Practice Guidelines for the Molecular Diagnosis of Haemophilia B.

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1.0 GENERAL RECOMMENDATIONS

Where guidance states this is *recommended* practice or text is given in italics then this should be followed to ensure quality of service.

It is recommended that genetic testing for haemophilia in the UK should be performed in a member laboratory of the UKHCDO Haemophilia Genetics Laboratory Network. This is a consortium of laboratories, within Comprehensive Care Haemophilia Centres and Regional Genetics Laboratories, which work to agreed peer-reviewed standards of quality.

Retention and storage of records and specimens relating to molecular diagnosis in the UK must conform with both the Human Tissue Act 2004 and the guidance from the Royal College of Pathologists and Institute of Biomedical Science on the retention and storage of pathological records and specimens, 4th edition, 2009.

NOMENCLATURE AND GENE ID

Table 1.

Gene Name (HUGO nomenclature)	Factor IX (*F9*)
OMIM Number	306900
Gene Cards Id	F9
Ensembl Gene ID	ENSG00000101981
Chromosomal location	Xq27.1-q27.2
Medline MESH term	Haemophilia B, factor-IX
NCBI LocusLink	HsF9 (Locus ID 2158)

2.0 DESCRIPTION OF THE DISEASE.

Haemophilia B or Christmas Disease is a recessively inherited X-linked bleeding disorder which results from deficiency of procoagulant factor IX (FIX). Factor IX deficiency is characterised by prolonged oozing after injuries, tooth extractions or surgery and renewed bleeding after initial bleeding has stopped. Severely affected males suffer from spontaneous joint and muscle bleeds and easy bruising. The age of diagnosis and frequency of bleeding episodes are usually related to the FIX clotting activity. Haemophilia severity is defined by the residual FIX coagulant activity (FIX:C) level in plasma, where severely affected individuals have <1 IU/dL (<1% of normal); moderate 1-5 IU/dL (1-5% of normal); and mild >5 - <40 IU/dL (>5 - <40% of normal) (White et al, 2001). Haemophilia B is less common than haemophilia A (1 in 25,000 males versus 1 in 5,000 males worldwide) (Bolton-Maggs and Pasi, 2003).

3.0 COMMON REASONS FOR REFERRAL

Potential new cases of haemophilia B may be referred as a result of prior family history of the disease. In such cases male cord blood may be tested at birth to determine FIX:C. However, approximately one third of new cases have no prior history of haemophilia B, these are referred to as having sporadic disease (Kasper and Lin 2007). Such cases may be first diagnosed at any time from birth to adulthood, depending on severity of the haemophilia and individual circumstances. Patients with severe haemophilia B are usually diagnosed during the first year of life. Without treatment, they have an average of two to five spontaneous bleeding episodes each month. Patients with moderately severe haemophilia B seldom have spontaneous bleeding; however, they do have prolonged oozing after relatively minor trauma and are usually diagnosed before the age of five to six years. The frequency of bleeding episodes varies from once a month to once a year. Patients with mild haemophilia B do not have spontaneous bleeding; however, without treatment, abnormal bleeding occurs with surgery, tooth extraction, and major injuries. The frequency of bleeding may vary from once a year to once every ten years. Patients with mild haemophilia B are often not diagnosed until later in life. In any patient, bleeding episodes may be more frequent in childhood and adolescence than in adulthood. *It is recommended that all children with haemophilia are investigated to establish the causative FIX gene (F9) mutation.* Female relatives may request carrier analysis when a male relative is first diagnosed as having haemophilia, when they wish to start a family, or frequently, when in early pregnancy. Genetic analysis is required to determine reliably female carrier status because the majority of female carriers have

normal plasma FIX:C levels. Carrier females with FIX clotting activity <30% are at risk for bleeding (approximately 10% of carrier females, independently of severity of disease in their family). Genetic counselling should be performed by suitably qualified professionals with in-depth knowledge of haemophilia. Ideally a professional with experience of managing and treating patients with haemophilia and their families should be involved. For detailed discussion of genetic service provision in inherited bleeding disorders, reference should be made to the UKHCDO document "Clinical Genetics Services for Haemophilia" (ISBN 901787 07 9) and the National Service Specification for Haemophilia and Other Inherited Bleeding Disorders (see Section 13.0 Web Resources).

