**C-peptide Testing in People with a Clinician-Diagnosis of Type 1 Diabetes**

A national programme of C-peptide testing in people with a clinician-diagnosis of Type 1 diabetes is being introduced in NHS Scotland. This programme is supported by the Scottish Diabetes Group and the Scottish Clinical Biochemistry Network. It is recommended that all people with a diagnosis of Type 1 diabetes of at least 3 year’s duration should have a one-off measurement of serum C-peptide. Further immunology and genetic testing may be required depending on the C-peptide results.

**What is C-peptide?**

C-peptide is part of the pro-insulin molecule and is secreted from the pancreas at the same time as insulin and in equal amounts. Synthetic insulin does not contain C-peptide. Therefore, C-peptide is a measure of **endogenous** insulin secretion.

**How is C-peptide measured?**

C-peptide can be measured in blood or urine.

Serum C-peptide samples can be taken at the time of the clinic visit (lithium heparin tube; separated and spun within 8 hours) and can be taken without any prior adjustment or omission of insulin therapy. There is no requirement for the individual to fast. Serum C-peptide is very stable and there are no special specimen handling requirements.

Hypoglycaemia reduces C-peptide secretion. Serum C-peptide is most predictive if measured when blood glucose is >4 mmol/l. Therefore, it is important that a sample for blood glucose measurement is taken at the same time. If blood glucose is <4 mmol/L at the time of testing, C-peptide should be repeated (especially if <200 pmol/L).

Urine for C-peptide testing must be collected in a boric acid container (red top) and is best measured in a sample taken 2 hrs after the largest meal of the day. Samples are analysed in Exeter. The main downside of urine C-peptide is that it requires individuals to hand or post back the sample after the clinic and, for that reason, serum C-peptide is the preferred option in Scotland.

**Why measure C-peptide in people with Type 1 diabetes?**

Historically, antibody testing was not performed as routine at diagnosis in people with suspected Type 1 diabetes (T1D). The standard criteria used to determine the aetiology of diabetes, e.g. age at diagnosis, body mass index, ethnicity, the presence/absence of ketonaemia/ketonuria are not perfect. As a consequence, we know that some people with a clinician-diagnosis of T1D actually have other forms of diabetes, e.g. Type 2 diabetes (T2D) or monogenic diabetes. The different forms of diabetes are increasingly managed in different ways and so establishing the correct aetiology of diabetes allows affected individuals to get the most appropriate treatment and follow-up. C-peptide testing helps identify people whose diabetes may have been mis-classified.

**Who should have a C-peptide test?**

**Routine C-peptide testing is recommended only for individuals with a clinician-diagnosis of T1D of 3 year’s duration or more.** The rationale is that this is the group in whom a potential change of diagnosis will have the greatest impact on quality of life, i.e. may potentially result in cessation of unnecessary insulin. **Routine C-peptide testing at diagnosis of diabetes or within the first three years is not currently recommended** (see below – ‘How should I assess individuals who start insulin within 12 months of a diagnosis of diabetes?’). The rationale for waiting 3 years is because insulin deficiency can occur due to glucose- and lipo-toxicity at diagnosis in people with T2D (resulting in low C-peptide at diagnosis), while the ‘honeymoon phase’ of T1D can result in significant detectable levels of C-peptide in people with T1D (which will fall with time in most people). Thus testing within the first 3 years, for diagnostic purposes, could result in an erroneous diagnosis. However, after 3 years from diagnosis, most people with T1D will have very low or low levels of C-peptide, in contrast to people with monogenic diabetes or T2D. Therefore, a C-peptide test at this point onwards should help in diagnosing the aetiology of the diabetes.

**How do I interpret a C-peptide result?**

Providing the blood glucose was >4 mmol/L at the time of testing, serum C-peptide should be interpreted using the algorithm in Appendix 1. The algorithm presumes that secondary forms of diabetes (e.g. secondary to disorders of the pancreas, such as chronic pancreatitis or cystic fibrosis) have already been considered and excluded.

Severe insulin deficiency (C-peptide <200 pmol/L) is found in most people with T1D, in some forms of monogenic diabetes and in some people with diabetes secondary to pancreatic disorders. Further investigation is generally not recommended, because severe insulin deficiency requires treatment with insulin and further diagnostic evaluation is unlikely to alter management. The exception to this is neonatal diabetes, as some forms of this are sulphonylurea-sensitive. All individuals with diabetes diagnosed within the first 12 months of life should have genetic testing for monogenic diabetes. For further information see [www.diabetesgenes.org](http://www.diabetesgenes.org).

