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

Submitting laboratory: London NE RGC GOSH

1. Disorder/condition – approved name (please provide UK spelling if different from US) and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website).
If NGS panel test, please provide a test name & the number of unique conditions across the whole of the panel test.

If this submission is for a panel test please complete the Excel spread sheet, Appendix 1, available for download from the UKGTN website, and list all of the conditions grouped by sub panels if applicable.

Eye Disorders, Congenital, 450 Gene Exome Panel (consisting of 8 sub-panels)

2. OMIM number for disorder/condition

If a panel test – see 1 above. If a number of subpanels exist with different clinical entry points e.g. cancer panel test but different subpanels for different types of cancer (breast cancer, colon, phaeochromocytoma), then please list the sub panels here – providing name of each sub panel.

Ocular birth defects can be highly heterogeneous conditions and may include phenotypic overlap resulting in difficulty making differential diagnoses. In addition, some ocular birth defects can be caused by several genes, which can mean reaching a diagnosis is difficult using single gene testing methodology. Analysis may be based on the following sub-panel classifications; however it may be necessary for testing to be undertaken for the entire panel in patients where the diagnosis is unclear:

1. Eye Malformations (includes anterior segment dysgenesis (ASD) and glaucoma, cataract, congenital, or lens malformations, congenital, and microphthalmia, anophthalmia and coloboma (MAC) spectrum and aniridia sub-panels and additional genes associated with eye malformations as part of syndromic presentations)
2. Microphthalmia, anophthalmia and coloboma (MAC) spectrum and aniridia
3. Anterior Segment Dysgenesis (ASD) and Glaucoma
4. Retinal Dystrophies
5. Ocular albinism, photophobia and nystagmus
6. Cataract, Congenital, or Lens Malformations, Congenital
7. Optic Atrophy, Childhood Onset
8. Eye Movement Disorders

3a. Disorder/condition – to help commissioners to understand the impact of this condition please provide, in laymen's terms (e.g tubes in the kidney (renal tubule) or low sugar in the blood (hypoglycaemia)), a brief (2-5 sentences/no more than 50 words) description of how the disorder(s) affect individuals and prognosis.

1 in 2,500 children in the UK are diagnosed as blind or severely visually impaired by the time they reach one year old. Many congenital eye disorders causing visual impairment or blindness at birth or progressive visual impairment also include syndromic conditions involving additional metabolic, developmental, physical or sensory abnormalities.

3b. Disorder/condition – if required please expand on the description of the disorder provided in answer to Q3a.

Phenotypic overlap presents additional difficulty in defining these conditions. Gene panels offer the enhanced probability of diagnosis as a very large number of genes can be interrogated. As many as half of these cases are likely to be inherited and remain undiagnosed due to the vast number of genes involved in these conditions.

4. Disorder/condition – mode of inheritance

If this submission is for a panel test, please complete the mode of inheritance for each condition in the Excel spread sheet appendix 1 and if there is only one mode of inheritance across all conditions, please

state it here or if it varies please provide proportion split here.
Ocular birth defects include all inheritance modalities. Autosomal dominant and recessive diseases as well as X-linked dominant and recessive diseases are seen. These conditions can also be caused by *de novo* mutations.
5. Gene – approved name(s) and symbol as published on HGNC database (alternative names will be listed on the UKGTN website) If this submission is for a panel test please complete the Excel spread sheet, Appendix 1, available for download from the UKGTN website, and list all of the genes grouped by sub panels if applicable.
See Appendix 1
6a. OMIM number(s) for gene(s) If a panel test – see 5. above
See Appendix 1
6b. HGNC number(s) for gene(s) If a panel test – see 5. above
See Appendix 1
7a. Gene – description(s) If this submission is for a panel test, please provide total number of genes and if there are subpanels, please also list the number genes per sub panel.
450 unique genes in total with overlapping subpanel numbers below: Eye malformations (204) (GenU band H) MAC and aniridia (40) (GenU band G) ASD and Glaucoma (59) (GenU band H) Retinal Dystrophies (235) (GenU band H) Ocular albinism, photophobia and nystagmus (15) (GenU band G) Cataract, Congenital or Lens Malformations, Congenital (91) (GenU band H) Optic Atrophy, Childhood Onset (13) (GenU band G) Eye Movement Disorders (10) (GenU band G)
7b. Number of amplicons to provide this test (molecular) or type of test (cytogenetic) (n/a for panel tests)
N/A
7c. GenU band (based on 2016 version) that this test is assigned to for index case testing. **For NGS panel tests if there are sub panels, please provide GenU per subpanel.**
See 7a
8. Mutational spectrum for which you test including details of known common mutations (n/a for panel tests)
N/A
9a. Technical method(s) – please describe the test.
This test uses SureSelect (Agilent) for enrichment of target regions followed by Illumina sequencing.
9b. For panel tests, please specify the strategy for dealing with gaps in coverage.
Gaps (i.e. bases in CCDS exons and flanking +/-20bp intronic regions with less than 30 unambiguously mapped reads) will not be routinely filled but coverage will be detailed in reports. Gaps may be filled on a case-by-case basis in response to results obtained, e.g. if a single heterozygous mutation is identified for a recessive condition where the gene shows incomplete coverage.