4.0 *F9*

F9 is located on the long arm of the X chromosome at Xq27, spans 33.5kb of DNA and comprises 8 exons. *F9* mRNA is 2.8kb and encodes a pre-pro-protein of 461 amino acids that is post-translationally processed to yield a mature protein of 415 amino acids. Haemophilia B results from heterogeneous mutations spread throughout *F9*. Unlike haemophilia A, no common repeat mutation has been identified. However, 20-30% of cases of mild haemophilia B are due to a small number of founder mutations. Exon 8 is the largest *F9* exon, being 1.9kb in length and representing almost half of the *F9* coding region. Approximately half of all *F9* mutations are found in this exon. Mutations in the *F9* promoter are relatively rare (~2% of the total) but important because they can give rise to the unique Haemophilia B Leyden phenotype, which may be severe pre-puberty, but ameliorates at puberty. Such patients may even become asymptomatic.

Table 2:

Type of sequence	NCBI RefSeq accession number
RNA (cDNA)	NM_000133.x
Protein	NP_000124.x
Genomic	NG_007994.x

(.x denotes version number)

5.0 APPROACHES AND PROTOCOLS

5.1 Previously Characterised Mutations

Historically many UK families with haemophilia B had their mutations characterised by The Division of Medical & Molecular Genetics at Guy's Hospital, London. However, this programme was never intended as a diagnostic service and is no longer active. It is recommended that mutations identified during this programme should be confirmed in the testing lab prior to being used for carrier status determination. Many UK laboratories are now screening their patients for mutations in *F9* (see the UKHCDO Directory of Molecular Diagnostic Services for Inherited Bleeding Disorders). The UKHCDO haemophilia patient database, an annually updated reference to all UK registered patients, notes whether a mutation has been detected in a particular patient. Details of the mutation characterised in a patient of interest are only available from their Haemophilia Centre Director (see listing on UK Haemophilia Society website). An international mutation database is maintained by Dr. Peter Green at Guy's Hospital, London. The human gene mutation database (HGMD) also acts as a repository for collated mutation data. Links to these various resources can be found in section 13.0 Web Resources.

5.2 Mutation Detection

Mutations are generally sought in affected males, where available, and then confirmed or excluded in female relatives. Where an affected male is not available, initial mutation analysis may be performed on a known obligate carrier female. In some circumstances, despite a family history of haemophilia, neither an index case nor a known obligate carrier may be available. In such cases mutation analysis may still be undertaken in a potential carrier but the report must stress that the failure to identify a causative mutation **does not** prove non-carrier status.

The method selected for mutation detection will be dependent on resources and expertise available in a particular laboratory. Most laboratories in the UK now perform direct DNA sequencing of the essential regions of *F9* with no pre-screening step. The only mutation pre-screening method currently is use by Network laboratories is High Resolution Melt analysis (HRM).

5.2.1 HRM

High-resolution melting curve analysis (HRM) is a closed-tube fluorescent technique that can be used for genotyping and heteroduplex detection after initial PCR. The sensitivity and specificity of the technique depend on the length of amplicons used but a sensitivity of 93% has been reported when applied to haemophilia A mutation analysis (Lin *et al* 2008).

Other mutation pre-screening methods may be used but are not currently employed by the Network. For more details of these see Mitchell *et al* 2004 (version 2 of these guidelines).

Where these pre-screening methods fail to detect a candidate mutation it is recommended to proceed to direct DNA sequencing of the essential regions of F9.

