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

TEST – DISORDER/CONDITION – POPULATION TRIAD			
Submitting laboratory:	**Cardiff SAS Porphyria**	**Approved:**	**Sept 2013**

1. Disorder/condition – approved name and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website)	ANGIOEDEMA, HEREDITARY, TYPE III; HAE3
2. OMIM number for disorder/condition	610618
3a. Disorder/condition – please provide, in laymen's terms, a brief (2-5 sentences) description of how the disorder(s) affect individuals and prognosis.	Hereditary angioedema type III[1] is a rare disorder characterized clinically by recurrent subcutaneous swelling which may affect the skin, gut or larynx. The latter is life-threatening as it may lead to airway obstruction. HAE III occurs almost exclusively in women and is often precipitated or worsened by oestrogens (e.g., during pregnancy, periods or treatment with oral contraceptives)[1]. It is caused by gain of function mutations in the coagulation factor XII gene[2].
3b Disorder/condition – if required please expand on the description of the disorder provided in answer to Q3a.	HAE Types I and II are caused by mutations in the SERPING1 gene, which encodes C1-inhibitor. HAE III can therefore be distinguished from HAE I and HAE II by demonstrating normal C3, C4, C1-inhibitor concentration and function in serum. Treatment of Type III HAE is different from Types I and II in terms of avoidance of oestrogens and the use of the bradykinin B2 receptor antagonist (Icatibant) and C1 esterase inhibitor. 1.Bork, K., Barnstedt, S.-E., Koch, P., Traupe, H[2000]. Hereditary angioedema with normal C1-inhibitor activity in women. Lancet 356: 213-217 2. Dewald G, Bork K [2006] Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. Biochem, Biophys, Res Commun 343: 1286-1289
4. Disorder/condition – mode of inheritance	Autosomal Dominant
5. Gene – approved name(s) and symbol as published on HGNC database (alternative names will be listed on the UKGTN website)	coagulation factor XII (Hageman factor); F12
6a. OMIM number for gene(s) **6b HGNC number for gene(s)**	610619 HGNC:3530
7a. Gene – description(s)	The F12 gene, located on chromosome 5q33-qter is 7.4kb long and contains 14 exons. The mutations associated with angioedema are restricted to exon 9.

7b. Number of amplicons to provide this test (molecular) or type of test (cytogenetic)	1
7c. GenU band that this test is assigned to for index case testing	Band B
8. Mutational spectrum for which you test including details of known common mutations	Two missense mutations that affect the same codon in exon 9 [c.983C>A; p.Thr328Lys, c.983C>G; p.Thr328Arg] have been identified in cases of Type III HAE[1]. In addition, a 72bp deletion [c.971_1018+24del72], which leads to the loss of 48 bp of exon 9 (coding amino acids 324 to 340) has been identified in one Turkish family[2] . 1. Dewald G, Bork K [2006] Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. Biochem, Biophys, Res Commun 343: 1286-1289. 2. Bork K, Wulff K, Meinke P, et al [2011] A novel mutation in the coagulation factor 12 gene in subjects with hereditary angioedema and normal C1-inhibitor. Clin Immunol. 141: 31-5.
9a. Technical method(s)	PCR amplification of exon 9, followed by bi-directional fluorescent sequencing.
9b If a panel test using NGS please state if it is a conventional panel or a targeted exome test.	
9c. Panel/targeted exome Tests **i) Do the genes have 100% coverage? If not what is the strategy for dealing with the gaps in coverage?**	
ii) Does the test include MLPA?	
iii) Does this use sanger sequencing or Next Generation Sequencing (NGS)?	
iv) If NGS is used, does the lab adhere to the Practice Guidelines for NGS?	
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.	
11. Validation process Please explain how this test has been validated for use in your laboratory or submit your internal validation documentation	Blast analysis of primer sequences. SNP analysis of primers. Primer sequences with low secondary structure. Optimisation of PCRs. Sequencing of normal individuals and a positive control with comparison to a reference sequence. Sequencing is used for mutation detection in the laboratory for a number of genes.

