# ERNDIM
# Quantitative Schemes
# Cystine in White Blood Cells

# ANNUAL REPORT 2020


| 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, for 1 of them an annual report could not be generated due to insufficient data submission.

## 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 provide a website for on-line submission of results and access to scheme reports.

### *Samples*

The scheme consisted of 2 series of lyophilised samples: one series containing 8 samples protein pellets and the other 8 samples supernatants of lysed white blood

[1] If these scheme instructions are not Version 1 for this scheme year, go to APPENDIX 1 for details of the changes made since the last version of this document

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 2020. 01 - 06 | Sample Pair 2020. 02 - 05 | Sample Pair 2020. 03 - 07 | Sample Pair 2020. 04 - 08 |
| Protein | Instruch. 11930 | 0.25 | 0.50 | 1.05 | 1.40 |
| Cystine | Sigma 49603 | 0.04 | 0.35 | 1.00 | 2.50 |

### *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 2020*

In this part the results as seen in the annual report 2020 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 0.767 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 93%, which is 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 the mean value for all labs. The mean CV for protein is 5.0% and for cystine (nmol/aliquot) is 13.9%.

### *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 0.995 for Cystine (nmol/aliquot) and 0.997 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 15.2% for protein, 23.8% for cystine (nmol/aliquot) and of 22.5% 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. 60% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 11% 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.

If your laboratory is assigned poor performance and you wish to appeal against this classification please email the ERNDIM Administration Office (admin@erndim.org), with full details of the reason for your appeal, within one month receiving your Performance Support Letter.

*Table 2. Percentage Flags*

| % Red Flags seen in Annual Report | Percentage Labs In this Category | Cumulative Percentage Of Labs |
|---|---|---|
| >25% | 11% | 11% |
| 25% | 3% | 14% |
| 20 – 25% | 0% | 14% |
| 15 – 20% | 12% | 26% |
| 10 – 15% | 0% | 26% |
| 5 – 10% | 14% | 40% |
| 0 – 5% | 0% | 40% |
| 0% | 60% | 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 the introduction of clinical information and interpretation of the results with some interesting results. The clinical scenarios and concentrations are similar to the situations encountered by our laboratory in the last 10 years. A summary of the results of the interpretative part of the scheme is presented below:

**Distribution 2020.01**. Clinical information: Both parents carriers of the cystinosis CTNS gene . Sample taken at 1 day of life.

The median cystine concentration (all laboratories) for this distribution was 0.415 nmol ½ cystine / mg protein in the range of carrier status. 82 % of the participants agreed that the concentration for this distribution was not consistent with cystinosis or alternatively it was consistent with carrier status. The majority of the laboratories concluded that the concentration in this sample was slightly increased but not to the values usually see in cystinosis but some laboratories pointed out that the child was too young at the time for testing to rule out cystinosis. Some laboratories recommended a repeat sample taken between 3-4 weeks to 3-6 months of age. A large proportion of laboratories stated that diagnosis should be confirmed by genetic study (CTNS gene).

Of the six laboratories that reported this distribution as consistent with Cystinosis, one laboratory made a calculation error; two laboratories measured the final concentration of cystine correctly (0.41 and 0.43 nmol ½ cystine / mg protein respectively) but selected the incorrect answer. Both laboratories recommend molecular sequencing for confirmation. The remaining three laboratories measure concentrations of cystine above 2 nmol ½ cystine / mg protein (range 2.17 to 5.61 nmol ½ cystine / mg protein).

**Distribution 2020.02**. Clinical information: 16 months old, poor weight gain

The median cystine concentration (all laboratories) for this distribution was 1.56 nmol ½ cystine / mg protein. Only twenty out of thirty two laboratories/methods (62.5 %) agreed that the concentration for this distribution was consistent with nephropathic cystinosis. This was a difficult clinical scenario with a late onset presentation and a concentration well below the expected values in typical nephropatic cystinosis.

The majority of the laboratories require a concentration above 2 nmol ½ cystine / mg protein to be consistent with nephropatic cystinosis what it is probably the case in children presenting at 6-9 months with renal involvement. Overall, the laboratories were very cautious in their interpretation and suggested genetic analysis to confirm the initial result.

Some laboratories measure cystine in granulocytes rather than mixed leucocytes. These laboratories tend to experience higher concentrations of cystine (nmol ½ cystine / mg protein) for carriers and affected patients and their reference ranges should reflect this. Some of these laboratories measured the concentration very accurately and pointed out that based their own experience and local data, the concentration for this distribution was within the carrier status range.