9c. Does the test include MLPA?
(For panel tests, please provide this information in appendix 1)

See Appendix 1

9d. If NGS is used, does the lab adhere to the Association of Clinical Genetic Science Best Practice Guidelines for NGS?

Yes

10. Is the assay to be provided by the lab or is it to be outsourced to another provider?
If to be outsourced, please provide the name of the laboratory and a copy of their ISO certificate or their CPA number.

The assay is provided in our laboratory.

11. Validation process

Please explain how this test has been validated for use in your laboratory, including calculations of the sensitivity and specificity for the types of mutations reported to cause the clinical phenotype. Note that the preferred threshold for validation and verification is ≥95% sensitivity (with 95% Confidence Intervals). Your internal validation documentation can be submitted as an appendix (and will be included in the published Gene Dossier available on the website). The validation information should include data on establishing minimum read depth and horizontal coverage for the regions of interest, reproducibility of the pipeline, accuracy of variant calling, filtering of common variants and artefacts.

If this submission is for a panel test, please provide a summary of evidence of instrument and pipeline validation and complete the tables below. If the performance of the sub panels is expected to vary significantly to the data provided, please provide further details.

This test utilises an off-the-shelf clinical exome (Agilent SureSelect Focussed Exome) with added custom content to target additional required regions of the genome. Bioinformatic analysis will restrict variant calling to genes relevant to the clinical presentation, as detailed elsewhere in this dossier.

Analysis of data from the MiSeq/NextSeq/HiSeq sequencing instruments is conducted using an in-house developed pipeline of open-source tools, providing read alignment (BWA-MEM; Burrows Wheeler Aligner v0.7.5-a: http://bio-bwa.sourceforge.net/), variant calling (FreeBayes v0.9.21; https://github.com/ekg/freebayes) and variant annotation (Alamut-Batch v1.3.1; http://www.interactive-biosoftware.com/alamut-batch/).

Pipeline output is limited to variants within 20 base pairs of the donor and acceptor splice sites of CCDS exons. Variants are filtered when present at 2% or greater in ExAC (overall frequency), exome variant server (EVS) or 1000 genomes datasets or in greater than three patients on a run.

The combination of Agilent SureSelect enrichment with Illumina sequencing, analysed with the in-house data analysis pipeline, has been validated using SNVs (n=152) and small indels (1-6bp)(n=3) detected by Sanger sequencing or by alternative NGS technology. In addition, nine positive control samples were run for validation and verification of the clinical exome (SNV n=4, Indel=7, CNV=3). All mutations, apart from a 22 base-pair duplication, were detected using the standard analysis pipeline. These figures have been included in the validation data below. Manual inspection showed that the 22 base-pair duplication was present in sequencing reads (28 out of 239 reads) but was below the variant calling quality threshold.

Three positive control CNVs were correctly called using ExomeDepth (http://cran.r-project.org/web/packages/ExomeDepth/index.html). Samples will routinely be checked for CNVs using this method and positives confirmed by qPCR, however, the number of positive controls is insufficient to conclude that this method will robustly detect all CNVs. Mutation negative reports will therefore not state that CNVs have been excluded.

Due to the large number of genes included, it is not possible to give an accurate figure across the whole or sub-panels as to the predicted distribution of mutation type.

Validation of the analysis pipeline has principally addressed the detection of germline variation, since mosaicism has not been described for the majority of conditions tested, however the potential for

mosaicism in autosomal dominant and X-linked conditions is noted. Mosaicism has been documented in retinoblastoma in around 10% of families, but this is reduced to around 6% when considering mosaicism in the proband alone (Sippel, K.C. et al, AJHG 1998, 62(3):610-619). The potential for reduced sensitivity in cases of retinoblastoma is noted and referral to highly specialised services for pathogenic variant negative cases is appropriate. Whilst the variant calling algorithm has no lower read depth threshold for calling variants, the sensitivity to detect genuine variants at low alternate read percentages representing mosaicism has not been determined.

