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

Submitting laboratory: Salisbury RGC

1. Disorder/condition – approved name and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website)

If this submission is for a panel test please complete appendix 1 listing all of the conditions included using approved OMIM name, symbol and OMIM number.

Disorders of Sexual Development

2. OMIM number for disorder/condition

If a panel test – see 1. above

see appendix 1

3a. Disorder/condition – please provide, in laymen's terms, a brief (2-5 sentences) description of how the disorder(s) affect individuals and prognosis.

Disorders of sexual development (DSDs) are a group of conditions where the reproductive organs and genitals do not develop normally and affect one in 4,500 infants. Sometimes it is not possible to tell whether the baby is a boy or girl by a clinical examination alone. These conditions can influence a child's future fertility, risk of cancer, gender identity, sexual function and their ability to grow normally. In some cases hormone replacement is required.

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

There are many different causes of DSD and knowing the correct one is important to ensure the appropriate information and treatment is given to individual patients. Identification of the genetic cause could help in several areas: gender assignment, diagnosis of genital anomaly and adrenal problems (which can be life-threatening), fertility counselling and diagnosis of gonadal dysgenesis with gonadal failure. Genetic information also allows genetic counselling of the family and accurate advice on the likelihood of the condition recurring.

There are many different causes of DSD and knowing the correct one is important to ensure the appropriate information and treatment is given to individual patients. Identification of the genetic cause could help in several areas: gender assignment, diagnosis of genital anomaly and adrenal problems (and diagnosis of gonadal dysgenesis with gonadal failure. Genetic information also allows genetic counselling of the family and accurate advice on the likelihood of the condition recurring.

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 table in appendix 1.

see appendix 1

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 appendix 1 listing all of the genes included using approved HGNC name, symbol, number and OMIM number.

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.

total number of genes is 28

7b. Number of amplicons to provide this test (molecular) or type of test (cytogenetic)

(n/a for panel tests)

n/a

7c. GenU band that this test is assigned to for index case testing.

G

8. Mutational spectrum for which you test including details of known common mutations

(n/a for panel tests)

If this application is for a panel test to be used for different clinical phenotypes and/or various sub panel tests – please contact the team for advice before completing a Gene Dossier

n/a

9a. Technical method(s) – please describe the test.

Probes for the gene panel will be selected from the Illumina Trusight One Clinical Exome Panel. Library preparation will use Nextera Rapid Capture Custom Enrichment (Illumina) for sequencing on an Illumina MiSeq.

The technical method can de divided into a number of steps as outlined below:

1. Tagmentation of input DNA
2. Indexing PCR using primers targeted to universal sequences added in step 1
3. Product pooling
4. Hybridisation and capture
5. Post capture amplification
6. Library QC
7. Sequencing (Illumina MiSeq / NextSeq)
8. Read alignment
9. Indel realignment
10. Base quality score recalibration
11. Variant calling
12. Variant filtering
13. Variant annotation

Samples are identified via a pair of 8bp identity sequences, one appended to each end of the finished library constructs by an automated assay. All samples and reagents are bar-coded for tracking and recording.

Data analysis is performed using a bespoke pipeline following recommendations from the Broad Institute, who provides the *de facto* tools and advice for Illumina sequencing analysis. Briefly, paired-end reads are globally aligned to the GRCh37 reference genome and filtered to exclude amplification duplicates based on identical mapping positions. For inclusion, a minimum mapping quality score (MQ) of 40 is used and a read pair's best alignment score must be at least three times greater than its second best score.

Secondly, reads mapped near known or suspected indels are re-aligned to minimise the number of mismatching bases across all reads. Thereafter, the base quality scores are recalibrated to better reflect their true likelihood of error. Recalibration is achieved by analysing co-variation such as reported base quality, cycle and sequence context associated with non-dbSNP138 reference mismatches. Finally, variants are identified and given a probability of error using a Bayesian approach within locally assembled regions followed by filtering and annotation.

