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

Test – Disease – Population Triad

Disease – name	Glycogen Storage Diseases – all 13 subtypes
OMIM number for disease	Various OMIM numbers GSD 0 – 240600, 611556 GSD 1 – 232200 GSD II- 232300 GSD III – 232400 GSD IV – 232500 GSD V- 232600 GSD VI - 232700 GSD VII- 232800 GSD IX- 306000, 261750, 613027, 300559, 300257 GSDX – 261670 GSDXI – 612933 GSDXII – 611881 GSDXIII - 612932
Disease – alternative names Please provide any alternative names you wish listed	Various including Type I - Von Gierke's disease Type II – Pompe disease Type III – Cori disease Type IV – Andersen disease Type V – Mc Ardle disease Type VI – Hers disease Type VII – Tarui disease
Disease – please provide a brief description of the disease characteristics	There are two main presentations of these group of disorders Muscle – These can be of varying severity with antenatal hypokinesia and arthrogryposis like neonatal presentation, severe hypotonia occasionally mimicking muscular dystrophy, persistent hypotonia, developmental delay, cardiomyopathy of varying severity. Liver – persistent and recurrent hypoglycaemia, hepatomegaly and hepatic dysfunction. This includes bleeding tendency, secondary lipid abnormalities and renal dysfunction.
Disease - mode of inheritance	This is a heterogenous group of conditions. Most are autosomal recessive although IIb and IX a are X linked.
Gene – name(s)	GSD 0 – GYS1, GYS2 GSD I – G6PC, G6PT GSD II- GAA, LAMP2 GSD III – GDE GSD IV- GBE1 GSD V- PYGM GSD VI- PYGL GSD VII- PFKM GSD IX – PHKA2, PHKB, PHKG2 GSD X – PGAMM GSD XI- LDHM

Approval Date: Sept 2011 Submitting Laboratory: GSTS Pathology, Guy's Hospital

<table>
<tr><td></td><td>GSD XII- ALDOA
GSD XIII- ENO3</td></tr>
<tr><td>OMIM number for gene(s)</td><td>
<table>
<tr><th>Gene</th><th>OMIM number</th></tr>
<tr><td>GYS2</td><td>138571</td></tr>
<tr><td>G6PC</td><td>232200</td></tr>
<tr><td>SLC37A4</td><td>602671</td></tr>
<tr><td>AGL</td><td>610860</td></tr>
<tr><td>GBE1</td><td>607839</td></tr>
<tr><td>PYGL</td><td>232700</td></tr>
<tr><td>PHKA2</td><td>300798</td></tr>
<tr><td>PHKB</td><td>172490</td></tr>
<tr><td>PHKG2</td><td>172471</td></tr>
<tr><td>PYGM</td><td>608455</td></tr>
<tr><td>PFKM</td><td>610681</td></tr>
<tr><td>PGAM2</td><td>612931</td></tr>
<tr><td>ALDOA</td><td>103850</td></tr>
<tr><td>ENO3</td><td>131370</td></tr>
<tr><td>GAA</td><td>606800</td></tr>
<tr><td>LAMP2</td><td>309060</td></tr>
<tr><td>GYS1</td><td>138570</td></tr>
<tr><td>LDHA</td><td>150000</td></tr>
</table>
</td></tr>
<tr><td>Gene – alternative names
Please provide any alternative names you wish listed</td><td>
<table>
<tr><th>Gene</th><th>Alternative names</th></tr>
<tr><td>GYS2</td><td></td></tr>
<tr><td>G6PC</td><td></td></tr>
<tr><td>SLC37A4</td><td>G6PT</td></tr>
<tr><td>AGL</td><td>GDE</td></tr>
<tr><td>GBE1</td><td></td></tr>
<tr><td>PYGL</td><td></td></tr>
<tr><td>PHKA2</td><td>PHK, PYK</td></tr>
<tr><td>PHKB</td><td></td></tr>
<tr><td>PHKG2</td><td></td></tr>
<tr><td>PYGM</td><td></td></tr>
<tr><td>PFKM</td><td>PFKX, PFK-1</td></tr>
<tr><td>PGAM2</td><td>PGAMM</td></tr>
<tr><td>ALDOA</td><td></td></tr>
<tr><td>ENO3</td><td></td></tr>
<tr><td>GAA</td><td></td></tr>
<tr><td>LAMP2</td><td>CD107b</td></tr>
<tr><td>GYS1</td><td>GSY</td></tr>
<tr><td>LDHA</td><td></td></tr>
</table>
</td></tr>
<tr><td>Gene – description(s) (including number of amplicons)</td><td>The 18 genes range in size from 3 to 33 exons, and collectively comprise a total of 284 exons.
The target regions to be sequenced are selected by in-solution hybridisation capture, using oligonucleotide probes complementary to the regions to be captured. The number of probes per gene are indicated below. The regions captured include all exons and a minimum of 50 bp of each flanking</td></tr>
</table>

<table>
<tr><td></td><td>intronic sequence.

