

Cystine concentrations in ocular cystinosis are generally lower than in classic nephropatic cystinosis with more overlap between carriers and affected patients and values ranging from 0.6-3.0 nmol ½ cystine / mg. These clinical scenarios are more challenging for the laboratories and it is not possible biochemically to distinguish between carriers and affected individuals.

One particular laboratory measuring cystine in granulocytes considers suspicious concentrations over 1.0 nmol ½ cystine / mg protein (equivalent to 0.5 nmol ½ cystine / mg protein using mixed-leucocytes) but it states that patients usually falls between 1-3 based on publicated data (Ocular nonnephropathic cystinosis : clinical, biochemical, and molecular correlations ; Pediatr Res ; 2000 Jan ; 47(1):17-23. PMID : 10625078). However, in this paper it is not clear if cystine is measured in granulocytes or mixed-leucocytes.

In another laboratory, in a patient with suspicion of ocular cystinosis, any value exceeding a cut-off of 0.5 nmol ½ cys / mg protein in isolated granulocytes would prompt them to perform further investigations into a possible diagnosis through CTNS genetic analysis. This cut-off value is based on Gertsman et al, Clinical Chemistry 62:5, 2016, 766-772.

Distribution 2021.03. Clinical information: 6 years old CKD cystinosis on QID cysteamine treatment. Sample taken 5-6 hours after last dose

The median cystine concentration (all laboratories) for this distribution was 1.80 nmol ½ cystine / mg protein. 94 % of the participants considered that the concentration for this distribution was above the desirable target treatment.

Most of the laboratories agreed that the desired concentration should be in the heterozygous range (less than 1.0 nmol ½ cystine / mg protein when using mixed-leucocytes and less than 2.0 nmol ½ cystine / mg protein when using granulocytes). A number of different sources for treatment target concentrations were provided by some laboratories which may be of benefit for all the participants.

(2014 consensus document on nephropatic cystinosis (Nephrol Dial Transplant (2014) 29: iv87–iv94 doi: 10.1093/ndt/gfu090)

Nephropathic cystinosis: an international consensus document." Nephrology Dialysis Transplantation 29(suppl_4): iv87-iv94.

Clinical Chemistry 62:5, 2016, 766-772

Diagnosis and Treatment", 6th Edition. Saudubray, Baumgartner, Walter Eds.

Nephropathic Cystinosis: Symptoms, Treatment, and Perspectives of a Systemic Disease (2018) Volume 6 Article 58 Frontiers in Paediatrics Baumner and Weber Orphan Europe Pharmaceutical Company (2009)

P. Niaudet: Cystinosis, In J.-M. Saudubray et al. (Eds.), Inborn Metabolic Diseases, 6. Ed., 2016.

Ariceta et al., 2015. Cistinosis en pacientes adolescentes y adultos:recomendaciones para la atención integral de la cistinosis. NEFROLOGIA 35(3):304-321

Distribution 2021.04. Clinical information: Both parents carriers of the cystinosis CTNS gene . Sample taken at 1 month of life.

The median cystine concentration (all laboratories) for this distribution was 7.15 nmol ½ cystine / mg protein, clearly abnormal and consistent with nephropatic cystinosis presentation. 100 % of the participants (34/34) agreed that the concentration for this distribution was consistent with nephropathic cystinosis.

Distribution 2021.05. Clinical information: 16 months old, no clinical information provided

The median cystine concentration (all laboratories) for this distribution was 5.28 nmol ½ cystine / mg protein, clearly abnormal and consistent with nephropatic cystinosis presentation. 100 % of the participants (34/34) agreed that the concentration for this distribution was consistent with nephropathic cystinosis. It is reassuring to see that all the laboratories are identifying clear cases of nephropatic cystinosis.

Distribution 2021.06. Clinical information: 15 years old CKD cystinosis post renal transplant on QID cysteamine treatment. Sample taken 5-6 hours after last dose.

The median cystine concentration (all laboratories) for this distribution was 7.59 nmol ½ cystine / mg protein. 100 % of the participants considered that the concentration for this distribution was above the desirable therapeutic target.

Distribution 2021.07. 16 years old, Patient had photophobia and proteinuria.