Serum C-peptide >900 pmo/L is consistent with significant insulin resistance and in most individuals establishes a diagnosis of T2D. Rarely, genetic disorders of insulin action can cause extremely high levels of C-peptide.

Individuals with serum C-peptide between 200 and 900 pmol/L could have T1D, T2D or monogenic diabetes and further evaluation by islet cell antibody testing and, if appropriate, genetic testing is indicated.

**How should I manage a serum C-peptide concentration that is near a cut-off?**

The 200 and 900 pmol/L cut-offs used in the algorithm in Appendix 1 are, to some extent, arbitrary and pragmatic. They are based on published literature, but there is clearly no difference between an individual with a C-peptide of 195 pmol/L and one with a C-peptide of 205 pmol/L. Therefore, it is important to stress that these cut-offs and the associated potential diagnoses must not be interpreted too rigidly; **clinical judgement must be used at all times**. The C-peptide test is a screening test only, not a diagnostic test.

Tables to aid interpretation of urine C-peptide results (expressed as a urine C-peptide to creatinine ratio; UCPCR) are also available on the ‘Diabetes Diagnostics’ App from the University of Exeter and on the Exeter MODY website ([www.diabetesgenes.org](http://www.diabetesgenes.org)).

**Does chronic kidney disease affect C-peptide?**

Yes, C-peptide is excreted by the kidneys and so CKD will result in higher C-peptide levels. The cut-offs were validated in people with normal kidney function and will not be reliable in people with advanced CKD. Clinical judgement will need to be used instead.

**Can I use the algorithm to interpret a serum C-peptide measured outside Scotland?**

In Scotland, there are two different C-peptide assays in use, which give very comparable results. However in Exeter, and many other parts of the UK, other C-Peptide assays are used, which may give numerically different results. The algorithm in Appendix 1 must be used cautiously if C-peptide has been measured outside Scotland and with a clear understanding of the differences between C-peptide methods.

**Does serum C-peptide need repeated after an interval?**

Providing serum C-peptide was measured after 3 or more year’s duration of diabetes, there should not be any need for this to be repeated as a matter of routine. C-peptide data are now automatically downloaded to SCI Diabetes and this should help avoid unnecessary repeat testing.

**How do I interpret islet cell antibody results?**

Elevated islet cell antibodies are a marker of autoimmunity and thus support a diagnosis of T1D. However, it is important to remember that true false positive antibody results can occur with any immunoassay, while positive islet cell antibodies may occur in the general population and not cause diabetes. On the other hand, approximately 5% of people with true T1D have negative islet cell antibodies, when three antibodies are tested (GAD, IA-2, ZnT8). Broadly speaking, higher antibody titres and/or multiple different positive antibodies are more likely to be ‘real’ results, indicative of a diagnosis of T1D.

For the purposes of the algorithm in Appendix 1, islet antibody titres >99th centile are considered ‘strongly’ positive, while antibody titres between the 97.5th and 99th centiles are considered ‘weakly’ positive. Specific antibody titre cut-offs are shown in Appendix 2. As with the C-peptide cut-offs, it is important that these cut-offs are not applied rigidly and that clinical judgement is shown. For the purposes of the algorithm, a diagnosis of T1D is considered robust if one or more antibody is ‘strongly’ positive or there is more than one antibody ‘weakly’ positive.

**Do I need to measure triple antibodies in all people with C-peptide 200-900 pmol/L?**

Measurement of anti GAD, IA-2 and ZnT8 antibody titres is not necessary in all such individuals, if titres of one or two antibodies are diagnostic for T1D. However, before considering an individual to be antibody negative or as having only one antibody ‘weakly’ positive, then titres of all three antibodies should have been measured.

**Can the algorithm be used for antibody titres measured outside Scotland or measured in the distant past?**

The titres quoted in Appendix 2 are based on data from Exeter using RSR immunoassays (also used currently in Scotland). Antibodies measured in the distant past and in other centres may have utilised other antibody assays and so these cut-offs are unlikely to be applicable. Therefore, clinical judgement will need to be used in the interpretation of such results. In general, though, if there is a positive islet cell antibody test in high titre, this is probably sufficient to establish a diagnosis of T1D, making further antibody testing unnecessary.