12a. Are you providing this test already?	☐ **No** ☒ **Yes**
12b. If yes, how many reports have you produced? Please provide the time period in which these reports have been produced and whether in a research or a full clinical diagnostic setting.	14
12c. Number of reports mutation positive	3
12d. Number of reports mutation negative	11
13. For how long have you been providing this service?	12 months
14a. Is there specialised local clinical/research expertise for this disorder?	☐ **No** ☒ **Yes**
14b. If yes, please provide details	The Immunology Department delivers the National Paediatric and Adult Primary Immunodeficiency (PID) services for Wales and has one of the largest cohorts of patients in the UK. This cohort includes approximately 40 patients with hereditary angioedema. The immunology laboratory is the hub laboratory in Wales and supports the diagnosis of PID patients. There is particular expertise in complement diagnostics at UHW with testing and advice offered nationally. Clinical services are provided as part of the Immunology Service as follows: 1) All patients are provided with information about their disorder at diagnosis. This includes patient support group information and all Wales PID patients' days. 2) Clinical and interpretative advice is available from Dr S Jolles, Dr T El-Shanawany or Dr P Williams. 3) Outpatient referrals are seen in either Paediatric or Adult weekly Immunodeficiency Clinics. 4) Inpatient assessment is available through shared care arrangements with Paediatric Infectious Disease and adult ID teams with the Department of Medicine. There has been a long-standing research interest in both the treatment and diagnosis of immunodeficiency including HAE in the department.
15. Are you testing for other genes/disorders/conditions closely allied to this one? Please give details	Hereditary Angioedema [HAE] Type I Hereditary Angioedema [HAE] Type II

16. Based on experience what will be the national (UK wide) activity, per annum, for:	These conditions are due to mutation of the SERPING1 gene. However as there has been no test for this condition available in the UK until now it is difficult to accurately predict the requirement. Based on the few estimates of HAE prevalence we calculate approximately 100 female patients nationwide.
16a. Index cases	10
16b. Family members where mutation is known	5
17a. Does the laboratory have capacity to provide the expected national activity?	Yes
17b. If your laboratory does not have capacity 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".	
18. Please justify the requirement for another laboratory to provide this test e.g. insufficient national capacity.	

EPIDEMIOLOGY	
19a. Estimated prevalence of condition in the general UK population	The prevalence of HAE in the UK is unknown, but estimates in Europe and America suggest disease prevalence is approximately 1:50000 [1,2]. HAE III was first reported in 2000 and relatively few cases have been reported since. Consequently, the frequency of HAE III compared to other types of HAE cannot be accurately determined. However, approximately 4% of patients with hereditary angioedema from the original cohort described by Bork had normal C4 concentration and C1-inhibitor concentration and function in plasma [3], providing an estimate of HAE III prevalence of 1:1250000. 1.Bygum A [2009]. Hereditary angio-oedema in Denmark: a nationwide survey. Br J Dermatol 161: 1153-8. 2.Weiler CR Van Dellen RG [2006] Genetic Test Indications and Interpretations in Patients With Hereditary Angioedema. Mayo Clin Proc 81:958-72. 3.Bork, K., Barnstedt, S.-E., Koch, P., Traupe, H [2000]. Hereditary angioedema with normal C1-inhibitor activity in women. Lancet 356: 213-217.
19b. Estimated incidence of condition in the general UK population Please identify the information on which this is based	Unknown albeit rare. Majority of cases are female
20. Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	This has not been formally established**. F12** mutations were identified in 3 of 15 (20%) of families in a French cohort of clinically and biologically defined HAE III cases [1], and in 6 of 20 (33%) unrelated cases of HAE III[2]. However, penetrance in males is much lower than in females and therefore gene frequency will be greater than disease frequency. 1. Vitrat-Hincky, Gompel A, Dumestre-Perard et al [2010] Type III hereditary angio-oedema: clinical and biological features in a French cohort. Allergy 65: 1331-1336. 2.Dewald G, Bork K [2006] Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. Biochem, Biophys, Res Commun 343: 1286-1289.
21. Estimated penetrance Please identify the information on which this is based	This has not been formally established since patients may have minimal or no symptoms for years before they become symptomatic or are exposed to oestrogens. Penetrance is higher in females than males. Affected males with p.Thr328Lys in the F12 gene have been confirmed in one family [1]. DNA analysis of 20 HAE III cases in 5 families identified two unaffected female and 8 male heterozygotes [2]. In another study, screening of 197 unrelated healthy