For panel tests:

Sensitivity 96.75-99.9% (95% CI)

Read depth minimum cut off: 30

	Previously tested	NGS test concordant results	NGS False negative
Number of patient samples			
Unique variants (total)	**169**	**168**	**1**
SNV	**156**	**156**	**0**
Indel (1bp to 22 bp)	**10**	**9**	**1**
CNV	**3**	**3**	**0**

Further validation using the 'Genome-in-a-bottle' (GIAB) sample NA12878 has been included below. Variant validation was restricted to regions which were of high confidence in the GIAB NIST dataset v3.3 and to regions covered by 30 or more reads in the clinical exome data. Since this dataset is significantly larger than the in-house dataset as described above, these data will be used for describing test sensitivity on clinical reports. Furthermore, since this process has highlighted the relatively poor sensitivity for the detection of indels, the single nucleotide variant and indel sensitivity figures will be quoted separately.

Average coverage across the target bases is 99.7%, therefore sensitivity figures have been adjusted to reflect this*.

	Known variants	NGS test concordant results	NGS False negative	Test sensitivity	*Adjusted test sensitivity	Test sensitivity (95% CI)
GIAB NA12878						
Unique variants (total)	**5494**	**5449**	**45**	**99.18%**	**98.88%**	**98.61--99.1%**
SNV	**5240**	**5240**	**0**	**100%**	**99.7%**	**99.63-99.7%**
Indel (1bp to 30 bp)	**254**	**209**	**45**	**82.82%**	**82.57%**	**76.79-86.51%**
CNV	**0**	**N/A**	**N/A**			

Specificity figures are not listed, since all clinically actionable reported variants are confirmed by Sanger sequencing. Therefore the combined specificity for these variants will be approaching 100%.

	Variant confirmed by other method	NGS False
Number of patient samples with a variant detected by NGS		
Unique variants (total)		
SNV		
Indel (1bp to X bp)		
CNV		

12a. Are you providing this test already?

Yes

12b. If yes, how many reports have you produced?

Sanger Based Tests	NGS Based Tests
	212

12c. Number of reports with a pathogenic (or likely pathogenic) mutation identified?

Sanger Based Tests	NGS Based Tests
	56

12d. Please provide the time period in which these reports have been produced and whether in a research or a full clinical diagnostic setting.

These tests were performed in a research setting in a period from 2015 to 2016 using a bespoke stand-alone panel, prior to transition to the diagnostic service using the clinical exome as described in this document.

13a. Is there specialised local clinical/research expertise for this disorder?

Yes

13b. If yes, please provide details

Prof Jane Sowden is Professor of Developmental Biology and Genetics in the UCL Great Ormond Street Institute of Child Health and NIHR Senior Investigator. Her research group works on eye development and repair and aim to define the molecular genetic pathways that regulate eye development, to understand how these pathways are disrupted in congenital eye disease, and to apply knowledge of retinal development to devise new strategies to repair and regenerate the diseased retina. The group conduct DNA analysis to identify the genetic causes of childhood blindness working closely with GOSH Ophthalmology and Clinical Genetics Departments and the North East Thames Genetics Laboratories such that this panel has now been translated into service. The GOSH Ophthalmology Department provide specialist expertise in a wide range of rare childhood disorders. By correlating molecular diagnoses with clinical phenotype and outcomes they aim to support clinical practice and management in paediatric ophthalmology.

14. If using this form as an Additional Provider application, please explain why you wish to provide this test as it is already available from another provider.

EPIDEMIOLOGY

15. Estimated prevalence and/or incidence of conditions in the general UK population

For panel tests, please provide estimates for the conditions grouped by phenotypes being tested.

Prevalence is total number of persons with the condition(s) in a defined population at a specific time (i.e. new and existing cases).

e.g. CF prevalence approx. 12 per 100,000 with UK population of approx. 63 million the prevalence of affected individuals in the UK is 7560

Incidence is total number of newly identified cases in a year in a defined population. e.g. CF incidence 1/2650 live births in a UK population with 724,000 live births in a year = 273 new cases a year

Please identify the information on which this is based.

