Carbohydrate-Deficient Transferrin

April 2013
1 GENERAL INFORMATION

1.1 Submitting Company, Institution or Organization

MUHC – Royal Victoria Hospital

1.2 Application Submitted: June 15, 2012

1.3 Notice Issued: April 2, 2013

Note:
This notice is based on the scientific and commercial information (submitted by the requestor(s) and on a complementary review of the literature) according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY AND LICENCE(S)

2.1 Name of the Technology: Capillary Zone Electrophoresis.

2.2 Brief Description of the Technology

Capillary zone electrophoresis (CZE) is a method for separating ionic species by their charge and the frictional forces acting on them. In other words, charged molecules are separated based on their electrophoretic mobility by pH and electro-osmotic flow. To detect congenital disorders of glycosylation, CZE can separate isoforms of human serum transferrin, a glycoprotein that transports iron, in a basic medium. The isoforms are separated into fractions based on the number of sialic acids present on the molecule. Low-sialylated forms (asialotransferrin, monosialotransferrin and disialotransferrin) are referred to as carbohydrate-deficient transferrin (CDT). They can be detected directly in the capillary by absorption spectrophotometry at 200 nm. The result quantifies the percentage of estimated CDT compared to the total amount of transferrin detected (from asialotransferrin to pentasialotransferrin). A high relative amount of CDT suggests a possible congenital disorder of glycosylation.

2.3 Company or Developer: Sebia Capillars CDT.

2.4 Licence(s): Sebia Capillars CDT.

2.5 Patent, if Applicable: Does not apply.

2.6 Approval status

Licensed by Health Canada: CAPILLARYS ELECTROPHORESIS SYSTEM — CDT KIT (CARBOHYDRATE-DEFICIENT TRANSFERRIN) by Sebia, licence #37197.

2.7 Weighted Value: 33.78.
3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patients

Patients with unexplained neurological syndromes or related family history (information submitted by the requestor) or for whom a congenital disorder of glycosylation is suspected.

3.2 Targeted Diseases

Congenital disorders of glycosylation (CDG) are a group of rare hereditary disorders caused by mutations in the genes coding for enzymes involved in the biosynthesis of glycoproteins and other glycoconjugates (Helander et al., 2004; Quintana et al., 2009). Clinical characteristics are variable but often include neurological, psychomotor, and growth impairments of early childhood onset.

Congenital disorders of glycosylation are the result of abnormal synthesis (assembly and transfer) (type I) or defective processing (type II) of N-glycans, causing defective incorporation of sialic acid, the negatively charged terminal sugar (Quintana et al., 2009; Jaeken and Matthijs, 2001), in proteins and lipids (Denecke, 2009; Lefeber et al., 2011; O'Brien, 2005; Sparks and Krasnewich, 2012). Depending on the defective enzyme, 18 subtypes of CDG I and 12 subtypes of CDG II have been identified (Sparks and Krasnewich, 2012). Carbohydrate-deficient transferrin (CDT) is the most common marker for early detection of CDG (Quintana et al., 2009).

Actual prevalence and incidence in Quebec are unknown. The prevalence of the most common type of CDG, PMM2-CDG (CDG-Ia) (phosphomannomutase [PMM] II deficiency), is estimated at 1 in 20,000 (Jaeken and Matthijs, 2001). These disorders have been found in more than 20 different populations: in Europe, Scandinavia (Jaeken and Matthijs, 2001), North and South America, Australia, and Asia (Sparks and Krasnewich, 2012; Jaeken and Matthijs, 2001).

Associated with a deficiency in phosphomannose isomerase (PMI), PMI-CDG-Ib can be treated by oral administration of mannose; it is the only CDG that can be treated. Its prevalence is less than 1 in 1,000,000 (Orphanet, 2013).

3.3 Number of Patients to Be Tested

The requestor estimates the expected provincial volume for the next three years at 900 samples, or approximately 300 samples per year. This estimate is based on test requests currently received at the MUHC – Royal Victoria Hospital in Montreal.

3.4 Medical Specialties Involved (and Other Professionals, if Applicable)

Medical biochemistry, neurology, pediatrics, genetics.

3.5 Testing Procedure

The test should be performed in a supraregional centre (based on the requestor’s proposal), given the rarity of these disorders.

Serum should be collected using the standard procedure for biomedical lab tests. Samples should not be stored at room temperature; they should be refrigerated (between 2°C and 8°C) for a maximum of 10 days. Samples should be kept between 2°C and 8°C during
For longer storage (one month), samples can be frozen quickly, within eight hours of collection (Sebia, 2008).