<table>
<tr><th>Gene</th><th>Number of exons</th><th>Number of probes</th></tr>
<tr><td>GYS2</td><td>16</td><td>1328</td></tr>
<tr><td>G6PC</td><td>5</td><td>209</td></tr>
<tr><td>SLC37A4</td><td>8</td><td>286</td></tr>
<tr><td>AGL</td><td>33</td><td>1439</td></tr>
<tr><td>GBE1</td><td>16</td><td>5767</td></tr>
<tr><td>PYGL</td><td>20</td><td>979</td></tr>
<tr><td>PHKA2</td><td>33</td><td>1911</td></tr>
<tr><td>PHKB</td><td>29</td><td>4382</td></tr>
<tr><td>PHKG2</td><td>9</td><td>254</td></tr>
<tr><td>PYGM</td><td>20</td><td>1112</td></tr>
<tr><td>PFKM</td><td>22</td><td>816</td></tr>
<tr><td>PGAM2</td><td>3</td><td>112</td></tr>
<tr><td>ALDOA</td><td>8</td><td>404</td></tr>
<tr><td>ENO3</td><td>11</td><td>193</td></tr>
<tr><td>GAA</td><td>19</td><td>533</td></tr>
<tr><td>LAMP2</td><td>9</td><td>1161</td></tr>
<tr><td>GYS1</td><td>16</td><td>461</td></tr>
<tr><td>LDHA</td><td>7</td><td>337</td></tr>
<tr><td>TOTALS</td><td>284</td><td>21684</td></tr>
</table></td></tr>
<tr><td>Mutational spectrum for which you test including details of known common mutations.</td><td>Our validations have shown that this technology detects all point mutations and small sequence variants including indels up to 38bp (see further details on validation below).
Whole exon deletions and duplications are also detected by evaluation of the number of sequence reads obtained.</td></tr>
<tr><td>Technical Method (s)</td><td>Massively parallel Illumina sequencing by synthesis, following genomic target capture by in-solution hybridisation.
Agilent SureSelect in-solution array is used to capture by hybridisation all coding regions from all 18 genes, plus a minimum of 50 bp of intronic sequence either side of each exon.
The capture array comprises RNA probes (120 bases) designed in a tiled array across the target genomic reference sequences such that each probe overlaps the adjacent probe by 96 bases. This results in every nucleotide in the target region being covered by 5 different probes (5x tiling array). The composition of the array has been optimised to ensure all targeted sequences are sufficiently captured.
In total, 969kb of genomic sequence are targeted for capture.
The captured DNA from each patient is prepared for sequencing which includes “indexing” using unique sequence tags for each sample so that the resulting sequence data can be identified as originating from a given sample. This enables efficiency savings through pooling of samples in the sequencing process. Pooled samples are subjected to Next generation sequencing using Illumina massively parallel sequencing by synthesis technology on a Genome Analyser 2.
Sequence reads are separated by sample, and aligned to</td></tr>
</table>

<table>
<tr><td></td><td>human genome reference sequences using NextGene software (SoftGenetics)
Variants are flagged using NextGene software. Known SNP's are ignored. The presence of all other identified variants (potentially pathogenic) detected in at least 10% of reads at a given locus are confirmed by fresh PCR amplification of individual exons and standard fluorescent capillary Sanger sequencing. Pathogenicity of confirmed variants is evaluated using the Alamut software application, as used for evaluation of variants detected by conventional capillary sequencing.
Copy number variants (CNVs) are searched for by comparing the log2 ratio of read depth for each exon from each patient against the corresponding average value obtained from all samples in the same run.
All target regions (coding exons + splice sites) will be covered by >30x read depth. Any targets that need to be analysed having less than 30 reads will be subjected to standard PCR amplification and fluorescent capillary Sanger sequencing.
Testing of relatives for identified familial mutations will be carried out by conventional PCR amplification and fluorescent capillary Sanger sequencing for point mutations, and by quantitative multiplex PCR assay for copy number variations (whole exon deletions or duplications), according to standard protocols used for other genes that we test in our laboratory.</td></tr>
<tr><td>Validation Process
Note: please explain how this test has been validated for use in your laboratory</td><td>Please see separate data below</td></tr>
<tr><td>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</td><td>Y
If Yes:
Number of reports issued: 16 new patients tested, 8 reports completed, 8 in preparation (see separate table below for full details).
Number of reports mutation positive: Diagnosis was confirmed in 12 patients.
Number of reports mutation negative: No pathogenic mutations were detected in 2 patients; At least one pathogenic variant was detected in 2 patients, supporting but not confirming a diagnosis.</td></tr>
<tr><td>For how long have you been providing this service?</td><td>First report issued January 2011</td></tr>
<tr><td>Is there specialised local clinical/research expertise for this disease?</td><td><table><tr><td>Yes ✓</td><td>No</td><td>Please provide details</td></tr></table>Consultant in Metabolic Medicine, Dr Mike Champion and colleagues in the clinical metabolic unit at Guy's & St Thomas' Trust.</td></tr>
<tr><td>Are you testing for other genes/diseases closely allied to this one? Please give details</td><td>Also establishing sequencing for nuclear mitochondrial genes using same technology</td></tr>
</table>