Prevalence:

MAC: 1 per 10,000 (Shah et al., 2011, Morrison et al., 2002)
ASD: Axenfeld Rieger Syndrome 1 per 200,000 (Orphanet)
Glaucoma 1 -9 per 100,000 (Orphanet)
Retinal dystrophies 1 per 3,500-4000 (Hartong et al., 2006)
Albinism 1 per 17,000 (http://www.evidence.nhs.uk/Search?ps=50&q=ocular+albinism)
Cataract 1 per 2,890 (Gillespie et al 2014)

Incidence

MAC: 72 new cases per year
ASD: Axenfeld Rieger Syndrome 36 new cases per year
Glaucoma 72 new cases per year
Retinal dystrophies: 241 new cases per year
Albinism: 42.5 new cases per year
Cataract: 250 new cases per year

References

Gillespie, R.L., et al., Personalized diagnosis and management of congenital cataract by next-generation sequencing. Ophthalmology, 2014. 121(11): p. 2124-37.e1-2.
Hartong DT, Berson EL, Dryja TP; Retinitis pigmentosa. Lancet. 2006 Nov 18;368(9549):1795-809.
Morrison, D., et al. National study of microphthalmia, anophthalmia, and coloboma (MAC) in Scotland: investigation of genetic aetiology. J Med Genet, 2002, 39, 16-22
Shah, S.P., et al. Anophthalmos, microphthalmos, and typical coloboma in the United Kingdom: a prospective study of incidence and risk. Invest Ophthalmol Vis Sci, 2011, 52, 558-64
http://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=782 Axenfeld Rieger accessed 27/07/16
http://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=98976 congenital glaucoma accessed 27/07/16
http://www.evidence.nhs.uk/Search?ps=50&q=ocular+albinism accessed 27/07/16

16. Estimated gene frequency (Carrier frequency or allele frequency)

Please identify the information on which this is based.
n/a for panel tests.

N/A

17. Estimated penetrance of the condition. Please identify the information on which this is based

n/a for panel tests

N/A

18. Estimated prevalence of conditions in the population of people that will be tested.

n/a for panel tests.

N/A

INTENDED USE

19. Please tick either yes or no for each clinical purpose listed.
Panel Tests: a panel test would not be used for pre symptomatic testing, carrier testing and pre natal testing as the familial mutation would already be known in this case and the full panel would not be required.

Diagnosis	☒ **Yes**	☐ **No**
Treatment	☒ **Yes**	☐ **No**
Prognosis & management	☒ **Yes**	☐ **No**
Presymptomatic testing (n/a for Panel Tests)	☐ **Yes**	☐ **No**
Carrier testing for family members (n/a for Panel Tests)	☐ **Yes**	☐ **No**
Prenatal testing (n/a for Panel Tests)	☐ **Yes**	☐ **No**

TEST CHARACTERISTICS

20. Analytical sensitivity and specificity
The *analytical sensitivity* of a test is the proportion of positive results correctly identified by the test (true positive/true positive + false negative). The *analytical specificity* of a test is the proportion of negative results correctly identified by the test (true negative/true negative + false positive).
This should be based on your own laboratory data for (a) the specific test being applied for or (b) the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up. Please specify any types of mutations reported to cause the clinical phenotype that cannot be detected by the test.
Note that the preferred threshold is ≥95% sensitivity (with 95% Confidence Intervals).

The analytical sensitivity as shown in Q11 is 96.75-99.9% (95% CI) for single base substitutions and small insertion/deletions. The sensitivity to detect larger indels and CNVs is uncertain. The specificity of the test for reported likely pathogenic mutations will be approaching 100% as these mutations will be confirmed by Sanger sequencing.

21. 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 disorder is known to be absent. The denominator in this case is the number with the disorder (for sensitivity) or the number without condition (for specificity).
Please provide the best estimate. UKGTN will request actual data after one year service.
For a panel test, the expected percentage diagnostic yield for the test in the target population can be presented as an alternative to clinical sensitivity and specificity?

The diagnostic yield was 26% across 212 paediatric samples when the panel was applied in a research setting. Yield varies between panels, as demonstrated in the breakdown below.

MAC 6/82 = 7.3%
ASD & Glaucoma 27/93 = 29%
Retinal 19/33 = 57.6%
Cataract, congenital or lens malformations, congenital 4/4 = 100%

The clinical sensitivity for all sub-panels will relate to the proportion of cases linked to loci which are not targeted by this test, due to the causative gene being as yet unidentified. Figures can be provided for all groups following provision of service. Although mosaicism has not been described for the majority of conditions tested for, this phenomenon must be considered as a potential source of reduced sensitivity in dominantly-acting and X-linked conditions.

