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

Test – Disease – Population Triad

Disease – name	**Branchio-oto-renal syndrome / Branchio-otic syndrome (BOR / BO)**
OMIM number for disease	#113650
Disease – alternative names please provide any alternative names you wish listed	Branchio-oto-renal dysplasia Melnick-Fraser syndrome
Disease – please provide a brief description of the disease characteristics	BOR is characterised by sensorineural, conductive or mixed hearing loss, structural defects of the outer, middle and inner ear, branchial fistulas or cysts and renal anomalies ranging from mild hypoplasia to complete absence. Reduced penetrance and variable expression have been observed. Several genes are believed to contribute to the phenotype.
Disease - mode of inheritance	Autosomal dominant
Gene – name(s)	SIX1 and SIX5
OMIM number for gene(s)	SIX1 - *601205 SIX5 - *600963
Gene – alternative names please provide any alternative names you wish listed	SIX1 - NONE SIX5 – DM locus-associated homeodomain protein (DMAHP)
Gene – description(s) (including number of amplicons).	SIX1 and SIX5 interact with EYA1 forming a homeodomain complex believed to be involved in initiating transcription. SIX1 – Gene map locus 14q23. Analysis would involve testing of both exons amplified in 6 amplicons SIX5 - Gene map locus 19q13.3. Analysis would involve testing of all three exons amplified in 8 amplicons
Mutational spectrum for which you test including details of known common mutations.	Missense, nonsense and small insertion and deletion mutations. No common mutations known
Technical Method (s)	Direct sequencing of all exons and intron/exon boundaries. Detection rate of sequence analysis method ~100% Family mutations: PCR and restriction enzyme digest or sequencing
Validation Process Note: please explain how this test has been validated for use in your laboratory	Sequencing is a standard analytical method used in the laboratory for a range of diagnostic services requiring mutation detection. The laboratory participates in external quality assessment for sequence analysis. All primers used are regularly checked for single nucleotide polymorphisms (SNPs).

Are you providing this test already? If yes, how many reports have you produced? Please give the number of mutation positive/negative samples you have reported	The existing service for BOR/BO consists of testing for EYA1 gene mutations. The proposed analysis is an extension of the existing service to include additional genes, mutations in which lead to the same phenotype.
For how long have you been providing this service?	Since 1999
Is there specialised local clinical/research expertise for this disease?	**Yes** √ | **No** | **Please provide details** Dr Maria Bitner-Glindzicz, Consulant Clinical Geneticist, Clinical Genetics, Great Ormond St Hospital, 4-5 Long Yard, London WC1N 3LU
Are you testing for other genes/diseases closely allied to this one? Please give details	EYA1 is the only test we provide at present for BOR/BO. We test for another syndromic deafness condition: Pendred syndrome; and for non syndromic hearing loss: Connexin 26
Your Activity If applicable - How many tests do you currently provide annually in your laboratory?	Index cases: 30-40pa Family members where mutation is known: ~8pa
Your Activity How many tests will you be able to provide annually in your laboratory if this gene dossier is approved and recommended for NHS funding?	Index cases: 20 Family members where mutation is known: 20
Based on experience how many tests will be required nationally (UK wide)? Please identify the information on which this is based	Index cases: 20 Family members where mutation is known: 20
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".	This laboratory will be able to provide a National Service

Epidemiology

Estimated prevalence of disease in the general UK population Please identify the information on which this is based	BOR occurs in approx 1 in 40,000 individuals and accounts for ~ 2% of profound deafness cases. (Chang et al (2004) Hum Mutat, 23:582-589). Mutations SIX1 and SIX5 are rare contributors to this disease with incidence data not currently available. However as we plan to do this screen in those who have no EYA1 mutations detected, this may enrich for those with SIX1/SIX5 mutations.
Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	40% in those patients with some symptoms of BOR have EYA1 mutations. The figure is closer to 70% in clinically confirmed cases. Gene frequency of SIX1 and SIX5 unknown
Estimated penetrance Please identify the information on which this is based	Penetrance is high but expressivity is very variable.
Target Population Description of the population to which this test will apply (i.e. description of the population as defined by the minimum criteria listed in the testing criteria)	The target population would be referred by clinical geneticists. The patients would have been screened and shown to be negative for mutations in the EYA1 gene. Minimum clinical criteria required: Hearing loss, pre-auricular esr pits/tags, branchial sinus and renal dysplasia.
Estimated prevalence of disease in the target population	Information on the prevalence is limited. For SIX5 - approx 1 in 19 mutation positives were found in a cohort of BOR patients negative for EYA1 (Hoskins et al (2007) Am J Hum Gent 80 800-804). FOR SIX1 – Mutations were found in 3 out of 4 kindreds that were linked to the SIX1 locus. (Ruf et al 2003 J Med Genet 40:515-519; Ruf et al 2004 Proc Nat Acad Sci 101:8090-8095.