An excel output is generated showing gaps in the horizontal coverage for core genes and any sequence variants (excluding known polymorphisms) that require confirmation, i.e. any region in a core gene not covered >=x30 and all detected variants to be reported are tested/confirmed by a second test.

Filtering for clinical significance and categorisation of variants for the purpose of reporting is performed in a semi-automated process with the aid of disease-specific databases (in-house and online) and Alamut software.

The complete DSD panel will be run on all patients. However, depending upon the specific referral criteria, the NGS sequencing data will be "mined" to create one of three overlapping sub-panels:

1. **Gender Assignment (13 genes):**

   AR, DHH, DMRT1, HSD17B3, HSD17B2, SRD5A2, SRY, SOX9, LHCGR, CBX2, CYP19A1, MAMLD1,

   RSPO1.

2. **Genital Anomalies and suspected Adrenal problems (12 genes):**

   CYP21A2, CYP11A1, CYP11B1, DHH, CYP17A1, DHCR7, SOX9, NR5A1, ATRX, POR, STAR, HSD3B2

3. **Gonadal Dysgenesis with Gonadal failure (12 genes):**

   WNT4, WT1, NR0B1, DMRT1, SRY, CBX2, SOX9, NR5A1, POR, STAR, ARX, MAP3K1.

9b. For panel tests, please specify the strategy for dealing with gaps in coverage.

Reportable diagnostic coverage is defined as read depth greater than or equal to 30x on a per base basis. In general the minimum region of interest comprises all coding exons +/- 10bp of intronic sequence to include the consensus splice sites.

Mean coverage for each gene calculated from the sub-panel verification data is given in the appendix.

Core genes can be defined as genes for which we currently perform a diagnostic test or critical genes whose screening provides most clinical utility; therefore a total of six genes across the sub-panels are core genes. For "core" genes all horizontal coverage gaps in relation to the minimum region of interest are filled in on a run by run basis to provide horizontal coverage greater than or equal to that provided by the previous conventional methodology.

Gap filling to ensure horizontal coverage is currently performed by Sanger sequencing. From 2015 we will transfer to a PCR based genotyping assay to amplify specific fragments and sequence on an Illumina MiSeq.

For non-core genes, no additional work is done to increase coverage above that provided by the panel test. Percentage coverage of the minimum region of interest is calculated per run for each gene analysed and indicated on the report.

9c. Does the test include MLPA?

(For panel tests, please provide this information in appendix 1)

Yes for some genes in the panel. 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.

Provided by the lab.

11. Validation process

Please explain how this test has been validated for use in your laboratory or submit your internal validation documentation. If this submission is for a panel test, please provide a summary of evidence of:

i) instrument and pipeline validation, and

ii) panel verification for the test

Please submit as appendices to the Gene Dossier (these will be included in the published Gene Dossier available on the website).

Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.

(1) Validation.

The full internal validation document is attached as a separate document.

The Illumina TruSightOne sequencing panel (P/N FC-141-1006 and FC-141-1007) is a hybridisation based capture kit that covers 4813 genes designed for preparation of targeted libraries for next generation sequencing on Illumina platforms. The kit and / or sub-panels derived from the kit will be used for mutation scanning in a variety of different disease conditions.

Validation of the Illumina TruSightOne sequencing panel was performed for NGS mutation scanning to calculate estimates of general sensitivity and specificity; individual sub-panels will be verified against the performance specifications defined by this validation before diagnostic use. The methodology was validated for the detection of both single nucleotide variations and small indels.

Three sequencing libraries were prepared according to the manufacturer's published protocol for the TruSightOne sequencing panel (Part # 15046431 Rev. A October 2013). Each library preparation included one cell line DNA sample (CEPH/UTAH PEDIGREE 146, sample NA12878) pooled with two other genomic DNA samples and sequenced on a separate MiSeq V3 2x150 sequencing run.

Data analysis was performed using a bespoke bio-informatics pipeline described in section 9a.