	controls identified heterozygous p.Thr328Lys in 2 males[3]. 1. Martin L, Raison-Peyron N, Nothen MM et al [2007] Hereditary angioedema with normal C1 inhibitor gene in a family with affected women and men is associated with the p.Thr328Lys mutation in the F12 gene. J Allergy Clin Immunol 120: 975-977. 2.Dewald G, Bork K [2006] Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. Biochem, Biophys, Res Commun 343: 1286-1289. 3. Nagy N, Greaves MW, Tanaka A et al [2009] Recurrent European missense mutations in the F12 gene in a British family with Type III Hereditary Angioedema J Dermat Sci 56: 58-73.
22. Estimated prevalence of condition in the population of people that meet the Testing Criteria.	Testing for mutations in patients with a family history of predominantly affected females, often with symptoms related to oestrogen and with normal C4 levels and normal C1-inhibitor concentration and activity, would result in mutations being detected in 20-33% of cases.

INTENDED USE

23. 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

24. 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.

The analytical sensitivity of fluorescent sequencing is considered to be greater than 99%.

25. 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).

Testing for mutations in patients with a family history of predominantly affected females, often with symptoms related to oestrogen and with normal C4 levels and normal C1-inhibitor concentration and activity, would result in mutations being detected in 20-33% of cases. Clinical specificity would be predicted to be high.

26. 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).

The clinical validity is high. The positive predictive value is different for females and males given the different oestrogen levels and the degree of penetrance and would therefore be lower in males than females. It is also clear that there remain as yet undiscovered causes of non urticarial angioedema with normal C4 and C1INH and fC1INH.

27. Testing pathway for tests where more than one gene is to be tested 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.

If there is an appropriate clinical history of hereditary angioedema with normal C4 and C1INH and fC1INH then only the analysis of FXII would be performed.

CLINICAL UTILITY

28. How will the test change the management of the patient and/or alter clinical outcome?

There is currently no biochemical test for Type III HAE as C3, C4, C1INH and fC1INH are normal. In addition the subsequent treatment of Type III HAE differs from Types I and II in advice in terms of oestrogen avoidance (progesterone only OCP etc) and the potential use of the bradykinin B2 receptor antagonist (Icatibant) and C1 esterase inhibitor. It may be difficult to obtain funding for these therapies in the absence of a clear diagnosis.

29. Benefits of the test for the patient & other family members Please provide a summary of the overall benefits of this test.

Diagnosis as well as family testing is of utility for patients, alongside the reduction in attacks through targeted advice and the reassurance of having an effective treatment in the event of severe acute attacks which are disabling, abdominal or life threatening attacks affecting the airway. This is likely to improve quality of life and avoid or reduce the frequency of abdominal pain and vomiting. Abdominal attacks may mimic an acute abdomen and have been known to result in inappropriate laparotomy where the diagnosis of HAE is not known. Peripheral attacks and airway attacks have also resulted in inappropriate prescription of antihistamines and adrenaline auto-injectors which are not effective in this condition. The diagnostic delay for Type I HAE is 9.6 years and Type II 17.7 years (S Jolles: UK National audit) with a 3-9 fold increase in death from laryngeal oedema in patients before diagnosis compared with following diagnosis.

Bork, K., J. Hardt, and G. Witzke, Fatal laryngeal attacks and mortality in hereditary angioedema due to C1-INH deficiency. J Allergy Clin Immunol, 2012. **130**(3): p. 692-7.

30. What will be the consequences for patients and family members if this test is not approved?

If this test is not available diagnosis of this condition will not be possible and this will significantly affect clinical management of these patients with poor clinical outcomes.

31. Is there an alternative means of diagnosis or prediction that does not involve molecular diagnosis? If so (and in particular if there is a biochemical test), please state the added advantage of the molecular test.

There is currently no other definitive test for Type III HAE.

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

None.

33. Only complete this question if there is previously approved Testing Criteria and you do not agree with it.

Please provide revised Testing Criteria on the Testing Criteria form and explain here the changes and the reasons for the changes.

34. List the diagnostic tests/procedures that an index case no longer needs if this genetic test is available.

Unlikely to be significant cost savings for index cases in lead up to being offered genetic test.