5.4 Direct Sequencing

DNA sequencing is considered the gold standard for mutation detection. *F9* is small enough to contemplate sequencing the coding region, splice junctions, and the 5' & 3' regions for previously unknown mutations (Vidal et al 2000), within the time constraints of a diagnostic service. Normally a candidate mutation would be identified in a hemizygous male haemophiliac before applying DNA sequencing to determine the presence or absence of a nucleotide alteration in at risk family members. Alternatively, a known obligate carrier female can be used for initial mutation identification. Once a mutation is identified in a family, direct sequencing is the preferred method with which to confirm or exclude its presence in other family members.

Note: direct sequencing is not infallible for detecting heterozygosity for a base change. Whilst 'unidirectional sequencing' is now generally accepted as adequate for gene screening (Ellard *et al*), *it is recommended that carrier status sequencing should preferably be performed in both a 5' (forward) and 3' (reverse) direction*. Design of a 'mutation specific test, for example restriction digestion or allele specific PCR are alternative approaches to consider. However, if using restriction digestion, it is important to ensure that the test is specific for the nucleotide change: a restriction site can be lost due to any nucleotide change within the recognition motif, therefore loss of a site is not specific for a given base change.

It is recommended to state on the diagnostic report which regions of F9 have been screened.

5.4.1 DNA Sequencing Best Practice

Refer to the CMGS Sequencing Best Practice Guidelines (section 13, web resources) for guidance on minimum sequence quality and interpretation standards. It is recommended that that the following points be given particular attention: Software analysis tools (e.g. tools which facilitate comparative sequence analysis such as the Staden Package or various commercial alternatives) should be employed when analysing large quantities of DNA sequence data. Sequence analysis should always be performed only on data of high quality. In line with CMGS guidance, in the absence of a robust tube transfer checking system to assure sample identity at each stage (e.g. barcode scanning, witnessed transfers), mutations identified for the first time in a given pedigree should be confirmed by a second test, using a fresh template. Testing for known familial mutations should include, where possible, the use of a family member as the positive control. In the case of PND it is recommended to confirm the identity of the sequence change in a sample from the mother. This may be from an archive sample or, where local quality control measures dictate, repeat analysis of an independent sample.
It is recommended that in cases where only the familial mutation is tested for that this is stated on the report.

5.5 *F9* Deletions

Partial or complete *F9* gene deletions are relatively rare, comprising ~3% of patients. Such deletions may be readily detectible in affected males, but not so in heterozygous female carriers. A multiplex ligation dependent probe amplification (MLPA) kit for the detection of deletions and duplications in *F9* is available (MRC Holland –section 13 web resources) (Casana et al 2009). Other methodologies for measuring gene dosage e.g. Quantitative PCR/RT-PCR or loss of heterozygosity are possible. Large duplications have not yet been reported in *F9*, but may be identified as the use of dosage analysis increases. *Dosage analysis should be undertaken in haemophilia B patients where no other mutation has been identified.*

5.6 Primer validation

Polymorphic variation may lead to allelic drop-out if a single nucleotide polymorphism (SNP) lies under a PCR primer, affecting its amplification efficiency. Since this possibility cannot be eliminated completely it should be borne in mind when screening query carrier females who prove negative for a *F9* mutation.
It is recommended to screen primer sequences for the presence of underlying polymorphism(s) when first designed, using an appropriate checking tool such as SNPCheck, and then when each new build of the SNP database (dbSNP) becomes available (section 13 Web Resources).
A judgement can then be made on the need to redesign a specific primer pair based on reported allele frequency data relating to the described polymorphic variation.