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology

Complementary (confirmation of diagnosis).

4.2 Brief Description of the Current Technological Context

Transferrin isoelectric focusing (IEF) is most commonly used to detect congenital disorders of glycosylation (Quintana et al., 2009; Jaeken and Matthijs, 2001; Carchon et al., 2004). It is even considered the gold standard (Parente et al., 2010). This method separates molecules based on differences in their isoelectric point (pI). The pI is defined as the pH at which the overall charge of a molecule is zero or, in other words, the pH at which the molecule is electrically neutral. However, IEF is labour intensive and time consuming and it is not suitable for automation, nor does it allow for accurate quantification of CDT (Carchon et al., 2004; Schellenberg et al., 2007; Quintana et al., 2009).

Semi-automated methods such as high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) have been used for this test for the last few years because they can provide reproducible separation and a measurement of the relative percentage of the various isoforms of transferrin (Quintana et al., 2009). They also allow for the graphical representation of all glycoforms of transferrin, thereby facilitating comparison of analysis profiles. When CDT values are borderline, a confirmation test should be performed using another method such as HPLC, which gives slightly higher estimates than CZE for low concentrations of CDT (Kenan et al., 2010; Quintana et al., 2009).

This test is not available in Quebec to detect congenital disorders of glycosylation. Tests are sent to laboratories outside Quebec as needed (information provided by the requestor).

4.3 Brief Description of the Advantages Cited for the New Technology

Capillary zone electrophoresis provides reproducible separation and a measurement of the relative percentage of the various isoforms of transferrin (Quintana et al., 2009). It also produces graphical representations that show the quality of transferrin separation (Bortolotti et al., 2005). Output can be stored for documentation purposes. Furthermore, this method is quicker and more easily quantifiable than IEF (Parente et al., 2010). Sample preparation is simpler with CZE than with HPLC, and CZE requires less time for analysis (10 minutes for CZE; 36 minutes for HPLC), including separation, cleaning and reconditioning of the system (Bortolotti et al., 2005).

According to the requestor, other advantages include the ability to use existing equipment, the availability of external quality control, and the provision of quantitative results.

Limitations of the technology are:

- Detection of CDT is not recommended in infants younger than three weeks of age because it cannot reliably exclude CDG (Denecke, 2009; Helander et al., 2004);
- This test can detect only a few subtypes of CDG (Denecke, 2009; Jaeken and Matthijs, 2001).
4.4 Cost of the Technology and Options

Information on instruments, and test and reagent kits is not available.

5 EVIDENCE

5.1 Clinical Relevance (Utility and Validity) and Analytical Validity

5.1.1 Other Tests Replaced

Does not apply.

5.1.2 Diagnostic and Prognostic Value

Clinical utility lies in oral treatment with D-mannose for patients with MPI-CDG (CDG-Ib). There is no treatment for other types of CDG (Jaeken and Matthijs, 2001). For patients with unexplained neurological syndromes, diagnosis can mean the end to an agonizing search for an unknown disorder (diagnostic delay) or reorientation toward appropriate health care.

5.1.3 Therapeutic Value

There are no treatment options associated with test results. Of the various types of CDG, MPI-CDG-Ib is the only form for which treatment is available.

5.2 Clinical Validity

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<thead>
<tr>
<th>Component</th>
<th>Presence</th>
<th>Absence</th>
<th>Not Applicable</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td>✓</td>
<td></td>
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<tr>
<td>Positive predictive value (PPV)</td>
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<td>✓</td>
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<tr>
<td>Negative predictive value (NPV)</td>
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<td>✓</td>
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<tr>
<td>Likelihood ratio (LR)</td>
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<tr>
<td>ROC curve</td>
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<td>✓</td>
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<tr>
<td>Accuracy</td>
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No data on clinical validity were found for detecting congenital disorders of glycosylation. Predictive values do not apply with these rare disorders. Moreover, most studies dealt with detecting CDT in cases of alcohol abuse. The manufacturer recommends that each laboratory set its own threshold values (Sebia, 2005).

Normal results do not rule out CDG, given the many subtypes still to be discovered (Jaeken and Matthijs, 2001) or classified (Denecke, 2009), in particular disorders of glycosylation not included in the nomenclature for type I and II CDG.

