Proposal form for the evaluation of a genetic test for NHS Service Gene Dossier/Additional Provider

TEST – DISEASE/CONDITION – POPULATION TRIAD			
Submitting laboratory:	**Bristol RGC**	**Approved:**	**September 2012**
1. Disease/condition – approved name and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website)	Types A & B are clinically indistinguishable COCKAYNE SYNDROME, TYPE A; CSA COCKAYNE SYNDROME, TYPE B; CSB		
2. OMIM number for disease/condition	Type A = 216400; Type B = 133540		
3. Disease/condition – please provide a brief description of the characteristics of the disease/condition and prognosis for affected individuals. Please provide this information in laymen's terms.	Cockayne Syndrome (CS) is an autosomal recessive multisystem disorder characterised principally by microcephaly (developing by age 2 years), mental retardation, cachectic growth failure, photosensitivity, and progressive neurological deterioration leading usually to premature death in childhood or early adulthood. Other symptoms can include cataracts, ataxia, retinal degeneration and sensorineural deafness. CS has a variable rate of progression. The spectrum of CS phenotypes can be divided into three general clinical presentations: • Cockayne Syndrome type I. "Classic" CS (early-onset) in which the major features of the disease become apparent by one or two years of age. This is the most common type. • Cockayne Syndrome type II. A more severe form with abnormalities recognised at birth or in the early neonatal period. Less common type. • Cockayne Syndrome type III. Milder/later-onset forms that are still poorly defined. Cockayne Syndrome has been associated with mutations in two genes; ERCC6 (also known as CSB) and ERCC8 (also known as CSA or CKN1). Although described as Type A and B, phenotypically there is no difference in presentation.		
4. Disease/condition – mode of inheritance	Autosomal Recessive		
5. Gene – approved name(s) and symbol as published on HUGO database (alternative names will be listed on the UKGTN website)	ERCC8 - excision repair cross-complementing rodent repair deficiency, complementation group 8 ERCC6 - excision repair cross-complementing rodent repair deficiency, complementation group 6		
6. OMIM number for gene(s)	609412 – ERCC8 ; 609413 – ERCC6		

7. Gene – description(s)	ERCC8 (located at 5q12.1) is a 71.2kb 12 exon gene with a cDNA of 2kb in length encoding a 396 amino acid protein product. Recessive mutations account for 25% of CS. The ERCC8 gene is part of the nucleotide excision repair (NER) pathway, a complex system that eliminates a broad spectrum of structural DNA lesions, including ultraviolet (UV)-induced cyclobutane pyrimidine dimers, bulky chemical adducts, and DNA cross-links. ERCC6 (located at 10q21) is an 83.6kb 21 exon gene with a cDNA of 8kb in length encoding a 1493 amino acid protein product. Recessive mutations account for 75% of CS.
7b. Number of amplicons to provide this test	ERCC8 – 12; ERCC6 - 22
7c. MolU/Cyto band that this test is assigned to	ERCC8 – Band D (score 10) 2012/13 & band E GenUs 2013/14 ERCC6 – Band E (score 15) 2012/13 & band F GenUs 2013/14
8. Mutational spectrum for which you test including details of known common mutations	Analysis of the entire coding regions of genes, splice sites and branch points for point mutations and small insertions deletions. All of the known pathogenic mutations in ERCC6 are point mutations or small insertion/deletions that are detectable by sequence analysis. A large majority of these are nonsense or frameshift mutations that are predicted to result in the formation of a truncated protein [Mallery et al 1998, Horibata et al 2004]. Approximately 70% of the pathogenic mutations reported in ERCC8 are missense, nonsense, or splice-site mutations that are detectable by sequence analysis. The remaining 30% are large, partial deletions of ERCC8 that may escape detection by sequence analysis. [Ren et al 2003, Cao et al 2004]. Refs Ren Y, et al (2003)Three novel mutations responsible for Cockayne syndrome group A. Genes Genet Syst. 78:93–102 Cao H, et al (2004) CKN1 (MIM 216400): mutations in Cockayne syndrome type A and a new common polymorphism. J Hum Genet.;49:61–3. Mallery DL, et al (1998) Molecular analysis of mutations in the CSB (ERCC6) gene in patients with Cockayne syndrome [published erratum in Am J Hum; Genet 1999 May;64(5):1491]. Am J Hum Genet;