Your Current Activity If applicable - How many tests do you currently provide annually in your laboratory?	Index cases: Family members where mutation is known:
Your Capacity if Gene Dossier approved How many tests will you be able to provide annually in your laboratory if this gene dossier is approved and recommended for NHS funding?	Index cases: 100+ Family members where mutation is known: 200+
Based on experience how many tests will be required nationally (UK wide)? Please identify the information on which this is based	Index cases: ~70 new births pa in UK Likely underdiagnosed, so <70 new cases pa expected Based on: UK birth rate ~700,000 pa All GSD's estimated freq ~ 1/10,000 Possible "backlog" of an unknown number of cases where mutations remain undetected. Family members where mutation is known: unknown, since data on uptake of carrier/prenatal diagnosis for all GSD's is not available (since comprehensive testing for all GSD's is not currently available).
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".	Sheffield Genetics laboratory offers testing for some of the subtypes but there is no comprehensive genetic testing available for all the GSDs in the UK. Using Next Generation Sequencing the capacity is large, and the cost per sample decreases as increasing numbers of samples are tested per batch.

Validation of Next Generation Sequencing for GSD

In total, 25 patient samples have been tested in whom 30 different mutations were detected in 11 different genes. The method was initially validated by testing 9 samples with previously identified pathogenic mutations in a number of genes, including neuromuscular disorders (the neuromuscular genes are not included in the final GSD target capture array). These mutations consisted of single nucleotide changes, deletions between 2 bp to 38 bp, and a multi-exon duplication (see table below for details). All 13 mutations in these samples were detected.
In addition, 33 known SNPs in normal control DNA gave concordant results between Illumina sequencing and Sanger sequencing.

Validation Samples

Sample	Mutation	Gene
1	Hom c.150_151delGT; p.(Trp50fs)	G6PC
2	Het c.247C>T; p. (Gln83X) Het c.925C>T; p.(Arg309Trp)	PHKG2 PHKG2
3	Het c.754G>C; p.(Ala252Pro) Het c.1477T>A; p.(Tyr493Asn)	GYS2 GYS2
4	Het c.1239C>G; p.(Asp413Glu) Het c.2228A>G; p.(Gln743Arg)	GAA
5	Hemi c.2281_2285del5; p.(Glu761fs)	DMD
6	Hemi c.7590_7596del7; p.(Ile2531fs)	DMD
7	Hemi c.4565_4577del13; p.(Val1522fs)	DMD
8	Het c.943G>A; p.(Gly315Ser) Het c.746_747+36del38	SEPN1 SEPN1
9	Hemi duplication exons 18 to 30	DMD

Evidence for 30x coverage being sufficient

In order to assess the level of coverage required to accurately call variants, we simulated low read depth for genes where mutations were identified, by taking random subsets of reads from the original run and re-aligning them to the reference. There were no instances where a mutation would have been missed in the validation samples when the coverage was 30 or above.

New referrals

16 patient samples were new referrals for GSD testing. In 12 of these patients pathogenic mutations were detected which enabled confirmation of the diagnosis of GSD (sees table below). In 2 patients (6 & 13) no pathogenic variants were detected. Two patients (2 & 11) had only one clearly pathogenic variant, supporting but not confirming a diagnosis.

Patient	Referral GSD subtype	Genotype	Gene	Confirmed subtype
1 (SC)	IX	Hemi c.1561A>G; p.(Thr521Ala)	PHKA2	IX
2 (ES)	0	Het c.2482C>G; p.(Pro828Ala) Het c.612G>A; p.(Tyr204Tyr) (unclassified variant)	PHKB PYGM	Not confirmed
3 (DM)	IX	Hemi c.3632C>G; p.(Thr1211Arg)	PHKA2	IX
4 (LS)	GSD?	Hom c.4260-12A>G	AGL	III
5 (UJ)	GSD?	Hom c.1518G>A; p.(Glu506Glu)	PYGL	VI
6 (YI)	GSD?	No pathogenic mutation		Not confirmed
7 (MN)	GSDI	Het c.287G>A; p.(Trp96X) Het c.1015G>T; p.(Gly339Cys)	SLC37A4 SLC37A4	Ib
8 (SBe)	Non-GSDI	Hemi c.3542C>G; p.(Ser1181X)	PHKA2	IX

9 (MS)	GSD?	Hemi c.3409C>T; p.(Arg1137Trp) Het c.96-11A>G	PHKA2 PHKG2	IX
10 (AJC)	GSDIX	Hom c.1969C>T; p.(Gln657X)	PHKB	IX
11 (LU)	GSDIIIb, VI, IX	Het c.1257T>A; p.(Tyr419X)	PHKB	Not confirmed
12 (CE)	GSD?	Hom c.2867T>A; p.(Ile956Asn)	PHKB	IX
13 (MG)	GSD?	No pathogenic mutation		Not confirmed
14 (SBy)	GSD?	Hemi c.1174C>G; p.(Arg392Gly)	PHKA2	IX
15 (CB)	GSDIII	Hom c. 3911dupA p.(Asn1304fs)	AGL	III
16 (AK)	GSDIX	Hom c.958C>T; p.(Arg320X)	PHKG2	IX