There was complete concordance for all variants detected in the genomic DNA samples compared with those expected form previous analysis of specific genes (BRCA1 and BRCA2). Detection rate was determined by comparing high confidence variation calls available from Genome in a bottle (GIAB platinum project) for the cell line sample NA12878 with calls made in the validation runs (data from ftp://ftp-trace.ncbi.nih.gov/giab/ftp/technical/platinum_genomes/IlluminaPlatinumGenomes_v7.0/merged_platinum/NA12878.vcf) . No depth cut-off was used to determine failed regions in the validation runs. Therefore gold standard calls include variants in regions not covered by the validation.

Results:

Table1. Validation across full TruSight One sequencing Panel

Variant Type	Gold standard[1,2]	Run 1 (A6257)	Run 2 (A63A6)	Run 3 (A639R)	True positive[3]	False negative[3,4]	Detection rate	95% confidence interval
Heterozygous SNVs	4,850	4,706	4,721	4,651	4,693	157	96.8%	96.3% - 97.2%
Homozygous SNVs	2,917	2,895	2,896	2,880	2,890	27	99.1%	98.7% - 99.3%
Heterozygous Indels	277	255	256	248	253	24	91.3%	88.0% - 93.9%
Homozygous Indels	149	145	142	143	143	6	96.2%	92.2% - 98.2%

1 Gold standard calls from ftp://ftp-trace.ncbi.nih.gov/giab/ftp/technical/platinum_genomes/IlluminaPlatinumGenomes_v7.0/merged_platinum/NA12878.vcf
2 No depth cutoff used to determine failed regions in the validation runs. Therefore gold standard calls include variants in regions not covered by the validation.
3 Totals for true positives and false negatives taken as mean values over 3 biological replicates (runs 1, 2 and 3)
4 False negatives include116 variants not detected in any of the 3 validation runs

Specificity: 97-98% (95%CI)

Repeatability: 97.8-98.4% (95% CI)

Therefore, the library preparation and sequencing methodologies and the data analysis pipeline have been shown to perform to a level of accuracy above the preferred threshold stipulated by ACGS guidelines of > 95% sensitivity with 95% Confidence Intervals. Therefore this process is validated for use in the WRGL for the purpose of mutation scanning for constitutional variants in samples extracted form peripheral blood or similar material.

(2) Verification.

All 4813 genes in the Illumina TruSight One are covered by the validation described above. From the validation data, the results are shown below for sequence variants specifically within the genes of this sub-panel:

Variant Type	Gold standard[1,2]	Run 1 (A6257)	Run 2 (A63A6)	Run 3 (A639R)	True positive[3]	False negative[3]	Detection rate
Heterozygous SNVs	19	19	19	19	19	-	100.0%
Homozygous SNVs	20	20	20	20	20	-	100.0%
Heterozygous Indels	2	2	2	2	2	-	100.0%
Homozygous Indels	2	2	2	2	2	-	100.0%

[1] Gold standard calls from ftp://ftp-trace.ncbi.nih.gov/giab/ftp/technical/platinum_genomes/IlluminaPlatinumGenomes_v7.0/merged_platinum/NA12878.vcf

[2] No depth cutoff used to determine failed regions in the validation runs. Therefore gold standard calls include variants in regions not covered by the validation.

[3] Totals for true positives and false negatives taken as mean values over 3 biological replicates (runs 1, 2 and 3)

The intended purpose of the validation was to determine the sensitivity of the technology over the full range of genes on the TruSight One panel, which includes all the genes in the derived sub-panel defined here. A real variant could be of any type and located anywhere and as such may indeed not be detectable, however it is not possible to determine the sensitivity for all possible variants – rather an 'estimate' must be made based on the representative selection available. Consequently all validations are estimated extrapolations of how accurate the test is likely to be in practice. The sensitivity calculations for this panel are based on a very large number of data points and thus take into account as much variation in the ability to detect all types of sequence variations as possible.

Whilst the full range of variant types was not actually seen in this particular gene set there is no reason to suspect that they would not behave as modelled by the large number of variants used for the validation. It is the number of variants tested in the validation that provides confidence that this is the case. We have performed a conventional gene by gene verification for BRCA1 and BRCA2 and for these genes performance and coverage in the derived sub-panel were equivalent to the full validation.