	62:77–85 Horibata K, et al (2004) Complete absence of Cockayne syndrome group B gene product gives rise to UV-sensitive syndrome but not Cockayne syndrome. Proc Natl Acad Sci U S A.;101:15410–5
9. Technical method(s)	High Throughput (HT) Automated sequence analysis. Gene screening by bidirectional automated sequence analysis (BiomekNX and ABI3730), the standard platform used for gene screening at BGL and analysis of the results with Mutation Surveyor software.
10. Validation process Please explain how this test has been validated for use in your laboratory	HT automated sequence analysis: This method is routinely used to screen for mutations at BGL and had been validated for many services. Beckman NX robotics /ABI 3730 capillary electrophoresis and mutation surveyor software analysis are routinely used in the laboratory. Positive and negative control samples are included. Primers (SNP checked) will be designed to cover the coding regions and intron-exon boundaries. BGL also participates in external quality assurance EMQN sequencing QA schemes (since the pilot scheme was introduced in 2002).
11a. Are you providing this test already?	Yes
11b. If yes, how many reports have you produced?	7
11c. Number of reports mutation positive	6
11d. Number of reports mutation negative	1
12. For how long have you been providing this service?	Since August 2011
13a. Is there specialised local clinical/research expertise for this disease?	Yes
13b. If yes, please provide details	Dr Peter Lunt, Consultant Clinical Geneticist, St. Michael's Hospital
14. Are you testing for other genes/diseases/conditions closely allied to this one? Please give details	No other associated genes are tested using molecular methods. Cytogenetic analysis for chromosome breakage /DNA repair disorders is available. Work is ongoing to develop a next generation sequencing test for chromosome breakage disorders.
Your current activity If applicable - How many tests do you currently provide annually in your laboratory?	Since August 2011 we have reported 7 cases. Additionally there are 2 cases in progress.
15a. Index cases	7
15b. Family members where mutation is known	4

Your capacity if Gene Dossier approved How many tests will you be able to provide annually in your laboratory if this gene dossier is approved and recommended for NHS funding?	The capacity in BGL is available to meet demand for UK and abroad.
16a. Index cases	
16b. Family members where mutation is known	
Based on experience how many tests will be required nationally (UK wide) per annum? Please identify the information on which this is based	Based on the number of referrals since August 2011 we estimate between 15 – 20 cases per year. The estimated incidence of CS in the UK population is 1.8 per million births so a UK birth rate of 700,000 per annum would suggest approx. 3 new diagnoses per year. In addition there are likely to be currently undiagnosed cases referred. Referrals are also expected from outside the UK. Ref: Kleijer WJ et al. (2008) Incidence of DNA repair deficiency disorders in western Europe: Xeroderma Pigmentosum, Cockayne Syndrome and trichothiodystrophy. DNA Repair 7 744-750
17a. Index cases	20
17b. Family members where mutation is known	40 (including prenatal tests)
18. National activity (England, Scotland, Wales & Northern Ireland) **If your laboratory is unable to provide the full national need please could you provide information on how the national requirement may be met.** For example, are you aware of any other labs (UKGTN members or otherwise) offering this test to NHS patients on a local area basis only? This question has been included in order to gauge if there could be any issues in equity of access for NHS patients. It is appreciated that some laboratories may not be able to answer this question. If this is the case please write "unknown".	There is no other UK laboratory currently listed by UKGTN providing this activity. We are able to provide for UK cases.