5.7 Mutation Validation

When a novel nucleotide change is found, caution should be exercised before deciding that it is the one responsible for disease. Whereas termination, deletion and insertion mutations may obviously be causative, missense and other changes may not. The UK and Dutch molecular genetic societies have produced practice guidelines for the interpretation and reporting of unclassified variants (UV Guideline - section 13, web resources). A standard questionnaire is used to assess whether or not the variant is predicted to affect protein function, principally by means of cross-species amino acid alignment algorithms, evaluation using splice-site prediction tools and documented searches to demonstrate that the variant has not been previously reported in the literature or on mutation databases. This analysis can be assisted by the use of software such as Alamut (Interactive Biosoftware). Note that some of the requirements of the UV guideline may be more rigorous than those required for a sex-linked recessive disorder where no other sequence change has been found in the gene of an index male. In addition there are *F9* specific resources which can be drawn upon when interpreting the significance, or otherwise, of a sequence variant. The Haemophilia B Database and other resources such as HGMD can be consulted to determine whether the change has been previously reported in a patient having similar disease severity. Note that entries on mutation databases need to be interpreted with a degree of caution because they may not have been rigorously peer reviewed.

Minimum checks should include:

• Has the change been previously reported as a causative mutation?
• Does the reported severity agree with the phenotype in the patient being analysed?
• Has the candidate mutation been reported previously as a polymorphism? Resources such as dbSNP and Seattle SNP should be utilised.
• Is the changed amino acid conserved across species?
• Is an amino acid change likely to be pathogenic e.g. is the change non-conservative in nature or is it within a recognized functionally important region of the protein?
• Could the candidate mutation affect splicing? The UV Guideline recommends that at least four such *in silico* predictions are run before making any conclusion on pathogenicity
• Could the ethnic origin of the patient affect interpretation of polymorphism/candidate mutation status for a given base change?

The family should be tested to determine whether the nucleotide alteration tracks with the disease and a panel of normal DNA samples of the same ethnic origin (where possible) examined to rule out a polymorphic change. Wherever possible candidate mutations should be confirmed in affected, or excluded in unaffected, males on the maternal side of the family. Remember that candidate mutations can still be used as bespoke genetic markers if they track appropriately within the family, irrespective of their disease association.

DNA sequencing of the *F9* coding and control regions (promoter & 3'UTR) should have a very high degree of sensitivity. However, no causative mutation was identified in a small number of patients (<3%; Ljung et al 2001, Tagariello et al 2007). This may be the result of the defect lying in a region not routinely analysed e.g. deep within introns, being undetectable by sequencing e.g. duplications of entire exons or be mediated by mutation mechanisms not yet elucidated. Whilst there is no evidence to suggest it is likely, the

possibility of non-linkage of the haemophilia B phenotype to *F9* should be considered – assuming a possible alternative diagnosis e.g. multiple vitamin K-dependent coagulation factor deficiency has been excluded (Weston and Monahan 2008).
A recent survey of *F9* mutation detection was performed in the UK and Ireland (Vince Jenkins, personal communication). Data was collated from 11 laboratories of *F9* analysis in 724 apparently unrelated patients diagnosed with haemophilia B. Causative mutations were identified in 704 of 724 cases a detection rate of 97.2% . However the sensitivity for patients with FIX levels between <1-40% was 98.2%. The majority of patients with no detectable genetic lesion were mild haemophilia B patients. The proportion of severely affected patients with no mutation comprised 0.5% of the total analysed. (Data collated and held by Haemophilia Genetics Laboratory Network).

5.8 Linkage Analysis
Historically, linkage analysis was the method most commonly used to determine female carrier status in families with haemophilia B. Linkage studies have been superseded by direct mutation analysis protocols. However, intragenic linked markers may still be useful in certain circumstances, such as:
• Where a family has previously been investigated by linked markers and the mutation has not been identified
• Where a mutation has not been verified
• Where a mutation has not been found
• Where mutation analysis is not available
Only the use of intragenic markers should be considered. For detailed information on intragenic markers applicable to haemophilia B carrier detection see the earlier published version of these guidelines (Mitchell *et al* 2005).
It should be noted that linkage analysis fails in a number of families for one of the following reasons;
• Lack of prior family history (although exclusion may still be possible).
• Key pedigree members not available.
• Polymorphisms uninformative in key female(s).
• Non-paternity.
• Linkage analysis cannot determine the carrier status of the mother of a haemophiliac.
Wherever possible, mutation detection should be used for genetic counselling in haemophilia B families. However, this may not always be practicable and, where direct mutation detection is not feasible, linkage analysis provides an acceptable alternative which offers a high degree of diagnostic confidence.
It is recommended that where a mutation has not been found and linkage analysis has to be used, intragenic F9 polymorphisms are analysed.
It is recommended that the diagnostic report should state that the possibility of non linkage of the phenotype to F9 cannot be excluded.