The purpose of verification is to show that the particular region of interest is likely to perform as per the overall validation and not to determine sensitivity for particular variants in the specific region of interest. The addition of one or two variants in these particular genes does not add to the statistical power to determine sensitivity for the region of interest. The best surrogate marker for performance equivalence to the validation is equivalent read coverage in the region of interest. We have therefore based the verification of this panel on whether or not we were able to achieve robust read coverage for the region of interest. Verification was performed using the same library preparation, sequencing and data analysis methods as the full validation; the proportion of bases covered >x30 over the region of interest for each gene during the verification run is shown in appendix 1.

The approach of a large scale technical validation to determine sensitivity followed by verification of derivative sub-panels against the parameters defined in the validation was approved during a CPA inspection of the WRGL's NGS service in December 2014.

To verify that the NGS technology and bioinformatic pipeline would perform as well as the validation, we have run a total of 32 retrospective patients. Each patient had at least one (pathogenic) sequence change and most had a number of other neutral sequence changes and/or a variant of unknown clinical significance that had been previously identified by conventional mutation scanning.

In 20 patients screened for BRCA1 and BRCA2, there were a total of 121 heterozygous sequence variants: 12 indels (size range 1 to 11 bp) and 109 single base substitutions (including nonsense

mutations and splice site mutations).

A further 12 patients were screened for a range of different genes: (NF1, PTPN11, RAF1, FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2 and MYH11). These patients had a total of three indels (size range 2 to 4 bp) and 22 SNPS.

All 146 sequence variants (15 indels and 131 SNPS) were successfully identified. (Sensitivity with 95% CI >97%). Therefore the NGS technology is verified to detect a range of mutation types across a wide range of genes.

12a. Are you providing this test already?

No.

We do offer testing for two of the genes individually: NR0B1 (DAX1 - sequencing and MLPA) and SRY (dosage testing only and not mutation scanning).

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

NR0B1 - approx 200

SRY - approx 380

12b(ii). Number of reports mutation positive?

NR0B1 - approx 60

SRY - not mutation scanning

12b(iii). Number of reports mutation negative?

NR0B1 - approx 140

SRY - not mutation scanning

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

NR0B1 since 2000

SRY - since 1995

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

Yes

13b. If yes, please provide details

Dr Katherine Lachlan is a Consultant Clinical Geneticist and Dr Justin Davies is a Consultant in Paediatric Endocrinology; both have a clinical and academic interest in DSD.

There is also an established regional DSD team based at Southampton composed of a geneticist, endocrinologist, psychiatrist, urologist and gynaecologist. The team is supported by a supra-regional steroid assay service based at Southampton with access to tandem mass spectrometry. Collaboration already exists between the Wessex Regional Genetics Laboratory and the Southampton DSD clinic.

14. Based on experience what will be the national (UK wide) activity, per annum, for:

Index cases = 7-10 in our region ~ 200 nationally / year

Family members where mutation is known = 24

15. 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".

We do have the capacity to test all DSD patients in UK.

16. 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.

n/a

EPIDEMIOLOGY

17a. Estimated prevalence of conditions in the general UK population
Prevalence is total number of persons with the condition(s) in a defined population at a specific time.
Please identify the information on which this is based.
For panel tests, please provide estimates for the conditions grouped by phenotypes being tested.

It is not known how common DSDs are, but they are estimated to affect 0.1-2% of the UK population.

17b. Estimated annual incidence of conditions in the general UK population
Incidence is total number of new cases in a year in a defined population.
Please identify the information on which this is based.
For panel tests, please provide for groups of conditions.

One in 4,500 infants

18. Estimated gene frequency (Carrier frequency or allele frequency)
Please identify the information on which this is based.
n/a for panel tests.

n/a

19. Estimated penetrance of the condition. Please identify the information on which this is based
n/a for panel tests

n/a

20. Estimated prevalence of conditions in the population of people that will be tested.
n/a for panel tests.

n/a

INTENDED USE (Please use the questions in Annex A to inform your answers)

21. 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

22. 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.

Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.

Sensitivity: >99% (95% CI) overall, >99% (95% CI) for SNVs and 95.7-98.4% (95% CI) for indels

Specificity: 97-98% (95%CI)

23. 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 two years service.

The overall clinical sensitivity is likely to be around 40%, although this will vary according to the referral reason and the sub-panel tested. No data are available for clinical specificity.

24. 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

25. 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

26. How will the test change the management of the patient and/or alter clinical outcome? Please describe associated benefits for patients and family members. If there are any cost savings AFTER the diagnosis, please detail them here.

Early identification of the specific genetic cause enables the correct management to be instituted promptly and will inform to gender assignment in some cases.

Identification of a causative mutation in an index case will provide the best outcome for the patient and family by enabling early management decisions for gender assignment, for example in gonad management where removal or screening for gonadal malignancy may be indicated. In certain cases there will be the opportunity to start hormonal treatments early to ameliorate the genital abnormality eg the institution of topical DHT. In other scenarios future screening for adrenal problems may be targeted. The early identification of a causative mutation may avoid the need for expensive biochemical investigations as well as nursing time and hospital admission.

Knowledge of the underlying genetic cause will also allow accurate genetic counselling of the family including assessment of any potential recurrence risk. Testing of the parents and future prenatal diagnosis can also be offered if appropriate.

27. If this test was not available, what would be the consequences for patients and family members?

The consequences of not providing the test would be continued surveillance and secondary care follow up as well as possible inappropriate surgical treatment.

Without genetic information it is not possible to accurately advise on the likelihood of the condition recurring in the family. Additional affected siblings are likely to be born where there is recurrence risk, for example genes with autosomal recessive inheritance.

28. 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.

No

29a. What unexpected findings could this test show? 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.

None

29b. Please list any genes where the main phenotype associated with that gene is unrelated to the phenotype being tested by the panel.

None

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

n/a

31. If a panel test, is this replacing an existing panel/multi gene test and/or other tests currently carried out through UKGTN using Sanger sequencing? If so, please provide details below.

No

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

None

IS IT A REASONABLE COST TO THE PUBLIC?

33. In order to establish the potential costs/savings that could be realised in the diagnostic care pathway, please list the tests/procedures that would be required in the index case to make a diagnosis if this genetic test was not available.

	Type of test	Cost (£)
Replacement of single gene genetic tests carried out by Sanger sequencing.		1500
Total cost of tests/procedures no longer required (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)		**1500**

34. Based on the expected annual activity of index cases (Q14), please calculate the estimated annual savings/investments based on information provided in Q33.

Number of index cases expected annually	(a) 200
Cost to provide tests for index cases if the genetic test in this Gene Dossier was not available (see Q32)	(b) 1500
Total annual costs pre genetic test	(a) x (b) = (c) 300,000
Total annual costs to provide genetic test	(a) x cost of genetic testing for index case of £900 =(d) 180,000 assuming one sub panel used
Additional savings for 100% positive rate for index cases	(d) – (c) = (e) 120,000
Percentage of index cases estimated to be negative	(f) 60%
Number of index cases estimated to be negative	(f) x number of index cases = (g) 120
Costs to provide additional tests for index cases testing negative	(g) x (b) = (h) The tests are for single sanger tests which you wouldn't need to do after the panel test.
Total savings for tests for index patient activity	(e) + (h) = (i) £120,000 savings
Total costs for family members	Costs for family member test x number of family members expected to test in a year (j) 175 x 20 = 3,500
If there is a genetic test already available and some of the family testing is already being provided, please advise the cost of the family testing already available	Cost for family member testing already available x estimated number of tests for family members already provided (k) 2,000
Total costs for family members minus any family member testing costs already provided	(j) – (k) = (l) 1,500
Additional savings for all activity expected in a year	(i) + (j) or (i) + (l) £118,500 savings