False positives

In the first 11 diagnostic samples analysed, 37 sequence variants were detected at low levels, of between 5 and 10% of sequence reads at those loci. All these loci were analysed by PCR and standard Sanger fluorescent sequencing to distinguish between low-level false positives and real heterozygotes giving disproportionate allele detection on the Next Generation Sequencing. Sanger sequencing did not detect any of these variants, so we therefore concluded that these were low-level false positives. Thus, for standard diagnostic heterozygote detection, all variants present in less than 10% of reads are filtered out, and all those found in 10% or more of reads are confirmed by Sanger sequencing. In specific cases if there is a possibility of otherwise missing a pathogenic variant, it may be appropriate to evaluate all variants detected at low level by NGS, but for most cases by using this cut-off level of 10% the rate of false positives is reduced to an average of 0.2 variants per sample.
Variants confirmed as being present heterozygously by Sanger sequencing were present in NGS data at between 22 – 54% of reads.

Sensitivity

During in-house validation, all expected variants were observed. In addition, in one patient sample a mutation was detected by NGS (and confirmed by Sanger sequencing) which had been missed by a previous screen at another accredited laboratory by PCR and Sanger sequencing. Our data therefore demonstrates that NGS has a higher level of sensitivity than Sanger sequencing, which was shown to be >99% in a collaborative CMGS study.

A recent application of this technology to screen for carrier status in recessive disorders demonstrated a specificity of >99% and sensitivity of ~95%. (Carrier Testing for Severe Childhood Recessive Diseases by Next-Generation Sequencing. CJ. Bell, et al. Sci Transl Med 3, 65ra4; 2011). Our method for GSD generates a higher read depth than in this study of Bell et al, since (a) we have a higher density of tiled array to capture the targets, and (b) we are capturing a smaller sized target. The sensitivity of this approach increases with read depth obtained, and since the read depth varies between different loci the sensitivity will vary, and for some loci where we obtain >1000 read depth the sensitivity will be extremely high.

Epidemiology

Estimated prevalence of disease in the general UK population Please identify the information on which this is based	This is difficult to estimate for all subtypes due to lack of published data for all subtypes. The overall frequency is estimated at 1 in 20,000 live births (Hers et al in Metabolic Basis of Inherited Disease, 6th Edition,McGraw-Hill 1989). Some subtypes e.g. Types I, II, III, VI and IX are commoner and make up 90% of the cases. The prevalence of subtype I in the Caucasian population is 1 in 40,000. It is estimated that the birth incidence of all GSDs

	is 1 in 10 000 although it may be higher in population where consanguinity is common (OMIM, Sanderson et al – Arch Dis Child 2006;91:896-899)
Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	Based on above prevalence- 1/75
Estimated penetrance Please identify the information on which this is based	Variable for each subtype.
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)	Clinical diagnosis of Glycogen Storage Disease, based on: • Persistent hypoglycaemia, raised lipids • Previous glucose loading test suggestive • or Persistent hepatomegaly in childhood- infectious causes excluded • or hypotonia and previous muscle biopsy suggestive • or Previous liver biopsy suggestive or Affected 1st degree relative
Estimated prevalence of disease in the target population	Unknown, since comprehensive molecular confirmation of diagnosis (by testing for all GSD genes) is not currently available. However, our initial testing of 16 patients confirmed a diagnosis of GSD in 12 (75%), and results were consistent with a diagnosis of GSD in a further 2 (having only one clearly pathogenic mutation detected) in a total of 87.5% patients (14/16) the results were consistent with, or confirmed a diagnosis of GSD.

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

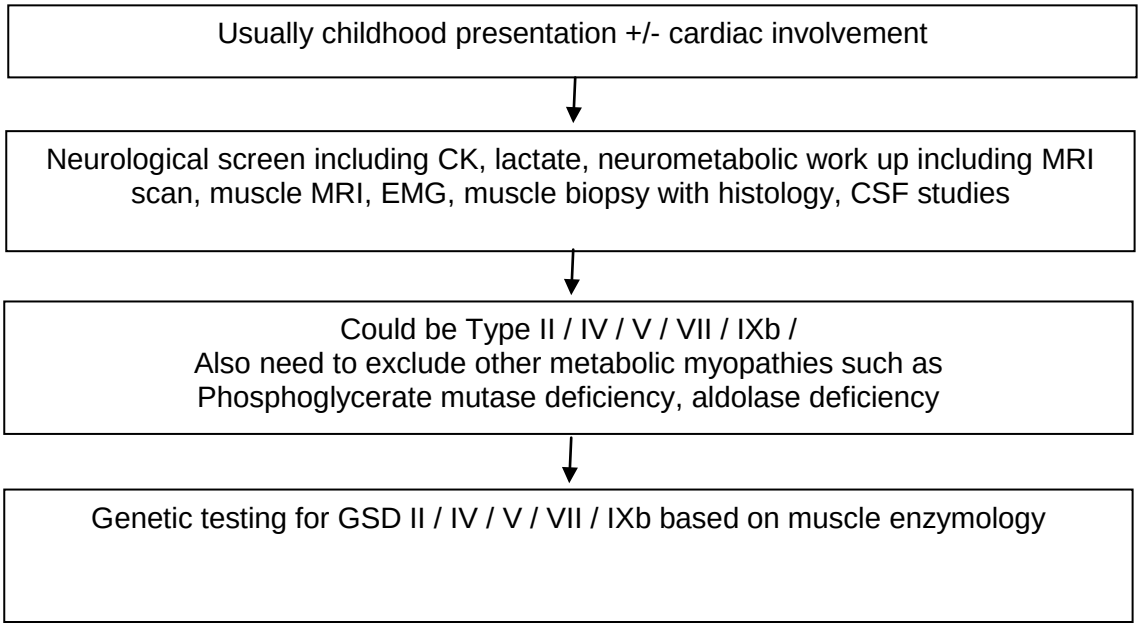
Please tick the relevant clinical purpose of testing	YES	NO
Diagnosis	✓	
Treatment	✓	
Prognosis & Management	✓	
Presymptomatic testing	✓	
Risk Assessment for family members	✓	
Risk Assessment – prenatal testing	✓	