**Are islet cell antibody titres a reliable measure of autoimmunity in people with diabetes of long duration?**

Islet cell antibody titres generally fall with increasing duration of T1D. Individuals with C-peptide 200-900 pmol/L have residual beta cells in the pancreas, so this should in theory provide a ready source of antigen that maintains antibody production. However, antibodies may revert to negative in any individual and this may be particularly more likely where C-peptide is <200 pmol/L. As noted above, approximately 5% of individuals with true T1D have triple negative antibodies at diagnosis. Therefore, negative antibodies do not exclude a diagnosis of T1D.

**What is the Type 1 diabetes genetic risk score (T1GRS)?**

The T1GRS measures an individual’s genetic susceptibility to T1D. It is derived by analysing variants in 10 genes that are associated with T1D. The T1GRS has been validated in adults aged 20-40 years and the result is expressed as a centile of genetic risk. Thus, for example, an individual with a T1GRS on the 95th centile has an extremely high genetic susceptibility of getting T1D, while an individual with a T1GRS on the 2nd centile has a very low genetic susceptibility of getting T1D. It is very important to remember that **the T1GRS does not give a probability estimate of the likelihood someone has T1D**, i.e. a score of 95 does not mean the person has a 95% chance of having T1D. This may seem counterintuitive, but in theory an individual with a T1GRS on the 95th centile may never develop diabetes if they do not encounter an environmental trigger throughout their life. By contrast, an individual on the 2nd centile may still develop T1D if, in theory, they encounter an environmental trigger in a sufficiently high load.

**How do I request a T1GRS?**

The T1GRS is currently measured in Exeter, but DNA samples (or a minimum of 5ml blood in an EDTA tube) should be sent to the East of Scotland Regional Genetics Laboratory (using the ‘genetic diabetes request form’, available from https://www.nhstayside.scot.nhs.uk/OurServicesA-Z/Genetics/PROD\_295543/index.htm). The ESRG laboratory will send the sample to the Exeter laboratory. If a sample is sent directly to Exeter, this will incur a direct cost for your service, which can be avoided by sending the sample to the ESRG laboratory. In due course, the T1GRS will be measured in the ESRG laboratory.

**How do I interpret the T1GRS?**

The T1GRS is helpful in establishing the likelihood that an individual has T1D, particularly where diagnostic uncertainty remains, i.e. those with negative antibodies or one ‘weakly’ positive antibody. Individuals with diabetes and a T1GRS <5th centile are very unlikely to have T1D, while individuals with a T1GRS >50th centile are very likely to have T1D. However, as discussed above, this test looks at genetic susceptibility to T1D and not overall risk. Therefore, when determining the likelihood that an individual has T1D, the T1GRS should be considered with other clinical data, such as age at diagnosis, ethnicity, body mass index and antibody status. The **Exeter T1DT2D Prediction Model** uses these variables to provide an estimate of the likelihood that an individual has T1D versus T2D (available at https://www.diabetesgenes.org/t1dt2d-prediction-model/). Healthcare professionals in Scotland are advised to use this, when interpreting the T1GRS. This model is only validated for white Europeans aged 18-50 years and so clinical judgement must be used in individuals who do not fit these parameters.

**How do I request testing for monogenic diabetes?**

A DNA sample (or a minimum of 5ml blood in an EDTA tube) should be sent to the East of Scotland Regional Genetics laboratory (using the ‘genetic diabetes request form’, available from https://www.nhstayside.scot.nhs.uk/OurServicesA-Z/Genetics/PROD\_295543/index.htm). Advice is available from tay.esrg@nhs.scot or via their website [www.esrg.scot.nhs.uk](http://www.esrg.scot.nhs.uk).

**What proportion of individuals are likely to be re-classified?**

The proportion of individuals re-classified will be determined by historic practices in the individual Scottish diabetes centres. There is likely to have been substantial variation in the criteria used to determine the cause of diabetes over the years. The study from the Western General Hospital, Edinburgh (PMID: 33131101) found that 13.2% of people with a clinician-diagnosis of T1D had C-peptide ≥200 pmol/L and that 6.8% were reclassified (5.1% to T2D and 1.6% to monogenic diabetes). Re-classification was most likely in individuals diagnosed with T1D when over 30 years of age and was least likely in individuals aged <18 years at the time of diagnosis of T1D.