**Distribution 2020.03**. Clinical information: 37 years old, bilateral crystalline keratopathy, no evidence of renal disease and/or proteinuria

The median cystine concentration (all laboratories) for this distribution was 1.92 nmol ½ cystine / mg protein. 100 % of the participants agreed that the concentration for this distribution was consistent with ocular cystinosis.

Laboratories acknowledged that non-nephropathic ocular cystinosis patients are known to have lower levels of leucocyte cystine than nephropathic cystinosis patients owing to the presence of one severe and one mild pathogenic variant in the CTNS gene for cystine but there was no consensus about the cut-off value as there is overlap between carriers and affected patients. One laboratory stated that “a result >2 nmol ½ cystine/mg protein is consistent with a homozygous affected patient. Heterozygous patients (parents and siblings) without renal dysfunction tend to have values <1 nmol ½ cystine/mg protein” Another laboratory stated that cystine values between 1 - 3 nmol 1/2Cys /mg protein are typical for ocular presentation.

**Distribution 2020.04**. Clinical information: 9 months old presenting with polydipsia, vomiting, failure to thrive

The median cystine concentration (all laboratories) for this distribution was 3.55 nmol ½ cystine / mg protein, clearly abnormal and consistent with nephropatic cystinosis presentation. 96 % of the participants (29/30) agreed that the concentration for this distribution was consistent with nephropathic cystinosis. One laboratory reported a cystine concentration of 2.15 nmol ½ cystine / mg protein and selected the option “other” stating that the concentration was not elevated according to their local reference interval besides consensus that lkc-cystine >2 ng ½ cystin/mg protein is indicating disease. Their recommendation was to expand the investigation with genetic analysis to clarify if the patient is a carrier or has the disease

Some of the comments added by the laboratories in their reports:

“In an untreated patient, a result <0.3 nmol ½ cystine/mg protein is consistent with the patient being unaffected. A result >2 nmol ½ cystine/mg protein is consistent with a homozygous affected patient. Heterozygous patients (parents and siblings) without renal dysfunction tend to have values <1 nmol ½ cystine/mg protein. This result may be affected by sample preparation; any delay in the preparation of the cell pellet will affect the result.”

“The cystine concentration in granulocytes significantly exceeds our cut-off value, which is consistent with a diagnosis of nephropathic cystinosis. We advise to perform mutational screening of the associated CTNS gene and refer the patient to a metabolic paediatrician or nephrologist so treatment with cysteamine can be initiated.”

“Leukocyte cystine levels are indicative of a diagnosis of nephropathic cystinosis due to autosomal recessive deficiency of the CTNS cystine lysosomal transport protein (CTNS1). Please note that the diagnosis of cystinosis can be confirmed in the majority of Black and mixed race South African patients by screening for the common African mutation CTNS - c. 971 – 12G>A which results in an estimated newborn incidence of 1/10 000 in this population. A molecular diagnosis is of value in that siblings of index cases can be screened and identified for early intervention that can improve the outcome in this disorder.”

**Distribution 2020.05**. Clinical information: 10 years old, crystalline corneal dystrophy

The median cystine concentration (all laboratories) for this distribution was 1.41 nmol ½ cystine / mg protein. 81 % of the participants (26/32) agreed that the concentration for this distribution was consistent with intermediate (late-onset) cystinosis. Three

laboratories considered that carrier status was the correct answer. One of those laboratories reported a final concentration of cystine of 0.37 nmol ½ cystine / mg protein what explains the selected option, however the remaining two laboratories measured the concentration of cystine accurately at 1.33 and 1.39 nmol ½ cystine / mg protein respectively and still considered that carrier status was most likely. Another laboratory considered that the cystine concentration reported of 1.13 nmol ½ cystine / mg protein was not consistent with intermediate (late-onset) cystinosis. Two laboratories selected the option "other". Those laboratories measured the cystine concentration with values of 1.1 and 1.12 nmol ½ cystine / mg protein respectively. One of these laboratories stated in its report that the cystine concentration in granulocytes could be indicative for late-onset cystinosis, however, based on the concentration found, no definite conclusion on diagnosis (or carriership) can be drawn and suggested mutational analysis.

Overall the laboratories agreed that based on clinical information and cystine concentration this clinical scenario is highly suggestive of intermediate (late-onset) cystinosis and genetic analysis of the CTNS gene is recommended.