5.3 Analytical (or Technical) Validity

<table>
<thead>
<tr>
<th>Component</th>
<th>Presence</th>
<th>Absence</th>
<th>Not Applicable</th>
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<tbody>
<tr>
<td>Repeatability</td>
<td>✓</td>
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<tr>
<td>Reproducibility</td>
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<td>Analytical sensitivity</td>
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<tr>
<td>Analytical specificity</td>
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<td>✓</td>
</tr>
<tr>
<td>Matrix effect</td>
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<td></td>
<td>✓</td>
</tr>
<tr>
<td>Concordance</td>
<td>✓</td>
<td></td>
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<tr>
<td>Correlation</td>
<td>✓</td>
<td></td>
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<tr>
<td>Other based on type of test</td>
<td></td>
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<td>✓</td>
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</tbody>
</table>
Repeatability

The intratrial coefficient of variation ranges from 4.6% (Parente et al., 2010) to 6.78% (Bortolotti et al., 2007); intertrial, it ranges from 5.2% (Schellenberg and Wielders, 2010) to 18.5% (Schellenberg et al., 2007). For mean values of CDT of 1.3% or less, the variation is less than 8% (Schellenberg and Wielders, 2010; Bortolotti et al., 2007; Schellenberg and Wielders, 2010) while for higher values, the variation is no more than 1.3% (Schellenberg and Wielders, 2010). In other words, there is greater variability for CDT values of 1.3% and less than for higher values.

Method validation data from the manufacturer (Sebia, 2005) for detecting CDT show a coefficient of variation that ranges from 2.1% to 10.6% with respect to intratrial, intertrial and reproducibility between lots. In terms of accuracy, the coefficient of correlation is 0.940. The sensitivity of the test is equal to a CDT value of 0.35%.

Reproducibility

The coefficient of variation for interlaboratory accuracy ranges from 3.22% to 8.44%. The ANOVA test reveals a significant difference in results from different laboratories (Schellenberg and Wielders, 2010).

Analytical Sensitivity

In two studies (Carchon et al., 2004; Parente et al., 2010), the results from CZE were compared visually with IEF. All sera from patients with CDG, confirmed by IEF, were identified by CZE. Another study (Bortolotti et al., 2005) compared the CZE and HPLC methods. In 14 out of 99 cases, asialotransferrin was detected by HPLC, but not by CZE.

Concordance

Concordance between CZE and HPLC was investigated and presented in Bland-Altman plots (Quintana et al., 2009; Kenan et al., 2010; Schellenberg et al., 2007). The values obtained by CZE are systematically lower than those obtained by HPLC (consistent underestimation). In one study, this bias was estimated at −0.5% (Schellenberg et al., 2007).

Correlation

Studies show a high linear correlation between CZE and HPLC with the various statistical tests: Passing-Bablok regression (Quintana et al., 2009; Schellenberg and Wielders, 2010), the Pearson test (Schellenberg and Wielders, 2010), the CUSUM test (Schellenberg and Wielders, 2010) and a linear correlation test (Bortolotti et al., 2005; Bortolotti et al., 2007, Quintana et al., 2009).

Interference

Interfering substances can produce additional peaks in the analytical profile, distorting estimation of relative amounts of transferrin isoforms. Before detecting CDG, other causes that could affect the amount of CDT should be ruled out. These include excessive alcohol (the half-life of CDT is 14 days), cystic fibrosis, chronic obstructive pulmonary disease and chronic viral hepatitis.
5.4 Recommendations for Listing in Other Jurisdictions

Information not available.

6 ANTICIPATED OUTCOMES OF INTRODUCING THIS TEST

6.1 Impact on Human and Material Resources

Introducing the test to detect CDT using capillary zone electrophoresis requires the purchase of the machine and its reagents, as well as regular internal and external quality control.

Any new biomedical test requires training for a sufficient number of technical and scientific staff.

6.2 Economic Consequences of Adding the Test to the Quebec Health and Social Services System

The unit cost of the test is not very high, and the expected number of tests is low. However, there are no economic assessments to allow us to comment on the economic impact on the Quebec health and social services system.

6.3 Main Organizational, Ethical and Other (Social, Legal, Political) Issues

Congenital disorders of glycosylation raise ethical issues associated with screening for disorders for which, with the exception of a single subtype, there is no treatment.
## Carbohydrate-Deficient Transferrin

### Status of the diagnostic technology

- **Established**
- **Innovative**
- **Experimental (for research only)**
- **Replacement for technology: _________________, which is becoming obsolete**

### INESSS recommendation

- **Include in the Index**
  
  To diagnose congenital disorders of glycosylation only.
- **Do not include in the Index**
- **Reassess**

### INESSS decision regarding any required work

- **Draw connection with listing of drugs, if it is a companion test**
- **Produce an optimal use manual**
- **Produce indicators, if close monitoring is required**
- **None**
REFERENCES