6.0 PND
PND is generally performed by chorionic villus sampling (CVS) at between 11 and 13 weeks of gestation, or amniocentesis around 15 weeks gestation. For both CVS and amniocentesis, direct karyotype analysis may be performed to determine fetal sex and to ensure that there are no chromosomal abnormalities – if requested. Rapid PCR based sexing protocols are in common usage. Female foetuses sexed by this method require confirmation that no maternal contamination is present in the sample. Both sexing and maternal contamination checks can be achieved by PCR amplification using commercially available kits that amplify, for example, multiple autosomal STRs to demonstrate the absence of maternal material in the foetal sample and also include sexing primers. A CMGS Practice Guideline on determination of maternal contamination is available (section 13, web resources). Female fetuses require no further analysis. Male haemophilia status can be determined by analysis of a previously determined familial mutation or informative marker. All of these analyses for *F9* involve rapid PCR-based analyses, therefore results should be provided within 2-3 days of the CVS sample being taken.
In practice, with the availability of non-invasive testing (see section 6.1) and direct karyotype sexing, the majority of PNDs that reach the lab are 'known' to be male.
Detailed discussion of issues of PND can be found in the UKHCDO document "Clinical Genetics Services for Haemophilia" (ISBN 901787 07 9).
6.1 Non-invasive prenatal testing
Non-invasive prenatal testing has been applied to haemophilia B. Fetal gender can be determined by ultrasound analysis which can be corroborated by analysis of either cell-free fetal DNA or intact fetal cells from the maternal circulation. Fetal sex has been determined in blood samples of haemophilia carriers using amelogenin or *SRY* genetic analysis (Santacroce et al 2006, Chi et al 2006). Non-invasive fetal sexing is available as a diagnostic service in the UK (see CMGS Lab Directory for up-to-date listing – section 13, web resources)
6.1.1 Pre-implantation genetic diagnosis
A proportion of women at risk of having a child with haemophilia do not wish to undergo invasive prenatal diagnosis and possible termination of pregnancy, or they may already be undergoing fertility treatment. Pre-implantation genetic diagnosis (PGD) may be an option in these cases (Lavery 2009).
6.1.2 Pre-implantation genetic haplotyping (PGH)
This is an alternative method for PGD enabling the selection of unaffected embryos in monogenic diseases in general. In PGH, a single blastomere is used for whole genome amplification (Renwick et al 2007). A panel of polymorphic markers surrounding the gene of interest are amplified and embryos screened for the affected allele(s) for which linkage has already been established in the family. Female embryos and males unaffected by haemophilia can then be implanted (Laurie et al 2010).
These non-invasive approaches are not yet well established in a routine diagnostic setting.

6.2 Late amniocentesis sampling
For women known to be haemophilia carriers who are carrying a male baby, but do not wish to pursue PND with a view to termination, options exist to inform clinical management of delivery. The haemophilia status of the male foetus can be determined by analysis of an amniotic sample taken late in pregnancy. This procedure should not be performed earlier than 35 weeks gestation in case it induces premature labour. Male fetuses shown not to carry the familial mutation can then be delivered in a setting of the mother's choice.

7.0 WORDING OF REPORTS

Reports must be clear, concise, accurate, fully interpretive, credible and authoritative. Best Practice Guidelines for Reporting have been produced by the CMGS (1997 – now 'retired') and the Swiss Society of Medical Genetics (2003 via EuroGentest) –section 13 .0 web resources.