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

A term baby was born and it was not possible to determine the sex of the baby from the clinical examination. The phallus measured 5 mm and no gonads were palpable. There were labioscrotal folds and no hyperpigmentation. The karyotype was 46,XY. The initial endocrine investigations showed that testicular tissue was present. The decision about sex of rearing at this point needs to take into account the following factors:

1. The need for a specific diagnosis to enable a discussion on a specific psychosexual outcome. This aids a discussion with the parents regarding future potential gender identity issues and sexual function
2. The prospect for future fertility
3. The need for surgical intervention and how this differs for male and female gender assignment. There is a gonadal malignancy risk for some of these disorders so this will inform decisions in some cases whether the gonads should be removed early or later.
4. The need for sex hormone replacement therapy at a later stage

Endocrine investigations in DSD, and especially during the neonatal period, often cannot delineate a specific DSD diagnosis as there is considerable variability in the results from hormonal studies even within individuals with the same specific DSD diagnosis. Unfortunately hormonal studies in the neonatal period are unreliable and subject to assay interference. Furthermore, the normal reference ranges for some of these assays are not known in this age group. This is further complicated by the use of tandem mass spectrometry (TMS) - a new and more accurate way to measure steroids. The majority of endocrine reference ranges and interpretation of tests and dynamic tests have been undertaken on standard immunoassays-whereas normative data from TMS for these tests is unavailable. This means that the interpretation of endocrine studies can be misleading and may inadvertently influence decisions on gender assignment incorrectly.

A DSD panel offers the clinician the opportunity to delineate a specific diagnosis early in the investigative process. In this case, if the diagnosis of 17-beta hydroxysteroid dehydrogenase deficiency had been known from the start that would have directly influenced the management as these babies can be raised male ie concordant with the karyotype, as they are able to respond to testosterone, which would be offered early to increase the penile length. Furthermore, some cases of DSD are at risk of adrenal failure outside the neonatal period, so ruling these causes out is extremely helpful for ongoing surveillance, whereas if such a case was detected, a potentially life-threatening adrenal crisis at a later stage could be avoided.

Currently the specific genetic diagnosis of many cases of DSD are not made till after gender assignment even though the diagnosis could have influenced decision making at presentation. Many parents and families find the uncertainty of not knowing the specific diagnosis traumatic, especially as they are involved in making life-changing decisions for their child. An early diagnosis in this case, using a DSD panel, could have significantly optimised the management.

UKGTN Testing Criteria

Test name: **Gender Assignment 13 Gene Panel**	
Approved name and symbol of disorder/condition(s): See website for disorders	**OMIM number(s):**
Approved name and symbol of gene(s): See website for genes	**OMIM number(s):**

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.**
Consultant Paediatric Endocrinologist	
Consultant Clinical Geneticist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Genital ambiguity	

Additional Information:
For panel tests:
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: **Genital Anomalies and suspected Adrenal problems 12 gene panel**	
Approved name and symbol of disorder/condition(s): See website for disorders **Approved name and symbol of gene(s):** See website for genes	**OMIM number(s):** **OMIM number(s):**

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.**
Consultant Paediatric Endocrinologist	
Consultant Clinical Geneticist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Genital ambiguity **AND**	
Adrenal dysfunction	

Additional Information:
For panel tests:
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: **Gonadal Dysgenesis with Gonadal failure 12 gene panel**	
Approved name and symbol of disorder/condition(s): See website for disorders	**OMIM number(s):**
Approved name and symbol of gene(s): See website for genes	**OMIM number(s):**

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.**
Consultant Endocrinologist (Adult or Paediatric)	
Consultant Clinical Geneticist	
Consultant Gynaecologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Evidence of gonadal dysgenesis or failure (e.g. uterus, raised FSH, low testosterone, low AMH, gonadal histology) **AND**	
46,XY karyotype	

Additional Information:
For panel tests:
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.

Appendix 1

Genes in panel test and associated conditions.

Gender assignment.