Test Characteristics

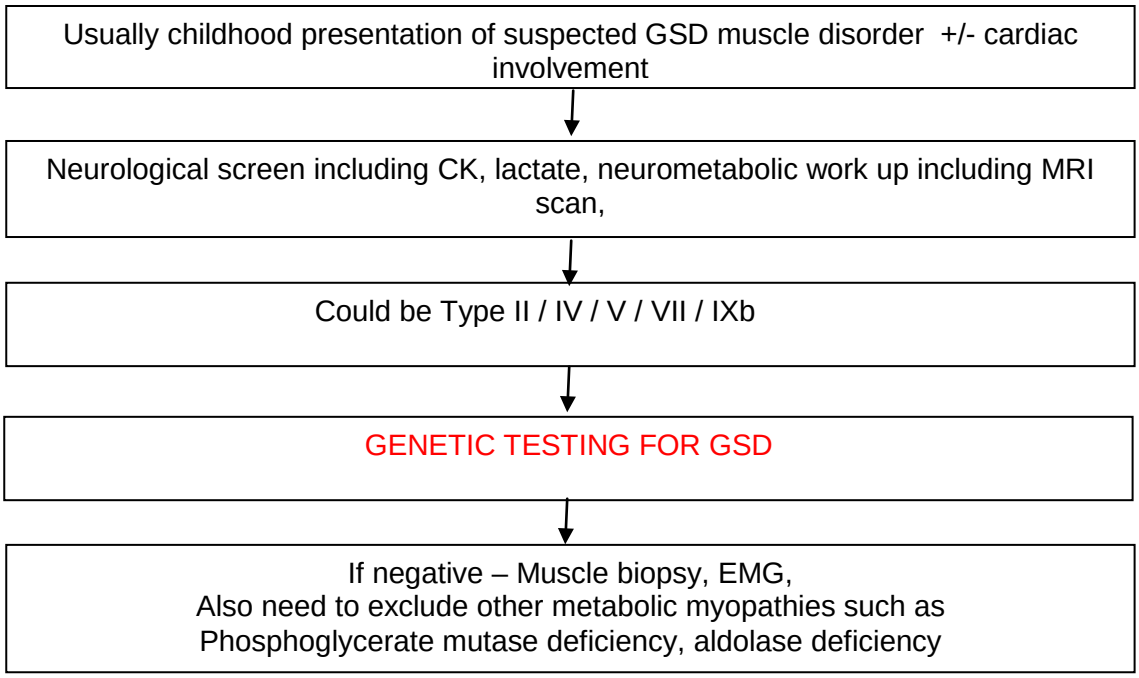
Analytical sensitivity and specificity This should be based on your own laboratory data for the specific test being applied for or the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up.	Please also see above for further details of analytical sensitivity and specificity. The analytical sensitivity of this technology approaches 100% for single nucleotide variants and small rearrangements. We have also demonstrated accurate detection of deletions up to 38 bp, and a duplication of several whole exons. The analytical sensitivity is dependant on reliable capture of all target regions, and obtaining sufficient read depth at all target loci. We will follow quality control measures to ensure at least 30 reads are obtained per sample for each locus, to ensure reliable detection of heterozygous alleles. nples where clearly pathogenic mutation(s) are not identified, any loci which do not meet the required quality threshold for sequence reads will be PCR amplified and sequenced using conventional capillary electrophoresis sequencing.
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)	Unknown, since comprehensive molecular confirmation of diagnosis is not currently available. For some subtypes eg GSD1a, there are common mutations that account for 90% of the mutations hence full sequencing not routinely offered. In our preliminary batch of patient samples tested the two cases each having a single pathogenic variant and the two cases without detectable mutations will be evaluated in more detail to correlate these results with the clinical phenotype.
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).	Unknown, since we do not yet know the full spectrum of sequence variants that will be detected by screening all these genes. However, these patients would have had baseline biochemical tests that would suggest GSD as an underlying diagnosis and we expect a high positive predictive value. In the event no mutation is identified, we propose to feedback to the referring clinician and arrange further functional studies including enzymology. However, we expect this to be very small numbers and most likely to be in GSD0 group.
Testing pathway 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 can be added to the document as a separate sheet if necessary.	For diagnostic referrals, all 18 GSD genes are sequenced simultaneously using Illumina next generation sequencing technology. To confirm the presence of mutations (detected by Illumina sequencing, or for known mutations in family members or prenatal diagnosis) standard PCR and fluorescent sequencing will be carried out.