The median cystine concentration (all laboratories) for this distribution was 1.84 nmol ½ cystine / mg protein. 91 % of the participants (29/32) considered that the concentration for this distribution was consistent with intermediate (late-onset) cystinosis. Three laboratories suggested that the concentration was more in agreement with carrier status but suggested further follow up by molecular analysis of the CTNS gene. These laboratories measured accurately the concentration of cystine in this distribution and at least one of these laboratories measured cystine in granulocytes. In this case a concentration below 2.0 nmol ½ cystine / mg protein may be well consistent with heterozygous status. Next year, we will be asking the laboratories to provide information about their white cell isolation protocol as this will aid to assess the performance of the laboratories.

The cut-off concentrations used in late-onset cystinosis varies between laboratories; it is lower than in nephropatic cystionosis and closer to the cut-off values used in ocular cystinosis. The cut-off concentrations provided by laboratories range from 0.4-1.0 nmol ½ cystine / mg protein with the majority of cases described in the literature having concentrations between 1-3 nmol ½ cystine / mg protein. Ideally, laboratories should establish the target cut-off concentrations for the different types of cystinosis based on their own methodology but in reality this is very difficult to achieve due to the small number of patients affected with this disorder.

Some of the literature sources provided by laboratories are shown below:

Schneider, J. A., et al. (1967). "Increased cystine in leukocytes from individuals homozygous and heterozygous for cystinosis." Science 157(3794): 1321-1322

Baumner S and Weber LT. Frontiers in Pediatrics. 2018; vol6;1:8

Cystinosis: practical tools for diagnosis and treatment (Wilmer et al, 2011) Paediatr. Nephrol Vol 26: 205-215

Wilmer et al. 2011 Pediatr Nephrol 26:205-215

Distribution 2021.08. Clinical information: 16 months old, poor weight gain

The median cystine concentration (all laboratories) for this distribution was 0.15 nmol ½ cystine / mg protein well below the range observed in cystinosis. 100 % of the participants agreed that the concentration for this distribution was not consistent with nephropatic cystinosis or consistent with carrier status. It is encouraging to see that low concentrations of cystine are measured well by laboratories and not unnecessary follow up it is required in these situations.

5. *Summary*

We feel that the scheme is well-established. The average performance of the labs is satisfactory but of course the performance of some individual laboratories requires improvement. The elevated Inter-laboratory CVs demonstrates lack of standardization which requires improvement. We would like to emphasize the need for all laboratories to use internal quality control. At its simplest this can be made from pooling surplus supernatants from assayed samples however we are considering to provide quality control material for the laboratories. We think that some of the aberrant results are still caused by simple calculating errors.

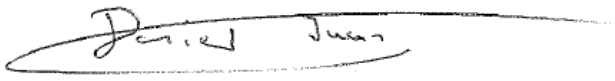
6. *Preview of the Scheme in 2022*

The design of the 2022-scheme is the same as in 2021, however from 2022, the interpretation score will be formally introduced. Laboratories are expected to participate in 6 out of 8 distributions with an score of at least 10 points out of 16 (2 points for correct interpretation, 0 points for incorrect interpretation) and not critical errors in order to attain satisfactory performance. The interpretation component will be scored and reflected in your yearly certificate.

7. Questions, Comments and Suggestions

If you have any questions, comments or suggestions please address to the scientific advisor of the Scheme Mr. D. Herrera (daniel.herrera@nhs.net) or the scheme organiser Dr. Eline van der Hagen (E.vanderHagen@skbwinterswijk.nl).

Leeds, 15 April 2022



Mr Daniel Juan Herrera
Scientific Advisor

Please note:
This annual report is intended for participants of the ERNDIM Cystine in White Blood Cells scheme. The contents should not be used for any publication without permission of the scheme advisor.

The fact that your laboratory participates in ERNDIM schemes is not confidential. However, the raw data and performance scores are confidential and will be shared within ERNDIM for the purpose of evaluating your laboratory performance, unless ERNDIM is required to disclose performance data by a relevant government agency. For details, please see the terms and conditions in the ERNDIM Privacy Policy on www.erndim.org.

APPENDIX 1. Change log (changes since the last version)

Version Number	Published	Amendments
1	25 Jan 2022	• 2021 annual report published
2	15 April 2022	• New ERNDIM logo, change of Administration Office address (heading) and change text footnote[1]

END