22. 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).
Not currently requested for panel tests

N/A

23. Testing pathway for tests where more than one gene is to be tested sequentially
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. This will be added to the published Testing Criteria.
n/a for panel tests

N/A

CLINICAL UTILITY

24. How will the test change the management of the patient and/or alter clinical outcome? Please summarise in 2-3 sentences – no more than 50 words.

Different ocular birth abnormalities can demonstrate overlapping phenotypes. This can result in incorrect clinical diagnoses. In affected children the early genetic diagnosis of syndromic conditions, following presentation of the congenital eye findings as an entry point for genetic testing, may reduce the time period before information, diagnosis and appropriate management can be provided for associated non ocular pathologies. More than 50% of the panel genes cause ocular conditions that can be associated with non ocular pathologies.

This test will help patients and their families to understand the cause of their condition and ensure that appropriate genetic counselling can be offered to them. This will allow accurate prognosis, estimate of recurrence risks and in some cases the potential for novel treatment options or inclusion in clinical trials.

25. Please provide full description on likely impact on management of patient and describe associated benefits for family members. If there are any cost savings AFTER the diagnosis, please detail them here.

Differential diagnoses can impact the patient management and results in benefits for family members as differential genetic results may lead to carrier testing or prenatal screening. Early genetic diagnosis for children with congenital eye conditions will reduce costs in some cases and in others will lead to more effective and more appropriate early treatment potentially preserving sight.

Sight loss has long term economic and social costs for the health service and affected families.
It is not possible to reliably estimate the cost savings per year based on the range of condition to be diagnosed. It will be possible once the test is in service.
Overall cost savings following positive genetic test results will include the following:
(i) Repeat ophthalmology appointments to monitor intraocular pressure become unnecessary in family members without the specific gene defects identified in a child with developmental glaucoma.
(ii) Appropriate early dietary management of metabolic congenital cataract can prevent the need for cataract surgery if implemented early.
(iii) Children with ocular conditions as part of rare syndromic condition will undergo multiple hospital visits and multiple tests (e.g biochemical, biopsy, physiology or imaging analysis), often over several years, commonly referred to as the diagnostic odyssey. Congenital eye conditions noted early in development and early panel testing will reduce the time and number of hospital visits before diagnosis particularly important for syndromic conditions.
(iv) Repeat genetic testing will not be necessary for complex childhood eye conditions because of the gene range provided on this panel

The results from the congenital eye disorders panel have so far have been provided as “research” findings under the supervision of diagnostic staff.
>30 reports generated so far show the clinical utility of this panel for paediatric ophthalmology patients by defining the genetic cause of syndromic conditions, and providing information that alters management, and counselling of patients**.**

26a. If this test was not available, what would be the consequences for patients and family members? Please describe in not more than 50 of words.

Most children and families with congenital eye conditions would be left untested if a panel test was not available to make genetic diagnosis an efficient process. Without a clear diagnosis, families cannot be counselled about recurrence risk.

26b. The consequences for patients and family members if this test was not available – if required please expand on the response provided in question 26a.

Patients and families would not have the opportunity to prepare for the future, which may include progressive blindness, dual sensory disorders, and delayed diagnosis of other medical complications. This preparation may include psychosocial issues and/or practical issues to assist the child in school and improve quality of life as far as possible. In other cases the result may mean that the patient’s condition

will not worsen (e.g. congenital stationery night blindness) or other siblings are not at risk and do not need repeated testing, for instance for glaucoma. Some genetic ocular conditions can benefit from gene/cell therapies if diagnosed.

Without knowing the genetic diagnosis, PGD, prenatal screening and carrier testing could not be offered.

27. 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.

There may be other means of diagnoses for some of these conditions. For example, intraocular pressure for glaucoma, electrophysiology and retinal images for retinal dystrophies. However, differential diagnosis cannot always be made without a molecular diagnosis and often a diagnosis is not made without a molecular test. Ophthalmic testing in young children is also often not possible and detailed examination may require a general anaesthetic. Molecular diagnosis can distinguish between dominant and recessive forms of glaucoma and between different phenotypic subtypes e.g. primary congenital glaucoma and glaucoma associated with Axenfeld-Rieger syndrome.