EPIDEMIOLOGY

19. Estimated prevalence of condition in the general UK population Please identify the information on which this is based	Prevalence less than 1 per 100,000 UK Cockayne Syndrome support group (Amy and Friends) estimate a UK prevalence of 1 in 1,870,000. Approx. 30 patients living with CS in the UK at the present time. http://www.amyandfriends.org/aboutcs.htm
20. Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	ERCC8 and ERCC6 carrier frequency in the UK population can only be estimated based on the incidence figures quoted above. Using Hardy-Weinberg this is approx 1/257 Founder mutations: ERCC8 p.Tyr322X mutation: Common in the Christian Arab population. Genetic screening of the ERCC8 gene in this population documented a carrier frequency of 6.79% (95% confidence interval: 3.84-9.74%). *(Khayat M et al (2010) Am J Med Genet A. Dec; 152A(12):3091-4)* ERCC6 c.1034-1035insT in exon 5 of the ERCC6 gene (p.Lys345Asnfs24). This mutation was identified in 1:15 healthy individuals from a Druze isolate in Northern Israel indicating an extremely high carrier frequency, in that population. *Fallik-Zaccai et al (2008) Am.J Med Genet A : 146A, : 11: 1423-1429*
21. Estimated penetrance Please identify the information on which this is based	There is no evidence to suggest non-penetrance of ERCC6/ERCC8 mutations in homozygotes or compound heterozygotes. Mouse models of Cockayne syndrome display 100% penetrance Andressoo et al. (2009) An Xpb Mouse Model for Combined Xeroderma Pigmentosum and Cockayne Syndrome Reveals Progeroid Features upon Further Attenuation of DNA Repair. Molecular and Cellular Biology, Mar. 2009, p. 1276–1290 vol. 29, no. 5
22. Estimated prevalence of condition in the target population. The target population is the group of people that meet the minimum criteria as listed in the Testing Criteria.	In patients who fully meet the Cockayne Syndrome testing criteria a mutation in either ERCC6 or ERCC8 should be identified in 100% of individuals.The clinical presentation of Cockayne Syndrome does however overlap with other disorders i.e. Bloom syndrome and Xeroderma Pigementosum. In patients who do not fully meet the testing criteria the prevalence will be lower, there is no data available to provide figures for this.

INTENDED USE

23. Please tick the relevant clinical purpose of testing		
Diagnosis	☒ Yes	☐ No
Treatment	☐ Yes	☒ No
Prognosis & management	☒ Yes	☐ No
Presymptomatic testing	☐ Yes	☒ No
Carrier testing for family members	☒ Yes	☐ No
Prenatal testing	☒ Yes	☐ No

TEST CHARACTERISTICS

24. Analytical sensitivity and specificity

This should be based on your own laboratory data for the specific test being applied for or the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up.

High Throughput Semi Automated Sequence analysis.

Sensitivity of sequencing assay is 99-100%.Current validation of unidirectional sequencing within SCOBEC indicates a sensitivity of 99%.

Specificity of >99%

25. Clinical sensitivity and specificity of test in target population

The *clinical sensitivity* of a test is the probability of a positive test result when condition is known to be present; the *clinical specificity* is the probability of a negative test result when disease is known to be absent. The denominator in this case is the number with the disease (for sensitivity) or the number without condition (for specificity).

Clinical Sensitivity:

30% of ERCC8 mutations are reported to be large partial deletions that may not be detected by sequence analysis. A quantitative approach is a future development for these cases.

Using a sequencing only approach we would expect the sensitivity to be ~92.5%.

Clinical Specificity :

Presumed over 95%, depending on the basis of interpretation of sequence variants as 'consensus' mutations, or as innocuous polymorphisms.

For testing of at-risk relatives: Clinical sensitivity and specificity: will both be 100% when the mutation is definitely pathogenic and the disease is fully penetrant.

26. Clinical validity (positive and negative predictive value in the target population)

The *clinical validity* of a genetic test is a measure of how well the test predicts the presence or absence of the phenotype, clinical condition or predisposition. It is measured by its *positive predictive value* (the probability of getting the condition given a positive test) and *negative predictive value* (the probability of not getting the condition given a negative test).

Clinical validity: In an index case, finding two pathogenic mutations confirms the diagnosis in all cases.

We estimate that for index cases-

Positive predictive value (PPV) = 100% for consensus mutations

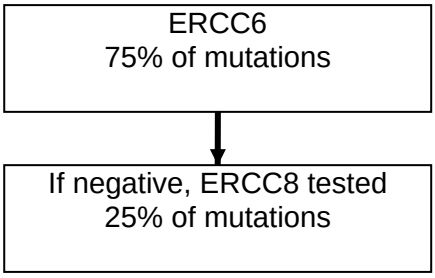
Negative predictive value (NPV) = greater uncertainty but likely to approach 100%

However, for testing family members, PPV and NPV are both effectively 100% for carrier status using consensus mutations.

27. Testing pathway for tests where more than one gene is to be tested

Please include your testing strategy if more than one gene will be tested and data on the expected proportions of positive results for each part of the process. Please illustrate this with a flow diagram.