7.1 Mutation Analysis Reporting
Wording will depend on the confidence placed in the interpretation of any candidate mutation, as discussed in section 5.6. Suggested wording for a mutation which has a high confidence attached to it may include**:**

7.1.1 Mutation analysis in males
"x has a F9 mutation (c.No.nt>nt, p.aaNo.aa), previously reported in F9 / not previously reported. The mutation is consistent with the severity of haemophilia B in x". A brief explanation as to why a novel mutation is considered causative should be included, especially for a missense mutation. For example, the altered amino acid is conserved across species, and/or is structurally/functionally important; this base change has been excluded as a common polymorphism by analysis of >100 normal alleles; etc.

7.1.2 Mutation analysis in females
"y carries a F9 mutation (c.No.nt>nt, p.aaNo.aa) which is consistent with the severity of haemophilia B seen in male relative x".

7.2 Linkage analysis using intragenic markers
The following wording is suggested; *The female can be diagnosed as a carrier/excluded from being a carrier, with a risk of error due to meiotic recombination of <1%.*

8.0 NOMENCLATURE AND MUTATION REPORTING

8.1 Mutation Nomenclature
Mutation reporting should ensure that the location of a given sequence change can be identified between laboratories. For guidance on nomenclature conventions used when reporting *F9* mutations refer to the recommendations of the Human Genome Variation Society (HGVS – section 13, web resources). Use of the HGVS system allows systematic identification of sequence alterations. In the case of *F9* the primary RefSeq in use (table 2) is the RNA (cDNA) sequence. This numbering system supersedes that of Yoshitake et al, 1985.

1. Use cDNA nucleotide numbering; +1 is the first base ("A") of the initiator methionine codon.

2. Amino acid numbering starts at +1 for the initiator methionine. Traditionally the system in use for FIX amino acid numbering has started from the beginning of the mature protein sequence (see *Legacy Nomenclature* section below). *It is recommended that, for consistency with other genes, and in observance with HGVS guidance, it should be standard practice to number, on the diagnostic report, FIX amino acids from the initiator methionine.*

3. Three letter amino acid codes should be used in the report to avoid confusion between amino acids and DNA bases.

4. The intron or exon containing the mutation should be stated.

5. A genomic reference sequence is available for *F9* (table 2). However, when reporting intronic changes, intronic numbering should be based on the cDNA nucleotide number of the closest end of the adjacent exon and should comply with HGVS guidelines: *"-" for intronic mutations upstream of an exon, where "-1" is the intronic nucleotide immediately 5' to the first exonic nucleotide; "+" for intronic mutations downstream of an exon end, where "+1" is the intronic nucleotide immediately 3' to the last exonic nucleotide.*

8.2 Legacy Nomenclature
Note that databases such as the Haemophilia B Mutation Database and HGMD still give the previous nomenclature convention (Yoshitake et al 1985) for amino acid numbering of FIX (accessed 31 October 2010).
To convert to this legacy amino acid numbering system which refers to the mature protein, subtract 46 from the HGVS convention amino acid number.
Given this legacy nomenclature it is essential practice to ensure that when reporting a mutation the nomenclature system in use is clearly stated.
It is recommended that the older numbering system should no longer be used, but where a family member has been previously reported using this legacy nomenclature, both old and new should be referred to in subsequent reports on the family (e.g. p.Arg75X, previous nomenclature Arg29X).

Including both numbering systems on the report is advisable, providing the old nomenclature does not obscure the HGVS nomenclature, e.g. by its inclusion as a footnote or table legend.

9.0 MOSAICISM
Germline and somatic mosaicism may complicate any genetic diagnosis in haemophilia B (Kasper and Buzin 2009). Particular attention should be given to the possibility of mosaicism in sporadic haemophilia where the mother of an affected male does not appear to carry the mutation in her leucocyte DNA, particularly (although not exclusively) where the apparently *de novo* mutation is a point mutation.
It is recommended not to state that the mother of a haemophiliac is not a carrier, even when the mutation is not identified in her somatic DNA. A specific reference to the possibility of germline mosaicism may be added.