28. Please list any genes where the main phenotype associated with that gene is unrelated to the phenotype being tested by the panel. For example, lung cancer susceptibility when testing for congenital cataract because ERCC6 gene (primarily associated with lung cancer) is included in a panel test for congenital cataract.

The following genes are also associated with phenotypes that are different from the phenotype being tested on the panel:

- ABCB6: Dyschromatosis universalis hereditaria 3; Pseudohyperkalemia, familial, 2, due to red cell leak
- C12ORF65: Spastic paraplegia 55, autosomal recessive
- COL2A1: Achondrogenesis, type II or hypochondrogenesis; Avascular necrosis of the femoral head; Czech dysplasia; Legg-Calve-Perthes disease; Osteoarthritis with mild chondrodysplasia; Otospondylomegaepiphyseal dysplasia; Platyspondylic skeletal dysplasia, Torrance type; SMED Strudwick type; Spondyloepiphyseal dysplasia, Stanescu type; Spondyloperipheral dysplasia.
- COL4A1: Angiopathy, hereditary, with nephropathy, aneurysms, and muscle cramps; Porencephaly 1.
- CRYAB: Cardiomyopathy, dilated, 1II; Myopathy, myofibrillar, 2; Myopathy, myofibrillar, fatal infantile hypertrophy, alpha-B crystallin-related.
- ELOVL4: Ichthyosis, spastic quadriplegia, and mental retardation; ?Spinocerebellar ataxia 34
- ERCC6: {Lung cancer, susceptibility to}; Cockayne syndrome, type B; De Sanctis-Cacchione syndrome; Premature ovarian failure 11; UV-sensitive syndrome 1.
- HK1: Hemolytic anemia due to hexokinase deficiency; Neuropathy, hereditary motor and sensory, Russe type
- MVK: Mevalonic aciduria; Hyper-IgD syndrome; Porokeratosis 3, multiple types.
- NEUROD1: Maturity-onset diabetes of the young 6
- RB1: Small cell cancer of the lung, somatic; Osteosarcoma, somatic; Bladder cancer, somatic.

29. If testing highlights a condition that is very different from that being tested for, please outline your strategy for dealing with this situation.

Pre and post-test genetic counselling, multidisciplinary management and referral to appropriate paediatric services

30. If a panel test, is this replacing an existing panel/multi gene test and/or other tests currently carried out by your lab e.g. Noonan Spectrum Disorders 12 Gene Panel replaced multigene Sanger test for KRAS, RAF1, PTPN11 and SOS1? If so, please provide details below.

No

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

There are no specific ethical, legal or social issues for this test.

32. REAL LIFE CASE STUDY
Please provide a case study that illustrates the benefits of this test

This case study describes a definitive diagnosis made where patient would not have received a diagnosis as no current molecular test exists either as a single gene test or within an NGS panel.

The patient's phenotype is congenital corneal opacity, right eye and left eye microphthalmia and optic disc coloboma, 4/5 hand syndactyly, 3/4 foot syndactyly with missing digit.
Following sequencing using the Oculome, two class 4 heterozygous variants were detected in *SMOC1* (SPARC-RELATED MODULAR CALCIUM-BINDING 1, OMIM: 608488): c.378G>C p.(Gln126His) heterozygote and c.379-2A>T p.(?) heterozygote.

Recessive mutations in this gene are associated with the rare condition Ophthalmo-Acromelic syndrome (OAS) also known as microphthalmia with limb anomalies. This disorder is 'characterised by bilateral microphthalmia or anophthalmia, synostosis, syndactyly, oligodactyly and/or polydactyly' (Orphanet ORPHA1106). Sanger sequencing in both parents confirmed their carrier status and that these mutations were on different alleles.

In this case the family can be offered genetic counselling to inform on the high (1/4) recurrence risk. Prenatal diagnosis can be offered to the family in any future pregnancy.

References:
Okada, Hamanoue et al. Am J Hum Genet. 2011 Jan 7; 88(1): 30–41
Rainger, van Beusekom et al. PLoS Genet. 2011 Jul;7(7):e1002114

UKGTN Testing Criteria

Test name: Retinal Dystrophy 235 Gene Exome Panel

Approved name and symbol of disorder/condition(s): *See website listing*	**OMIM number(s):**
Approved name and symbol of gene(s): *See website listing*	**OMIM number(s):**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Paediatric Ophthalmologist	
Consultant Clinical Geneticist	
Consultant Adult Ophthalmologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Patients present with decreased rod/cone responses on an ISCEV ERG **AND**	
Poor night vision from infancy **OR**	
Nystagmus **OR**	
Reduced vision from infancy **OR**	
Phenotypic evidence of retinal degeneration as shown by clinical features, e.g. retinal bone spicules, retinal thinning on OCT scans, typical visual field defects such as mid peripheral retinal scotoma, foveal abnormality.	