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments & Other labs that provided single gene testing
AR Androgen receptor gene	644	313700	AIS Androgen Insensitivity	XL	300068	Gottlieb et al (2004)	mean coverage 97 %	No	Sheffield and Cambridge Core gene
CBX2 Chromobox protein homolog 2 gene	1552	602770	46 XY sex reversal 5	AD	613080	Biason-Lauber et al (2009)	mean coverage 94 %	No	
DHH Desert Hedgehog gene	2856	605423	46XY partial gonadal dysgenesis	AR	607080 233420	Umehara et al (2000) Canto et al (2004)	mean coverage 88 %	No	
DMRT1 Double sex and mab-3 related transcription factor 1	2934	602424	46 XY sex reversal	AD	154230	Matson et al (2011)	mean coverage 93 %	Yes	
HSD17B3 Testosterone 17-beta-dehydrogenase 3 gene	5212	605573	Pseudohermaphroditism	AD	264300	Lindqvist et al (2001)	mean coverage 99 %	Yes	UCLH Biochemistry & Cambridge Core gene
HSD17B2 Testosterone 17-beta-dehydrogenase 2 gene	5211	109685	Pseudohermaphroditism	AD	264080	Lindqvist et al (2001)	mean coverage 96 %	No	UCLH Biochemistry
LHCGR Luteinizing hormone/choriogonadotrophin receptor gene	6585	152790	Leydig cell adenoma/hypopla. Precocious Puberty, male	AD	176410 238320	Latronica et al (1998) Laue et al (1995) Kremer et al (1999)	mean coverage 96 %	No	
MAMLD1 Master-	2568	300120	Hypospadias 2	X-L	300758	Fukami et al (2006)	mean	No	

like domain containing 1							coverage 94 %		
RSPO1 R-spondin homolog gene	21679	609595	Palmoplantar hermaphroditism	AD	610644	Parma et al (2006)	mean coverage 96 %	No	
SOX9 SRY (sex determining region Y)-box 9	11204	608160	Campomelic dysplasia with autosomal sex reversal	AD	114290	Foster et al (1994)	mean coverage 89 %	Yes	
SRD5A2 3-oxo-5-alpha steroid 4-dehydrogenase 2	11285	607306	Pseudovaginal perineoscrotal hypospadias	AD	264600	Sasaki (2000)	mean coverage 86 %	Yes	UCLH biochemistry and Cambridge Core gene
SRY Sex-determining region Y	11311	480000	Sex reversal1	Y-linked	400045 400044	Cameron and Sinclair (1997)	mean coverage 92 %	Yes	Core gene
CYP19A1 Cytochrome P450, family 19, subfamily A, polypeptide 1	2594	107910	Aromatase deficiency Aromatase excess syndrome	AR	613546 139300	Ito et al. (1993) Harada et al. (1992)	mean coverage 95 %	No	

Genital Anomalies and suspected Adrenal problems.

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments & Other labs that provided single gene testing
ATRX ATP dependent helicase ATRX	866	300032	Alpha Thal. Myelodysplasia syn.	XL	300448	Gibbons and Higgs (2000) Badens et al (2006)	mean coverage 99 %	No	
CYP21A2 CAH gene, P450 family	2600	613815	CAH Adrenal Hypoplasia	AD	201910	Sido et al (2005)	mean coverage 84 %	Yes	Manchester and Birmingham in sub-panel 2
CYP11A1 Cholesterol side-chain cleavage family (P450scc)	2590	118485	Adrenal Insufficiency with partial or complete sex reversal	AD	613743	Gharani et al (1997)	mean coverage 93 %	No	Cambridge & Aberdeen but targeted only. in sub-panel 2
CYP11B1 CAH gene, cytochrome P450 11B1	2591	610613	CAH Adrenal Hypoplasia	AR	202010	Loidi et al (1999)	mean coverage 89 %	No	Cambridge & Aberdeen but targeted only. in sub-panel 2
CYP17A1 Steroid 17-alpha-monooxygenase P450 17A1,	2593	609300	17-alphahydroxylase/ 17,20 lyase deficiency	AD	202110	Geller et al (1997)	mean coverage 88 %	Yes	UCLH biochemistry in sub-panel 2
DHCR7 7-dehydrocholesterol reductase	2860	602858	SLOS Smith-Lemli-Opiz syndrome	AD	270400	Yu et al (2000)	mean coverage 93 %	No	Birmingham, Bristol and Guys in sub-panel 2
DHH Desert Hedgehog gene	2856	605423	46XY partial gonadal dysgenesis	AR	607080 233420	Umehara et al (2000) Canto et al (2004)	mean coverage 88 %	No	
NR5A1 Nuclear receptor subfamily 5, group A, member 1	7983	184757	46XYsex reversal3	AD	612965	Lin et al (2007)	mean coverage 92 %	Yes	Cambridge in sub-panel 3
POR Cytochrome oxidoreductase gene, P450 9	9208	124015	Disordered Steroid genesis	AD	613571	Arlt et al (2004)	mean coverage 100 %	No	