10.0 TURN-AROUND TIMES
Analysis of samples for diagnostic cases should be given a defined turn-around time, so that both patients and clinicians have a reasonable, defined date to work to. The recommendations of the Genetics White Paper (http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_4019239.pdf) i.e. 8 weeks for screening in a single gene disorder, 2 weeks for a known mutation and 3 working days for a prenatal diagnosis should be used as a guideline but clinically appropriate turnaround times may be defined / agreed locally.

11.0 EXTERNAL QUALITY ASSESSMENT & REFERENCE SAMPLES FOR TEST OPTIMISATION AND VALIDATION
An EQA scheme has been established for haemophilia genetic investigation (Perry et al 2006). Details are available from UK NEQAS for Blood Coagulation - section 13, web resources.
The scheme examines clerical accuracy, correct identification

of the nucleotide change and its effect if any on the protein according to HGVS guidance and the quality of interpretation offered.
Participation in EQA is essential for UK laboratories performing haemophilia genetic analysis.
Reference samples have yet to be established for haemophilia B.

12.0 SPECIALIST REFERRAL LABORATORIES
A listing of services available for haemophilia A and B testing offered by UK laboratories is found in the UKHCDO Directory of Molecular Diagnostic Services for Inherited Bleeding Disorders
http://www.ukhcdo.org/GeneticsNetworkNW/directory.htm

13.0 WEB RESOURCES

CMGS Best Practice Guidelines, including guidance on DNA sequencing and interpretation of unclassified variants
http://www.cmgs.org/BPGs/Best_Practice_Guidelines.htm

Clinical Molecular Genetics Society, Lab Directory
http://www.cmgs.org/Lab%20Directory/lab_directory.htm

EuroGentest – Best Practice Guidelines for Molecular Genetic testing.
http://www.eurogentest.org/web/info/public/unit1/eqa/molecular_genetics/guidelines.xhtml

Haemophilia B database: Annually updated list of reported mutations in FIX.
http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html

HGMD. The Human Gene Mutation Database. This collates mutation data on many disease genes. Access to the most up to date data requires a subscription.
http://www.hgmd.cf.ac.uk/ac/index.php,

Human Genome Variation Society (HGVS) guidance on mutation nomenclature
http://www.genomic.unimelb.edu.au/mdi/mutnomen/

MRC Holland – MLPA information and kits
http://www.mrc-holland.com/WebForms/WebFormMain.aspx

NCBI RefSeq database. This contains current and archive versions of reference sequences given in table 2.
http://www.ncbi.nlm.nih.gov/RefSeq/

NCBI Single Nucleotide Polymorphism database (dbSNP)
http://www.ncbi.nlm.nih.gov/SNP/

NGRL Manchester UK genetic service network support. Includes MLPA data interpretation aids and primer validation tools (SNPCheck):
http://www.ngrl.org.uk/Manchester/index.html

Seattle SNPs: single nucleotide polymorphism resource.
http://pga.gs.washington.edu/

UK Haemophilia Society. Represents those affected by bleeding disorders in the UK and lists all UK Haemophilia Centres, with Directors names and addresses.
http://www.haemophilia.org.uk/

UKHCDO. A National Service Specification for Haemophilia and Other Inherited Bleeding Disorders
http://www.ukhcdo.org/docs/HaemAlliance-NatSvsSpec2006.pdf

UKHCDO Directory of Molecular Diagnostic Services for Inherited Bleeding Disorders: http://www.ukhcdo.org/

UK NEQAS for Blood Coagulation. External quality assessment scheme for genetic and phenotypic analysis of haemostatic disorders; http://www.ukneqasbc.org/

World Federation for Hemophilia. Contains information for patients and healthcare professionals
http://www.wfh.org/index.asp?lang=EN

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