Additional Information:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

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.

UKGTN Testing Criteria

Test name: **Cataract, Congenital, or Lens Malformations, Congenital 91 Gene Exome Panel**	
Approved name and symbol of disorder/condition(s): *For panel tests: See website listing*	**OMIM number(s):**
Approved name and symbol of gene(s): *For panel tests: See website listing*	**OMIM number(s):**

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

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Patients present with one of the following:	
• Congenital Bilateral Cataracts	
• Aphakia	
• Congenital Lens Malformations	

Additional Information:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

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.

UKGTN Testing Criteria

Test name: Eye Movement Disorders 10 Gene Exome Panel	
Approved name and symbol of disorder/condition(s): *For panel tests: See website listing*	**OMIM number(s):** **OMIM number(s):**
Approved name and symbol of gene(s): *For panel tests: See website listing*	

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

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Problems with visual acuity need to be excluded **AND**	
Patients present with a squint (eye misalignment) at a young age	
OR Diagnosis of congenital cranial dysinnervation disorders including congenital fibrosis of extraocular muscles, Duane syndrome, Moebius syndrome and horizontal gaze palsy with progressive scoliosis	

Additional Information:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

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.

UKGTN Testing Criteria

Test name: Anterior Segment Dysgenesis and Glaucoma 59 Gene Exome Panel	
Approved name and symbol of disorder/condition(s): *For panel tests: See website listing*	**OMIM number(s):**
Approved name and symbol of gene(s): *For panel tests: See website listing*	**OMIM number(s):**

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

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Patients present with congenital anterior segment abnormalities (e.g. aniridia, sclerocornea, anterior synechiae, corneal opacity, iris hypoplasia, microcornea) **OR**	
Diagnosis of developmental glaucoma **OR**	
Diagnosis of corneal dystrophy, corneal abnormality	

Additional Information:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

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.

UKGTN Testing Criteria

Test name: Microphthalmia, Anophthalmia and Coloboma (MAC) spectrum and Aniridia 40 Gene Exome Panel	
Approved name and symbol of disorder/condition(s): *For panel tests: See website listing*	**OMIM number(s):**
Approved name and symbol of gene(s): *For panel tests: See website listing*	**OMIM number(s):**

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

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Normal Karyotype or array CGH **AND** one or more of the following eye phenotype as a *prominent feature* of the clinical presentation:	
Anophthalmia **(please specify-Left or Right eye or Both)**	
Microphthalmia **(please specify-Left or Right eye or Both)**	
Coloboma **(please specify-Left or Right eye or Both)**	
Aniridia (typical of Classical Aniridia MIM #106210) **(please specify-Left or Right eye or Both)**	
Iris hypoplasia (typical of Gillespie syndrome MIM #206700) **(please specify-Left or Right eye or Both)**	

Additional Information:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

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.

UKGTN Testing Criteria

Test name: Ocular albinism, photophobia and nystagmus 15 Gene Exome Panel	
Approved name and symbol of disorder/condition(s): *For panel tests: See website listing*	**OMIM number(s):**
Approved name and symbol of gene(s): *For panel tests: See website listing*	**OMIM number(s):**

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

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Ocular albinism **AND**	
Photophobia **AND**	
Nystagmus	

Additional Information:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

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.

UKGTN Testing Criteria

Test name: Optic Atrophy, Childhood Onset 13 Gene Exome Panel	
Approved name and symbol of disorder/condition(s): *For panel tests: See website listing*	**OMIM number(s):**
Approved name and symbol of gene(s): *For panel tests: See website listing*	**OMIM number(s):**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Paediatric Ophthalmologist	
Consultant Clinical Geneticist	
Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Patients present with evidence of optic nerve dysfunction on clinical examination **AND** exclusion of optic atrophy secondary to inherited outer retinal disease **AND**	
Evidence of primary retinal ganglion cell dysfunction with optical coherence tomography imaging **OR**	
Visual electrophysiology performed to ISCEV standards	

Additional Information:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

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.