CLINICAL ALGORITHMS FOR DIAGNOSIS OF GLYCOGEN STORAGE DISORDERS

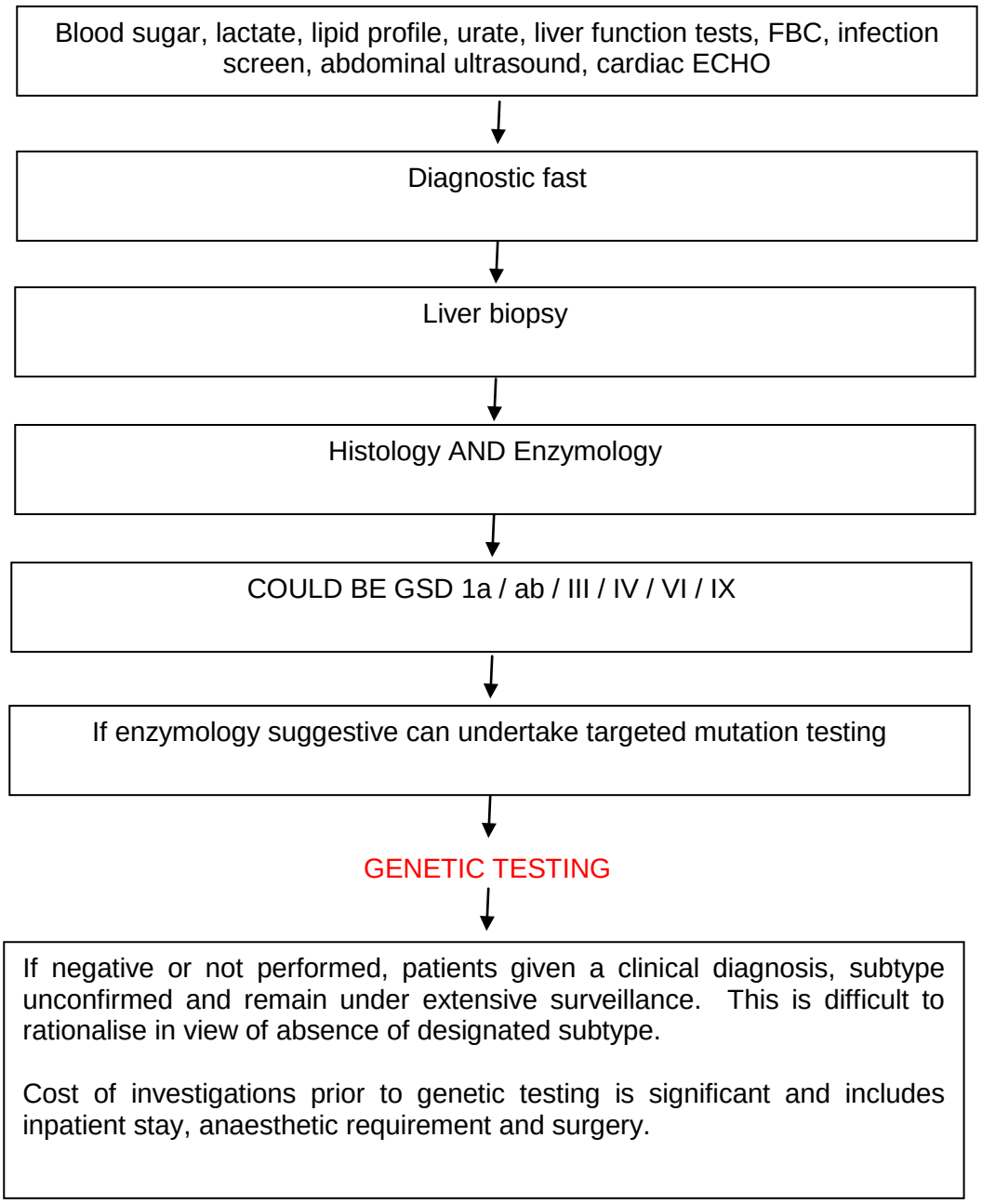
A. CURRENT Algorithm for muscle GSDs (presenting as myopathy)