SOX9 SRY (sex determining region Y)-box 9	11204	608160	Campomelic dysplasia with autosomal sex reversal	AD	114290	Foster et al (1994)	mean coverage 89 %	Yes	
StAR Steroidogenic acute regulatory gene	11359	600617	Lipoidadrenal hypersplasia	AR	201710	Bose et al (2000)	mean coverage 95 %	No	Core gene for sub-panel 3
HSD3B2 3-beta-hydroxysteroid dehydrogenase 2	5218	613890	3-beta-hydroxysteroid dehydrogenase, type II, deficiency	AD	201810	Rheaume et al. (1992)	mean coverage 100%	No	Core gene

Gonadal Dysgenesis with Gonadal failure.

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments & Other labs that provided single gene testing
ARX Aristaless related homeobox gene	18060	300382	LISX2 EIEE1	XL	300215 308350	Fullston et al (2010)	mean coverage 97 %	No	Cardiff in sub-panel 3
CBX2 Chromobox protein homolog 2 gene	1552	602770	46 XY sex reversal 5	AD	613080	Biason-Lauber et al (2009)	mean coverage 94 %	No	
DMRT1 Double sex and mab-3 related transcription factor 1	2934	602424	46 XY sex reversal	AD	154230	Matson et al (2011)	mean coverage 93 %	Yes	
MAP3K1 Mitogen activated protein kinase kinase 1	6848	600982	46XYsex reversal 6	AD	613762	Pearlman et al (2010)	mean coverage 98 %	No	
NR5A1 Nuclear receptor subfamily 5, group A, member 1	7983	184757	46XYsex reversal3	AD	612965	Lin et al (2007)	mean coverage 92 %	Yes	Cambridge in sub-panel 3
NR0B1 (DAX1) Nuclear receptor subfamily 0, group B, member 1	7960	300473	46XYsex reversal2 X-L Adrenal Hypoplasia	X-L	300018	Phelan and McCabe (2001)	mean coverage 100 %	Yes	Core gene for sub-panel 3
POR Cytochrome oxidoreductase gene, P450 9	9208	124015	Disordered Steroid genesis	AD	613571	Arlt et al (2004)	mean coverage 100 %	No	
SOX9 SRY (sex determining region Y)-box 9	11204	608160	Campomelic dysplasia with autosomal sex reversal	AD	114290	Foster et al (1994)	mean coverage 89 %	Yes	
SRY Sex-determining region Y	11311	480000	Sex reversal1	Y-linked	400045 400044	Cameron and Sinclair (1997)	mean coverage	Yes	Core gene

							92 %		
StAR Steroidogenic acute regulatory gene	11359	600617	Lipoidadrenal hypersplasia	AR	201710	Bose et al (2000)	mean coverage 95 %	No	Core gene
WNT4 Wingless-type MMTV integration site family, 4	12783	603490	Mullerian aplasia and hyperandrogenism	AD	158330	Biason-Lauber et al (2004)	mean coverage 85 %	Yes	
WT1 Wilm's tumour suppressor gene 1(WT1)	12796	607102	Denys-Drash Male peudohermaphroditism	AD	194080	Hastie (1992) Mueller (1994)	mean coverage 99 %	No	Sheffield