UKGTN Testing Criteria

Test name: Eye Malformations, Congenital, 204 gene exome panel

Approved name and symbol of disorder/condition(s): *For panel tests: See website listing*	**OMIM number(s):**
Approved name and symbol of gene(s): *For panel tests: See website listing*	**OMIM number(s):**

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

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Patients presenting with eye malformations as one of the following (singly or in combination and with or without additional syndromic features):	
• Anophthalmia (clinical absence of the eye)	
• Severe microphthalmia (congenital reduction in the overall size of the globe)	
• Ocular coloboma	
• Congenital anterior segment abnormalities (e.g. aniridia, sclerocornea, anterior synechiae, corneal opacity, iris hypoplasia, microcornea)	
• Congenital/Developmental glaucoma	
• Congenital bilateral cataracts or aphakia	
• Cryptophthalmos	
• Eyelid coloboma	

Additional Information:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

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.

IS IT A REASONABLE COST TO THE PUBLIC?

36. Based on experience what will be the national (UK wide) expected activity for requesting this test, per annum, for:

Index cases: 700+ (based on incidence – see Q15). Expected referral to our service: 150

Family members where mutation is known : 100

If a NGS panel test, it is recognised that the full panel will not be used to test family members where the familial mutation is known. Please provide expected number of tests to inform completion of Q40

37. If your laboratory does not have capacity to provide the full national need please suggest 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. If you are unable to answer this question please write "unknown".

The national need is relatively large, but other centres already offer some analogous eye disorder panels and others will probably be made available via similar clinical exome sequencing virtual panels. We would not therefore expect the full national need to be tested via our service, and we would be able to meet the needs of the expected referral numbers for the panel here (~150 samples).

38. In order to establish the potential costs/savings that could be realised in the diagnostic care pathway, please list the tests/procedures that are no longer required to make a diagnosis for index cases where index cases would have the molecular genetic test proposed in this gene dossier at an earlier stage in the pathway. It is the tests/procedures that would be stopped for patients that are eligible for the gene test.

This information will be used to calculate the overall investment / savings required in Q39

Example:
The introduction of a 95 gene panel for syndromic and non syndromic hearing loss would allow those patients who are recognised early enough in their pathway to diagnosis to be offered the genetic test instead of having sequential gene tests for individual genes already available and repeated ECGs, ERGs & renal ultrasounds as part of the diagnostic pathway although these may still be required as part of management after diagnosis.

	Type of test	Cost (£)
Imaging procedures	Repeated MRI	£250
Laboratory pathology tests (other than molecular/cyto genetic test proposed in this Gene Dossier)	Single gene tests	£1000
Physiological tests (e.g. ECG)		
Other investigations/procedures (e.g. biopsy)	Biopsy / Anaesthetic (Paeds)	£1000
Associated inpatient stays in the diagnostic pathway		
Total cost of tests/procedures to be stopped (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)	[redacted]	**£2250**
If any of the tests/procedures listed above would be carried out on individuals after having the genetic test because the genetic test did not pick up a pathogenic mutation (i.e. negatives), please indicate the costs for these tests to continue to diagnosis. ***For example a panel test replaces single gene tests that have been included above, but after the panel test an individual that tests negative would not need to have these single gene tests, because the genes were on the NGS panel.***	[redacted]	**MRI/Biopsy £1250**

39. Please complete the Excel spread sheet available to download from the UKGTN website to calculate the estimated investment or savings, based on the expected annual activity of index & family cases (Q36 above) and using the information provided in Q38.

Cost neutral

40. Please indicate the healthcare outcomes that apply to this test after diagnosis. It is recognised that all tests recommended by the UKGTN for NHS service improve clinical management and, if a familial mutation is found, allows for prenatal testing and therefore these are not included in the list below. This information provides a useful guide to commissioners on the utility of the test.

Healthcare outcomes	Does this apply to this test?
1. Alerts significant clinical co-morbidities	Yes
2. Reduces mortality/saves lives	Yes (treatment of Retinoblastoma)
3. Avoids irreversible harm	Yes
4. Avoids diagnostic procedures/tests (some of which may be invasive) and/or multiple hospital appointments	Yes
5. Avoids incorrect management (e.g. medication or treatment) that could be harmful	Yes
6. Confirms targeted therapy/management	Yes
7. Earlier diagnosis allowing commencement of treatment earlier with associated improved prognosis	Yes
8. Enables access to educational and social support	Yes
9. At risk family members that test negative for a familial mutation can be discharged from follow up	Yes
10. At risk family members that test positive for a familial mutation have appropriate follow up	Yes