PROPOSED Algorithm for diagnosis of muscle GSDs



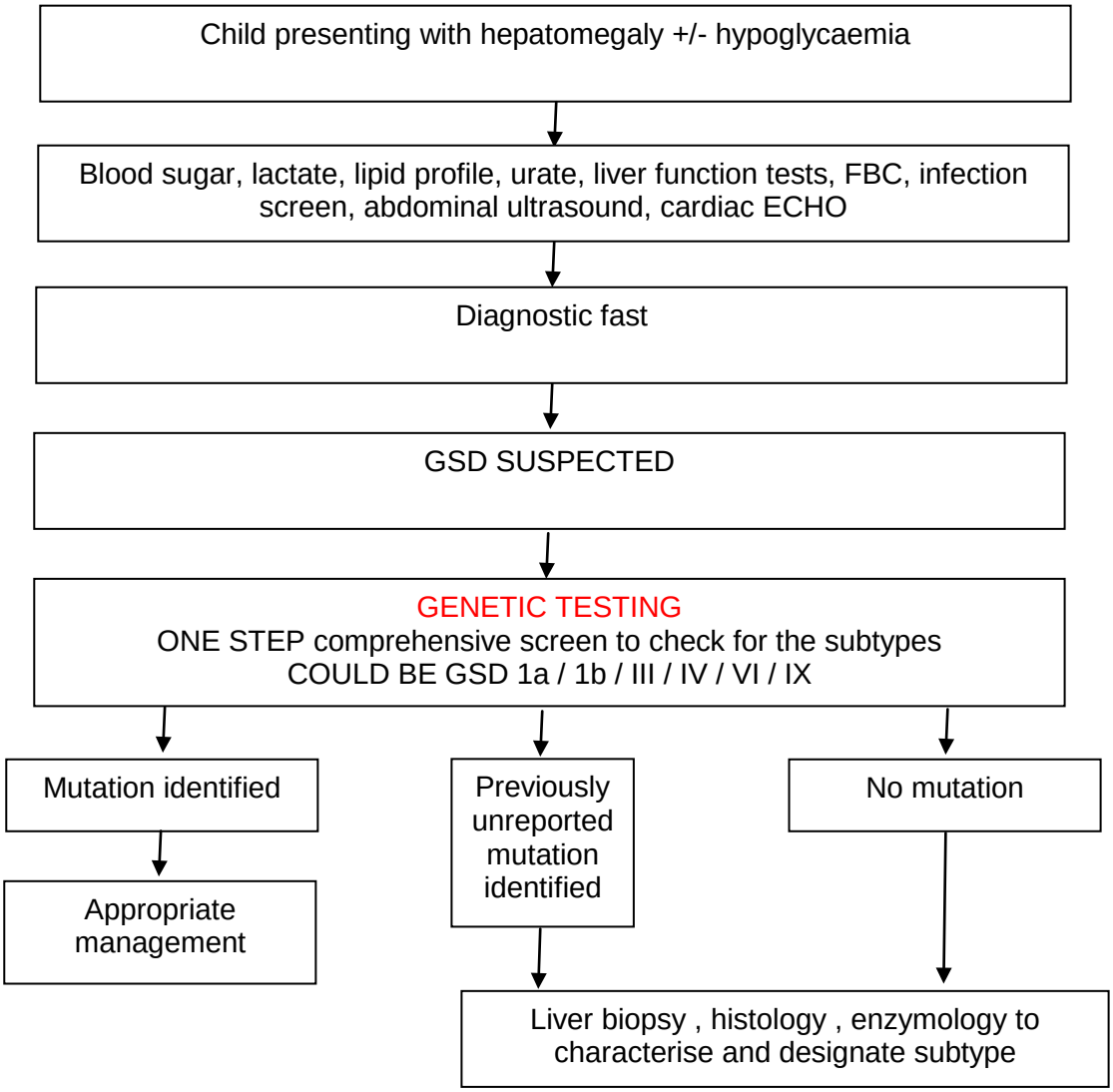
B. CURRENT algorithm - Hepatomegaly (+/- neonatal hypoglycaemia)



If negative or not performed, patients given a clinical diagnosis, subtype unconfirmed and remain under extensive surveillance. This is difficult to rationalise in view of absence of designated subtype.

Cost of investigations prior to genetic testing is significant and includes inpatient stay, anaesthetic requirement and surgery.

PROPOSED algorithm - Hepatomegaly (+/- neonatal hypoglycaemia)



Clinical utility of test in target population (Please refer to Appendix A) Please provide a description of the clinical care pathway.	Confirmation of clinical diagnosis as a "one-stop" test rather than requesting multiple gene analysis. Cost effective-for example if type IX is suspected on enzyme studies, genetic testing for the 3 genes is carried out sequentially costing £3000. Also, it is difficult to separate out types III/VI and IX and may necessitate further testing – see case study 1 as illustration. Small quantity of DNA required Ability to offer carrier testing to first degree relatives Possibility of offering prenatal The current and proposed patient care pathway is attached-Appendix I
How will the test add to the management of the patient or alter clinical outcome?	Avoid unnecessary hospital follow up appointments (GSD 0) Avoid invasive tests such as muscle and liver biopsy Decrease the need for nerve conduction studies and muscle MRI in cases of muscle presentation Appropriate management for patients presenting with cardiomyopathy in childhood Plan appropriate surveillance eg cardiac and liver surveillance for type VI
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? Please provide evidence from your own service.	At present, initial testing is by enzymology. The substrates are not stable and testing requires prompt transport of sample to the lab. Any delay in transit results in sample degradation and requires repeat testing. Multiple sampling requires repeat hospital visits and adds to the cost of patient care. Reduce total cost of molecular confirmation of diagnosis compared with current method of sequentially screening individual genes until pathogenic mutation(s) identified. Molecular testing will decrease the need for additional invasive tests such as muscle and liver biopsy. Decrease additional tests such as nerve conduction and MRI. Please also see example case studies at the end of this Gene Dossier.
What are the consequences of not doing this genetic test? Commissioners have asked for specific information to support introduction of tests.	Continued high cost to sequentially sequence individual genes. Possibility of wrong gene sequenced as not always possible to assign correct subtype on enzymology. Continuation of expensive invasive tests to assist in confirming diagnosis while awaiting molecular confirmation. Patients may have to wait extremely long times before obtaining molecular confirmation of diagnosis. Delay in instituting specific management. Prenatal testing is not available until a diagnosis is confirmed in an affected relative; a second affected sibling could be born while awaiting diagnosis. Sequential sequencing of individual genes in different centres may exhaust a unique, irreplaceable DNA sample, possibly making diagnosis impossible.