**How should results be communicated to patients?**

Clearly, it is a matter for individual services to determine how C-peptide testing will be integrated into their service. We would advise that patients are given the information leaflet on C-peptide testing (Appendix 3), so they are aware that testing is being undertaken. Most patients with a clinician-diagnosis of T1D will have a C-peptide <200 pmol/L and no further action will be required. For those who are re-classified, considerable sensitivity must be employed when discussing the results and this is best done by a senior member of the clinical team. An important point to convey is that the historic diagnosis of T1D was made with the best available information at the time and that re-classification has occurred because of better diagnostic techniques.

**Is there any specific guidance on insulin withdrawal in re-classified individuals?**

Insulin withdrawal should be considered in individuals with sulphonylurea-sensitive forms of monogenic diabetes and individuals re-classified to T2D. As a broad principle, alternative glucose-lowering therapy should initially be added in on top of the existing insulin therapy. Insulin doses should be progressively reduced as the additional co-therapies are up-titrated. In some individuals, complete insulin withdrawal may not be possible, but the addition of co-therapies may improve glycaemic control. The decision to initiate co-therapies should be made by a senior clinician and the possible risks and benefits must be discussed with the patient.

Caution should be exercised when attempting insulin withdrawal in people with T2D and a C-peptide of <600 pmol/L. The potential risk of ketoacidosis with SGLT-2 inhibitors and the potential reduced efficacy of GLP-1 receptor agonists must be considered. Close supervision of the patient is required, with regular monitoring of blood glucose and, if appropriate, blood ketones.

**How should I assess individuals started on insulin within 12 months of diagnosis of diabetes?**

As noted previously, C-peptide testing is not recommended as a matter of routine in people with a clinician-diagnosis of T1D of less than 3 year’s duration. If an individual has been commenced on insulin within 12 months of a diagnosis of diabetes and has a duration of diabetes of less than 3 years, it is recommended that the protocol in Appendix 4 is followed.

This protocol recommends antibody testing as the first diagnostic step. Individuals who meet the antibody criteria for T1D, specified earlier, need no further investigation. Individuals who have a single ‘weakly’ positive islet cell antibody should have a T1GRS measured if they do not have a high ‘pre-test probability of T1D’, e.g. have phenotypic features of T2D and/or presentation at an older age. Individuals who have a high pre-test probability of T1D, e.g. an individual aged < 30 years with no phenotypic features of T2D, do not need further testing at this stage, but should have C-peptide measured at 3 years. Individuals who are antibody negative (or have a low T1GRS) with no features of T2D or secondary diabetes, should be considered for investigation for monogenic diabetes. The MODY probability calculator can be very useful determining who should have testing for monogenic diabetes (available from <https://www.diabetesgenes.org/exeter-diabetes-app/ModyCalculator>), although it is only validated in individuals aged <35 years at diagnosis (see appendix 5 for how to interpret the output from the calculator).

C-peptide testing is not recommended as a matter of routine if duration of diabetes is <3 years, but should be measured if a trial of insulin withdrawal is being considered, or if a monogenic or syndromic form of insulin resistance is suspected. C-peptide testing is still recommended in all individuals with T1D at 3 years, even if a robust diagnosis has been made, as individuals with significant detectable C-peptide have reduced glucose variability, reduced frequency of hypoglycaemia and better long term outcomes.

**Where can I get further advice on C-peptide and diabetes diagnostic investigation?**

Within SCI Diabetes, a module is available that helps support healthcare professionals through the diagnostic pathways. It is entitled ‘Investigate Diabetes Type’. This module is not intended to provide clinicians with a correct diagnosis, but rather to lead clinicians through the investigation algorithms. Videos demonstrating the functionality of this module are available on the 'Help' menu on SCI Diabetes.

Mark Strachan (mark.strachan@nhslothian.scot.nhs.uk) and Ewan Pearson (ewan.pearson@nhs.scot) are also happy to provide advice to diabetes healthcare professionals on interpretation of C-peptide and related matters.