	Type of test	Cost (£)
Costs and type of imaging procedures		
Costs and types of laboratory pathology tests (other than molecular/cyto genetic test proposed in this gene dossier)		
Costs and types of physiological tests (e.g. ECG)		
Cost and types of other investigations/procedures (e.g. biopsy)		
Total cost tests/procedures no longer required		

35. Based on the expected annual activity of index cases (Q15a), please calculate the estimated annual savings/investments based on information provided in Q33.

Number of index cases expected annually	(a)
Cost to provide tests for index cases if the genetic test in this gene dossier was not available (see Q34)	(b)
Total annual costs pre genetic test	(a) x (b) = (c)
Total annual costs to provide genetic test	(a) x cost of genetic testing for index case = (d)
Total savings/investment	(c) – (d)

36. REAL LIFE CASE STUDY
In collaboration with the clinical lead, describe TWO real case examples:

1. **prior to availability of genetic test**
2. **post availability of genetic test**

to illustrate how the test improves patient experience and the costs involved.

Case example one – pre genetic test

A family of 3 females (mother and 2 daughters) presented in 2002 with recurrent episodes of facial and laryngeal oedema triggered by the use of the oral contraceptive pill and pregnancy. All 3 patients had normal C3, C4 and C1 esterase inhibitor (one daughter had borderline C1 esterase inhibitor levels on one occasion). HAE III was suspected but no confirmatory tests were available at the time. The management was limited to avoidance of oestrogen-containing pills and acute attacks were managed conservatively.
The family was lost to follow up but returned in 2012 when one of the daughters became pregnant. Testing for Factor XII mutations was then available and promptly identified the sequence variant c.983C>A (p.Thr328Lys) in all 3 females, confirming the diagnosis of HAE III. All 3 patients now have access to emergency bradykinin 2 receptor blocker (Icatibant) for their acute attacks, which has been shown to be effective in HAE III.
Genetic testing and the development of a bradykinin 2 receptor blocker have transformed the management of these patients over the past decade.

PRE GENETIC TEST COSTS

	Type of test	**Cost**
Costs and type of imaging procedures		
Costs and type of laboratory pathology tests		
Costs and type of physiological tests (e.g. ECG)		
Cost and type of other investigations/procedures (e.g. biopsy)		
Cost outpatient consultations (genetics and non genetics)		
Total cost pre genetic test		**£**

Case example two – post genetic test

POST GENETIC TEST COSTS

	Type of test	**Cost**
Costs and type of imaging procedures		
Costs and types laboratory pathology tests (other than molecular/cyto genetic proposed in this gene dossier)		
Cost of genetic test proposing in this gene dossier	DNA sequencing	£150
Costs and type of physiological tests (e.g. ECG)		
Cost and type of other investigations/procedures (e.g. biopsy)		
Cost outpatient consultations (genetics and non genetics)		
Total cost post genetic test		**£**

In the example above effective early treatment of attacks is likely to result in reduced need for hospitalisation due to attacks and clarity of diagnosis also means that there is a reduction in the prescription and use of ineffective or inappropriate therapies. The benefits in terms of improvement in QoL are more difficult to measure given the current lack of disease specific QoL tool, however the 10 years diagnostic delay is over and the risk of death from laryngeal oedema is now minimal given the introduction of effective therapy. Costs of therapy are approximately £1200 per icatibant injection.

37. Estimated savings between two case examples described £ n/a as the patients are from another centre.

UKGTN Testing Criteria

Test name: **Angioedema, Hereditary, Type III**	
Approved name and symbol of disease/condition(s): Angioedema, Hereditary, Type III; HAE3	**OMIM number(s):**
Approved name and symbol of gene(s): coagulation factor XII (Hageman factor); F12	**OMIM number(s):** 610619

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

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

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Clinical history of non-urticarial angioedema, particularly with a family history of similarly affected females and exacerbation of swellings due to oestrogens **AND**	
Exclusion of other causes – Drugs (eg ACE inhibitors) and Type I and II HAE by normal C4 and C1INH and fC1INH	
At risk family members where familial mutation is known.	

Additional Information:

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 2013 Submitting Laboratory: Cardiff SAS Porphyria