Some of the comments added by the laboratories in their reports:

"Elevated cystine concentration in white blood cells. Together with the clinical presentation this indicates that the patient is suffering from intermediate (late-onset) cystinosis"

"The cystine concentration in granulocytes could be indicative for late-onset cystinosis, however, based on the concentration found, no definite conclusion on diagnosis (or carriership) can be drawn. Mutational analysis of the associated CTNS gene should be performed to provide further insight in this."

"Heterozygotes: up to 1.0 nmol ½ cystine per mg protein. Cystinosis patients: usually greater than 2.0 nmol ½ cystine per mg protein. The white cell cystine level (nmol cystine/mg protein) is consistent with late-onset cystinosis, however there is some overlap between affected patients and carrier status. Suggest confirmation of result by molecular analysis of the CTNS gene."

**Distribution 2020.06**. Clinical information: 14 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 0.38 nmol ½ cystine / mg protein. 94 % of the participants (32/34) agreed that the concentration for this distribution was within therapeutic target. The two laboratories that selected the option "cystine concentration above therapeutic target" measured the concentration of cystine for this distribution high at 1.08 and 5.81 nmol ½ cystine / mg protein respectively.

Some of the comments added by the laboratories in their reports:

"Therapeutic target: less than 1.0 (ideal) or 2.0 (adequate). Monitoring samples should be taken as trough levels, i.e. pre-dose, whilst maintaining the normal dosage pattern."

"This pattern is suggestive of cystine concentration slightly above therapeutic target of 0.5 nmol/mg protein" (value reported by the laboratory 1.08 nmol ½ cystine / mg protein)

“Cystine in leukocytes within the reference range, excellent metabolic control. We can offer measurement of cysteamine concentration in blood, the dosage of cysteamine might be reduced.”

**Distribution 2020.07**. 20 years old, Patient had photophobia and proteinuria, crystals found in the cornea.

The median cystine concentration (all laboratories) for this distribution was 2.06 nmol ½ cystine / mg protein. 100 % of the participants (30/30) agreed that the concentration for this distribution was consistent with intermediate (late-onset) cystinosis. Most of the laboratories suggested genetic analysis of the CTSN gene.

Some of the comments added by the laboratories in their reports:

“The cystin leukocyte concentration is consistent with late-onset (intermediate) cystinosis.”

“The clinical symptoms and increased level of cystin in leukocytes are consistent with intermediate (late-onset) cystinosis. The diagnosis should be confirmed genetically and therapy with cysteamine (orally and eyedrops) should be considered.”

“Sugested CTNS gene molecular study. Non-nephropathic form.”

**Distribution 2020.08**. Both parents carriers of the cystinosis  CTNS gene . Sample taken at 1 day of life

The median cystine concentration (all laboratories) for this distribution was 3.82 nmol ½ cystine / mg protein, clearly abnormal at 1 day of age and consistent with cystinosis. 97 % of the participants (30/31) agreed that the concentration for this distribution was consistent with cystinosis. One laboratory reported a cystine concentration of 2.01 nmol ½ cystine / mg protein for this distribution and considered that it was consistent with carrier status rather than with cystinosis. Most of the laboratories suggested the need for confirmation by molecular analysis of the CTNS gene.

Some of the comments added by the laboratories:

“Leucocyte cystine consistent with cystinosis. Suggest confirmation of result using genotyping or a repeat white cell cystine at 2-4 weeks of age, and referral to a specialist centre.”

“Elevated leucocyte cystine would be consistent with a diagnosis of cystinosis. This should be confirmed by mutation analysis”

“The leukocyte cysteine concentration is above the reference range and consistent with a diagnosis of cystinosis. DNA sequencing of CTNS is available at XXX Health Sciences Centre Biochemical Genetics Laboratory.”

“The cystine content in leukocytes is indicative of a cystinosis. Please send us a control sample for confirmation and consider genetic testing of the CTNS gene”
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## 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 2021*

The design of the 2021-scheme is the same as in 2020; the scheme will pilot for another year the introduction of clinical details and interpretation of the results for each of the distributions. There are a few issues we need to resolve before its final introduction as part of the WCC scheme such as the overall scoring system, the option “other” that it is currently creating problems with scoring and assessment by the scientific advisors and the differences in reference ranges for laboratories using granulocytes versus mixed leucocytes. We would also like to seek feedback from participants at the next user survey about the introduction of clinical details and interpretation of the result in this scheme

The interpretation component of the scheme will not be scored in 2021. If the new design adds value to the scheme, it will be fully implemented in 2022 and 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, 11 January 2021

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 | 11 January 2021 | 2020 annual report published |
| 2 | 8 February 2021 | Page 4, Poor Performance Policy, information for appeal of poor performance added. |
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**END**