Utility of test in the NHS In a couple of sentences explain the utility of this test for the disease(s)	A one-stop molecular screen for all types of glycogen storage disease will avoid alternative invasive attempts at diagnosis; rapid molecular diagnosis will inform appropriate clinical management of affected patients and allow genetic counselling and prenatal diagnosis
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	Biochemical testing is possible in some subtypes but for others the substrate is not stable enough to perform enzyme assay to give a reliable result. With this new technique, we expect that the demand for this will decrease as a more reliable test is available. However, there is a role for biochemical tests in mutation negative patients.
Please describe any specific ethical, legal or social issues with this particular test?	Antenatal testing for some milder phenotypes e.g. muscle only GSDs

UKGTN Testing Criteria

Name of Disease(s): GLYCOGEN STORAGE DISORDERS

Name of gene(s): 18 GSD GENES

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 Metabolic physician	
Consultant Hepatologist	
Consultant Cardiologist - paediatric/ adult	
Consultant Neurologist - paediatric/adult	
Consultant Clinical Geneticist	
Consultant Paediatrician	

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

Criteria	Tick if this patient meets criteria
Persistent hypoglycaemia with other metabolic causes excluded	
OR Persistent hepatomegaly in childhood	
OR Previous liver biopsy suggestive	
OR Neuromuscular presentation suggestive of GSD (see algorithm) **OR** previous muscle biopsy suggestive	
OR Affected 1st degree relative	
OR At risk family member where mutation is known	

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

Approval Date: Sept 2011 Submitting Laboratory: GSTS Pathology, Guy's Hospital

EXAMPLE OF IMPACT ON THE NHS

Glycogen Storage Disorders (GSDS)

Case Study 1 (Patient 5)

Patient 5 in our initial study cohort was first referred to the metabolic physician at 3 years of age. He was seen by his local paediatrician for small stature and slow weight gain. Examination revealed hepatomegaly and nil else.

He had baseline biochemical tests that suggested a possible diagnosis of Glycogen Storage Disorder. Clinically, it was thought the presentation would be compatible with type III/ VI/ IX. Enzyme studies were performed on peripheral blood (£400) but were inconclusive. He was then reviewed regularly by the local team as well as the metabolic team and required intensive input from the dietician and the community paediatrics team. He remained small and hepatomegaly persisted. He then had repeat enzymology and as there was a delay in transit, the results could not be interpreted. It had to be repeated again but was still inconclusive (£800) He had a liver biopsy subsequently and histology (£800) as well as enzymology studies were done on liver tissue (£800). However, although a diagnosis of GSD was substantiated, it was not possible to assign the correct subtype.

He has remained under regular 6 monthly follow up with the metabolic team as well as his local paediatrician. This is because there is a high risk of cardiac complications with Type VI.

As molecular testing became available using conventional Sanger sequencing, he had mutation testing for all three GSD IX genes (£3000) but no mutation was identified. He is now 16 years old and remains without a definitive diagnosis. There was increasing concern by the family as his older sister was due to be married soon and they wanted accurate risks for her children.

Mutation analysis using the GSD NGS test has at last confirmed he has autosomal recessive GSD VI after 12 years. The total costs of tests alone for this patient exceed £8,000 over this period. This new test would have saved this as well as need for repeated hospital appointments, additional radiology investigations and invasive high risk procedure

Case Study 2 (Patient 15)

Patient 15 was seen in the metabolic clinic at 18 months with hepatomegaly. Clinical features as well as investigations including enzymology suggested this was GSD III. Mutation testing using conventional Sanger sequencing did not reveal any mutations in AGL (aka GDE) gene. This was disappointing as his mother was pregnant and enquired regarding a possible prenatal. Using the GSD NGS test we were able to identify the mutation in this patient confirming his clinical diagnosis but were not in time to offer a prenatal test. The sibling is due to be born shortly and will now be tested at birth.

We would have saved this patient's parents a lot of anxiety during the pregnancy and possibly avoid a recurrence had this test been available at the outset.