75% of mutations are detected in ERCC6 and is therefore tested first, if negative testing for ERCC8 is undertaken (25% of mutations)



CLINICAL UTILITY

28. How will the test add to the management of the patient or alter clinical outcome?

1. Currently diagnosis in UK requires a cellular assay based on cultured skin biopsy – typically taking 6-8 wks from clinical suspicion to lab confirmation, and involving an invasive procedure. The DNA test can take less than 2 weeks in an urgent (e.g. prenatal) situation
2. Rapid confirmation of diagnosis allows DNA testing to be the 1st line test in suspected cases, whereas previously many different conditions may have been tested for and excluded (from a blood sample) (eg. cytogenetics for DNA repair disorders, microarray CGH, congenital infection, metabolic tests) prior to arranging a skin biopsy due to its more invasive nature.
3. Prenatal diagnosis as a rapid test to give a result by 12wks from an 11wk CVS is a reality if the DNA mutations are known. The cellular based assay requires CVS culture and takes several weeks.

29. How will the availability of this test impact on patient and family life?

1. Rapid confirmation of diagnosis relieves anxiety of uncertainty
2. Enables realistic family planning knowing the long term prognosis for the affected child
3. Enabling rapid prenatal diagnosis allows more reproductive choice for some families.
4. Enables carrier testing in consanguineous families from high risk ethnic populations and hence reproductive risk assessment and choice in wider family

30. Benefits of the test
Please provide a summary of the overall benefits of this test.

1. Rapid 1st trimester prenatal diagnosis is made possible, rather than awaiting CVS culture and UV-testing of m-RNA synthesis on cultured cells (taking 6-8 wks from CVS)
2. Allows DNA testing to be the 1st line test in suspected cases, whereas previously many different conditions may have been tested for and excluded (from a blood sample) (eg. cytogenetics for DNA repair disorders, microarray CGH, congenital infection, metabolic tests) prior to arranging a skin biopsy due to its more invasive nature.
3. Avoids the child having a skin biopsy as an invasive procedure
4. Enables carrier testing in consanguineous families

31. Is there an alternative means of diagnosis or prediction that does not involve molecular diagnosis? If so (and in particular if there is a biochemical test), please state the added advantage of the molecular test.

1. DNA repair assay. Assays of DNA repair are performed on skin fibroblasts. The most consistent findings in CS fibroblasts are: marked sensitivity to UV radiation; deficient recovery of RNA synthesis following UV damage; and impaired repair of actively transcribed genes, or "transcription-coupled repair". Testing is available at the Genome Damage and Stability Centre, University of Sussex, but requires an invasive procedure (skin biopsy) and a wait of 6-8wks for the result.
2. Complementation groups. Cells from individuals with CS can be divided into two complementation groups based on the protein that corrects the DNA repair defect, but this also requires the same functional cellular assay.

32. Please describe any specific ethical, legal or social issues with this particular test.

None known

33. The Testing Criteria must be completed where Testing Criteria are not already available. If Testing Criteria are available, do you agree with them? If No: Please propose alternative Testing Criteria AND please explain here the reasons for the changes.

Criteria adapted from Gene Reviews:

Major criteria

- Postnatal growth failure (height and weight <5th centile by age 2 years), but usually profound

(<<0.4th centile)

- Postnatal progressive microcephaly (usually by age 2 yrs)
- Progressive neurologic dysfunction manifested as early developmental delay in most individuals, followed by progressive behavioral and intellectual deterioration in all individuals; brain MRI reveals leukodystrophy [Boltshauser et al 1989, Sugita et al 1992]. Intracranial calcifications are seen in some individuals.

Minor criteria

- Cutaneous photosensitivity with or without thin or dry skin or hair (~75%)
- Demyelinating peripheral neuropathy diagnosed by electromyography, nerve conduction testing, and/or nerve biopsy
- Pigmentary retinopathy (~55%) and/or cataracts (~36%)
- Sensorineural hearing loss (~60%)
- Dental caries (~86%)
- A characteristic physical appearance of "cachectic dwarfism" with thinning of the skin and hair, sunken eyes, and a stooped standing posture
- Characteristic radiographic findings of thickening of the calvarium, sclerotic epiphyses, vertebral and pelvic abnormalities
- The presence of a similarly affected sib can be useful for diagnosis.
- Ataxia

Connatal Cockayne syndrome (CS type II) is suspected:

- In infants with growth failure at birth and little postnatal increase in height, weight, or head circumference
- When there is little or no postnatal neurologic development
- When congenital cataracts with other structural defects of the eye (microphthalmos, microcornea, iris hypoplasia) are present

34. Savings or investment per annum in the diagnostic pathway based on national expected activity, cost of diagnostics avoided and cost of genetic test. Please show calculations.