**Professor Mark Strachan and Professor Ewan Pearson**

**April 2020**

**Appendix 1**

****

**Appendix 2: Islet Cell Antibody Titre Cut-Offs**

|  |  |  |
| --- | --- | --- |
|  | ‘Weakly Positive’ | ‘Strongly Positive’ |
|  | 97.5th centile | 99th centile |
| GAD U/ml | ≥11 | ≥64 |
| IA-2 U/ml | ≥15 | ≥15 |
| ZnT8 U/ml | Age <30  | ≥65 | ≥126 |
| ZnT8 U/ml | Age ≥ 30 | ≥10 | ≥20 |

Based upon data from the Exeter labs; produced by Ewan Pearson, on advice from Tim McDonald, February 2020

**Appendix 3: Patient Information Leaflet**

**C-Peptide Blood Test**

The purpose of this leaflet is to inform you about a new blood test which may be offered to you by the doctor you see in clinic today.

**What is the blood test?**

The blood test measures a substance called C-peptide. C-peptide gives us an indication of the amount of insulin that your pancreas is able to make itself.

**What is the purpose of the blood test?**

It is usually very easy to make a diagnosis of diabetes, as the diagnosis is based simply on the affected person having a raised blood glucose (sugar) level.

Diabetes can have many different causes, but the most common causes are Type 2 and Type 1 diabetes.

Establishing the cause of diabetes is usually straightforward. People with Type 2 diabetes usually tend to be older, carry extra weight and have raised blood pressure and cholesterol. By contrast, people with Type 1 diabetes tend to present at a younger age, often with a preceding history of weight loss, and may develop diabetic ketoacidosis.

However, some people fall into a ‘grey area’, with features of both types of diabetes, which can make it difficult for us to determine if they have Type 1 or Type 2 diabetes.

The purpose of the blood test is to help us establish if your diagnosis of Type 1 diabetes is accurate. The doctor or nurse can discuss the test with you in the clinic and can answer any questions.

**What would happen if the C-peptide test showed that I did not have Type 1 diabetes?**

Most people who have a diagnosis of Type 1 diabetes will have that diagnosis confirmed by the C-peptide blood test. However, a small number of people may be found not to have Type 1 diabetes. In that situation, we would arrange to see you and discuss the results further. We may need to carry out additional blood tests to determine the underlying cause of your diabetes. Once the cause of your diabetes is established, we may be able to alter the treatment that you need to control your blood sugar levels.

**Do I need to have the C-peptide blood test?**

We are introducing the C-peptide blood test as an improvement to the service that we provide to people with Type 1 diabetes. It is not part of a research study, although we may wish to describe in a scientific paper the impact of introducing this blood test on our service and the people who attend our clinic.

There is absolutely no obligation for you to have this blood test; if you do not wish to have your C-peptide measured then you do not need to agree to the test being performed. It will not affect in any way your future care at our clinic.

**Appendix 4**

****

**Appendix 5**

**Interpretation of MODY probability scores**

The MODY probability calculator used in SCI-Diabetes is the same as that developed by Exeter, found at [www.diabetesgenes.org/exeter-diabetes-app/](http://www.diabetesgenes.org/exeter-diabetes-app/)

This calculator is based upon referrals to the Exeter Genetics Laboratory and gives a probability that a patient has MODY (in someone whom the clinician has suspected the diagnosis).  It is developed and validated in those diagnosed with diabetes under the age of 35 and of white ethnicity.

When used in the context of the diagnostic pathways incorporated into SCI-Diabetes, i.e. with appropriate testing for C-peptide and antibodies in individuals who start insulin within 12 months of diagnosis, or in young onset non-insulin treated individuals, then the following thresholds should be considered for referral for genetic testing:

* *Probability ≥33% (1 in 3 chance of having a diagnostic genetic test)*

Refer for genetic testing

* *Probability ≥5% to <33% (1 in 20 to 1 in 3 chance of a diagnostic genetic test)*

Evaluate for other features that suggest monogenic diabetes and refer for genetic testing if clinical suspicion is high -

e.g  Sulphonylurea sensitivity, renal cysts, congenital heart disease, deafness etc.

e.g. More than 2 generation family history

* *Probability <5% (<1 in 20 chance of having a diagnostic genetic test)*

Do not refer for genetic testing unless many other clinical features raise the clinical suspicion dramatically

If SCI-Diabetes does not have a record of family history, then two scores will be provided, one with no family history (first degree relative affected) and one with a family history.  Select the score that is appropriate.

If the MODY probability calculator is used where there is no clinical suspicion of MODY then the probability will be overestimated and a higher threshold should be used to guide testing.

When evaluating MODY risk in non-white populations, the MODY probability calculator will overestimate probability and a higher probability threshold should be used to guide testing.