Intended Use **(Please use the questions in Annex A to inform your answers)**

Please tick the relevant clinical purpose of testing	**YES**	**NO**
Diagnosis	√	
Treatment		√
Prognosis & Management		√
Presymptomatic testing		
Risk Assessment for family members	√	
Risk Assessment – prenatal testing	√	

Test Characteristics

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. If more than one gene will be tested, please include your testing strategy and data on the expected proportions of positive results for each part of the process. Please illustrate this with a flow diagram.	Direct sequencing has a high sensitivity in this laboratory. We use Big Dye chemistry, ABI analysers (3100, 3130XL, 3730) and analyse using Mutation Surveyor software. We participate and perform successfully in EQA programmes for sequence analysis. Bidirectional sequence analysis has specificity approaching 100% although large insertions / deletions and deep intronic mutations will not be detected. Following negative testing for EYA1 mutations and deletions, SIX1 and SIX5 will be set up concurrently using robot technology for efficiency and economic viability.
Clinical sensitivity and specificity of test in target population The *clinical sensitivity* of a test is the probability of a positive test result when disease 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 disease (for specificity)	There is limited data on the contribution of these genes to the BOR phenotype and hence clinical sensitivity. For SIX5 - approx 1 in 19 mutation positives were found in a cohort of BOR patients negative for EYA1 (Hoskins et al (2007) Am J Hum Gent 80 800-804). FOR SIX1 – Mutations were found in 3 out of 4 kindreds previously shown to be linked to the SIX1 locus. (Ruf et al 2003 J Med Genet 40:515-519; Ruf et al 2004 Proc Nat Acad Sci 101:8090-8095 Clinical specificity is high.
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 disease or predisposition. It is measured by its *positive predictive value* (the probability of getting the disease given a positive test) and *negative predictive value* (the probability of not getting the disease given a negative test).	The PPV is high, however NPV is lower as other genes may yet be identified that lead to the BOR phenotype.

Clinical utility of test in target population (Please refer to Appendix A) Please provide a description of the clinical care pathway.	Testing will allow more accurate genetic counselling, particularly offspring risk. Occasionally there may be individuals in families with signs/symptoms that mimic BOR and testing will clarify their disease status and offspring risks and remove the need for monitoring of hearing and renal status. In Kochlar et al (2008), 6 SIX1 mutations were detected from 247 EYA1 negative probands (~2.4% pickup). Ruf et al (2004) detected 4 mutations in 91 EYA1 negative families (4.3%) Hoskins et al (2007) found 5 SIX5 mutations out of 95 probands (5.2% pick up rate) No laboratory pilot study data is as yet available
How will the test add to the management of the patient or alter clinical outcome?	Main benefit will be to clarify offspring risk in those whose symptoms are consistent with but not diagnostic of BOR.
What impact will this test have on the NHS i.e. by removing the need for alternative management and/or investigations for this clinical population?	Further genetic testing will be unnecessary if positive outcome. Clinical screening (for example renal and audiological) may be avoided in individuals from known BOR families who screen negative for the family mutation.
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	Clinical assessment is the only alternative to molecular testing, but because of very variable expressivity may be difficult. Molecular analysis allows the assessment of recurrence risk within a family and allows offer of prenatal diagnosis where appropriate. Ongoing clinical screening (eg renal) may be indicated in mutation positive individuals
Please describe any specific ethical, legal or social issues with this particular test?	N/A

Please complete the testing criteria form.

UKGTN Testing criteria

Name of Disease(s): BRANCHIOOTORENAL SYNDROME 1; BOR1 (113650)
Name of gene(s): SIX homeobox 1; SIX1 (601205)
SIX homeobox 5; SIX5 (600963)

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	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	Tick if this patient meets criteria
Hearing loss AND one of the following:	
• Preauricular ear pits;	
• Branchial sinuses (including branchial pits, fistula and tags);	
• Renal dysplasia;	
AND EYA1 mutation negative:	

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.