- Skin biopsy - £250
- Fibroblast culture - £200
- UV-sensitivity testing of mRNA synthesis (on cultured fibroblasts) - £500
- Metabolilc tests - £300

Total cost of tests no longer required per index case £1250 (as per Q35)

Cost of 20 tests per annum is 20 x £900 = £18000

Cost of tests no longer required per annum is 20 x £1250 = £25000

Annual cost savings is £25000 - £18000 = £7000

35. List the diagnostic tests/procedures that would no longer be required with costs.

Costs and type of imaging procedures	
Costs and types of laboratory pathology tests (other than molecular/cyto genetic proposed in this gene dossier)	UV sens £500 Fibroblast culture £200 Metabolic tests £300
Costs and types of physiological tests (e.g. ECG)	
Cost and types of other investigations/procedures (e.g. biopsy)	Skin biopsy £250
Total cost tests/procedures no longer required	**£1250**

36. REAL LIFE CASE STUDY
In collaboration with the clinical lead, describe a real case example to illustrate how the test would improve patient experience.

A 4-month old baby was referred to genetics by the community paediatrician with congenital cataracts, microcephaly, failure to thrive, and contractures; but due to failure to attend appointments was not seen until age 12 months, when a clinical diagnosis of Cockayne Syndrome was strongly suspected. Parents refused skin biopsy. They recontacted 5 months later by when the mother was 10 wks pregnant. Skin biopsy on the child, to which they now agreed, would take 6-8 wks to yield a result, and CVS would not be analysed until after that (ie. mother would be 18-21 wks gestation by time of result). The Bristol lab set up and ran the CS DNA testing on the child and on a pre-taken CVS in 10 days, giving a (unaffected) result by 12 wks gestation.

37. For the case example, if there are cost savings, please provide these below:
The main saving in this particular example is time and hence anxiety, since in this case a skin biopsy was run as a back up. This would not be needed in future.

PRE GENETIC TEST

Costs and type of imaging procedures	
Costs and type of laboratory pathology tests (other than molecular/cyto genetic proposed in this gene dossier)	
Costs and type of physiological tests (e.g. ECG)	
Cost and type of other investigations/procedures (e.g. biopsy)	
Cost outpatient consultations (genetics and non genetics)	
Total cost pre genetic test	**£**

POST GENETIC TEST

Costs and type of imaging procedures	
Costs and types laboratory pathology tests (other than molecular/cyto genetic proposed in this gene dossier)	
Cost of genetic test proposing in this gene dossier	
Costs and type of physiological tests (e.g. ECG)	
Cost and type of other investigations/procedures (e.g. biopsy)	
Cost outpatient consultations (genetics and non genetics)	
Total cost post genetic test	**£**

38. Estimated savings for case example described £

UKGTN Testing Criteria

Approved name and symbol of disease/condition(s):	**OMIM number(s):**
Cockayne Syndrome, Type A; CSA	216400
Cockayne Syndrome, Type B; CSB	133540
Approved name and symbol of gene(s):	**OMIM number(s):**
excision repair cross-complementing rodent repair deficiency, complementation group 6; ERCC6	609412 – ERCC8 609413 – ERCC6
excision repair cross-complementing rodent repair deficiency, complementation group 8; ERCC8	

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Clinical Geneticist	
Paediatric Neurologist	
Metabolic Paediatrician	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
1. Growth failure by 2 yrs (usually profound)	
AND 2. Microcephaly by 2 yrs (usually profound)	
AND 3. Progressive neurological dysfunction	
AND 4. Characteristic appearance (eg. thin skin, and/or sunken eyes, and/or stooped posture) **AND ONE OR MORE OF :** Cataracts Pig. Retinopathy S-N hearing loss Contractures Severe dental caries Characteristic radiological findings (eg. thickened calvaria) Skin sensitivity to UV-light (sunlight) Ataxia **OR :** proven affected sibling or son/daughter	
At risk family members where familial mutation is known	

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample