

# Clinical Practice Guidelines for the management of atypical Haemolytic Uraemic Syndrome in the United Kingdom

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## Summary

Atypical haemolytic uraemic syndrome (aHUS) is associated with a poor prognosis with regard to survival at presentation, recovery of renal function and transplantation. It is now established that aHUS is a disease of complement dysregulation with mutations in the genes encoding both complement regulators and activators, and autoantibodies against the complement regulator factor H. Identification of the underlying molecular abnormality in an individual patient can now help to guide their future management. In these guidelines we make recommendations for the investigation and management of aHUS patients both at presentation and in the long-term. We particularly address the role of renal transplantation alone and combined liver-kidney transplantation.

**Keywords:** haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura, complement, genetic investigation, transplantation.

## 1. Aim, scope and audience of the guidelines

These guidelines make recommendations for the investigation and management of patients within the United Kingdom who present with features compatible with a diagnosis of atypical haemolytic uraemic syndrome (aHUS). The guidelines are intended for use by healthcare professionals involved in the management of such patients. The recommendations in the guidelines are in keeping with other guidelines recently published by the European Paediatric Study Group for HUS (Ariceta *et al*, 2009) and the Consensus Study Group for liver-kidney transplantation in aHUS (Saland *et al*, 2009). The GRADE (Grading of Recommendations Assessment, Development and Evaluation) system (<http://www.gradeworkinggroup.org>) has been used to classify the strength of recommendations (strong or weak) and the quality of

evidence (high, moderate, low and very low) (Guyatt *et al*, 2008).

## 2. Classification, nomenclature and background

Haemolytic Uraemic Syndrome (HUS) is a disease characterised by the triad of acute renal failure, thrombocytopenia and microangiopathic haemolytic anaemia (Coombs test negative). The demonstration of a role for enterohaemorrhagic *Escherichia coli* and verotoxin (shiga-like toxin) in HUS associated with diarrhoea (D+HUS) (Karmali *et al*, 1983) confirmed the differentiation between diarrhoea-associated and non-diarrhoeal forms (D-) forms (Barratt *et al*, 1987). The D- form subsequently also became known as atypical HUS (aHUS). In a small number of patients the disease was also shown to be familial (Kaplan *et al*, 1975) and/or recurrent (Kaplan, 1977).

Our increased understanding of the molecular mechanisms responsible for both HUS and thrombotic thrombocytopenic purpura (TTP) has led to the need to re-examine the classification of these diseases. A recent publication from the European Paediatric Research Group for HUS has suggested that a new classification based on aetiology be adopted (Besbas *et al*, 2006). Table I shows this new classification and those diseases that might currently be considered under the heading of aHUS are highlighted. The term 'aHUS' will be used throughout the rest of these guidelines and the forms which comprise this group are the focus of this document.

### 2.1 Infection-induced HUS

Infection-induced HUS includes shiga and shiga-like (toxin)-producing bacteria (classically associated with D+HUS) and *Streptococcus pneumoniae*. Typically D+HUS is triggered by enterohaemorrhagic *Escherichia coli*, manifests as an acute self-limiting disease and >90% of childhood cases recover independent renal function spontaneously. Invasive *Streptococcus pneumoniae* produces neuraminidase that cleaves sialic acid residues from various glycoproteins and, on red cell surfaces, exposes the Thomsen-Freidenreich antigen (T-antigen). Desialation is thought to predispose to a thrombotic microangiopathy (Klein *et al*, 1977).

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**Table I.** Classification of thrombotic microangiopathies. Adapted by permission from Macmillan Publishers Ltd: Besbas *et al* (2006). Those conditions which might be included under the heading of aHUS are highlighted.

Advanced understanding of aetiology
Infection induced
Shiga and verocytotoxin (shiga-like toxin)-producing bacteria <i>Streptococcus pneumoniae</i>
Disorders of complement regulation
Genetic disorders of complement regulation
Acquired disorders of complement regulation, for example anti-FH antibody
ADAMTS13 abnormalities
ADAMTS13 deficiency secondary to mutations
Autoantibodies against ADAMTS13
Defective cobalamine metabolism
Quinine induced
Aetiology not fully understood
HIV
Malignancy
Drugs
Pregnancy
Systemic lupus erythematosus and antiphospholipid antibody syndrome

## 2.2 Disorders of complement regulation

Disorders of complement regulation include mutations and copy number variation in the genes encoding factor H (*CFH*), CD46 (*CD46*, previously termed membrane cofactor protein [*MCP*]), factor I (*CFI*), factor B (*CFB*), C3 (*C3*) and factor H related proteins 1-5 (*CFHR1-5*).

In *c.* 30% of patients mutations will be found in the gene encoding the soluble complement regulator factor H (Saunders *et al*, 2006). A hybrid complement gene, which encodes a protein identical to a mutant form of factor H has also been described (Venables *et al*, 2006).

Autoantibodies against factor H have been reported in *c.* 10% of aHUS patients, most of whom are children, (Dragon-Durey *et al*, 2005; Jozsi *et al*, 2007) and are associated with deficiency of factor H related protein 1 (Jozsi *et al*, 2007).

In *c.* 10% of patients mutations are found in CD46 (Richards *et al*, 2007).

In another 10% mutations are found in the serine protease factor I which is responsible for cleaving complement convertases with the aid of cofactors such as factor H and MCP. Mutations in factor I that affect both secretion and function have been reported (Fremeaux-Bacchi *et al*, 2004; Kavanagh *et al*, 2005; Caprioli *et al*, 2006).

Activating mutations in complement factor B and C3 have also recently been reported in a small number of patients (Goicoechea de Jorge *et al*, 2007; Fremeaux-Bacchi *et al*, 2008). The penetrance of mutations in all these genes is *c.* 50% and it has been shown that various factors, including

copy number of other complement genes (Zipfel *et al*, 2007) and susceptibility haplotypes in factor H and MCP (Caprioli *et al*, 2003; Esparza-Gordillo *et al*, 2005), are responsible. It has also been shown that mutations in more than one complement gene can influence the predisposition to developing HUS (Esparza-Gordillo *et al*, 2006).

## 2.3 ADAMTS13 deficiency

On rare occasions, patients presenting with the clinical phenotype of aHUS have a profound deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), either due to a congenital ADAMTS13 defect (eg. in young children) or acquired antibody to ADAMTS13. It is often difficult to distinguish between aHUS and TTP, but acquired TTP very rarely presents with dialysis-dependant renal failure. Congenital TTP cases however, may have end-stage renal failure, but typically as a result of recurrent 'HUS' episodes. These patients are better described as having TTP and should be managed according to the 'Guidelines on the Diagnosis and Management of the Thrombotic Microangiopathic Haemolytic Anaemias' (Allford *et al*, 2003).

## 2.4 Disorders of defective cobalamine metabolism

Disorders of intracellular cobalamine metabolism, usually Cobalamin C (cblC) disease, predispose to a thrombotic microangiopathy (cblC) (Geraghty *et al*, 1992). This is characterised by methylmalonic aciduria and homocystinuria. HUS with cblC has also been associated with a factor H mutation (Guignonis *et al*, 2005).

## 2.5 Quinine-induced thrombotic microangiopathy

Quinine-induced thrombotic microangiopathy is associated with antibodies against glycoproteins on platelets.

## 2.6 Other diseases associated with a thrombotic microangiopathy

Diseases associated with a thrombotic microangiopathy where the aetiology is not so well defined includes human immunodeficiency virus (HIV), malignancy, drugs (including cytotoxics, calcineurin inhibitors, oral contraceptives and antiplatelet agents) (Dlott *et al*, 2004), pregnancy, systemic lupus erythematosus (SLE) and antiphospholipid antibody syndrome.

## 3. Demography of aHUS

aHUS is a rare disease (incidence *c.* 2 per million population per year) that affects all ages but primarily children and young adults. The incidence of aHUS in children is approximately 5% that of typical HUS. Updated national

data on paediatric HUS established for some European countries (France, Germany, Austria and Italy) enable an estimated prevalence of aHUS of 7 per million children in the whole of the European Community.

#### 4. Investigations in patients presenting with aHUS

##### 4.1 Measurement and interpretation of serum complement components

Blood should be taken for the measurement of C3, C4, factor H and factor I concentration, and for antibodies against complement components (see 4.4) before either plasma exchange or plasma infusions are commenced. C4 levels are, in most cases, normal. C3 levels can be either normal or low in patients with a mutation in *CFH*, *CFI* and *CD46*. The percentage of patients who have a normal C3 level in the presence of such a change ranges from 50% for *CFH* to 30% for *CFI* and 70% for *CD46* (Caprioli *et al*, 2006; Kavanagh *et al*, 2006). Approximately 10% of patients who do not have an identified mutation or autoantibodies to factor H will have a low C3 level, suggesting that other, as of yet unidentified, complement components are involved.

Factor H and factor I concentrations will be normal in those patients with a mutation that does not affect secretion of the mutant protein. This includes both missense mutations and deletions (Saunders *et al*, 2006, 2007). Factor H and factor I concentrations are lower in neonates (de Paula *et al*, 2003) and this has to be taken into account when interpreting results in this age group. There is also a large variation in factor H concentrations in normal adults and use of 'normal ranges' to interpret individual results must be cautious. Most *CFH* mutations that result in impaired secretion are heterozygous and one would therefore expect that in such individuals the factor H concentration would be *c.* 50% of normal. However, because the population normal range is so large a 50% concentration for an individual may still lie within the population normal range. Of the *CFH* mutations listed on the FH-HUS mutation database (<http://www.fh-hus.org>) 37% are associated with a normal factor H level and 22% a low level. For the *CFI* mutations listed on the same database 42% are associated with a low factor I level and 25% a normal level. As for *CFH*, most of the mutations in *CFI* that result in impaired secretion are heterozygous.

##### Recommendation

**In all patients presenting with clinical features compatible with a diagnosis of aHUS serum levels of C3, C4, factor H and factor I should be measured as the results guide prognosis and transplantation options. (strong, moderate).**

##### 4.2 Fluorescent-activated cell sorting (FACS) analysis

Because CD46 is a transmembrane protein, FACS analysis provides a means to quickly screen for mutations that result in decreased expression. A wide range of antibodies are commercially available. Expression of MCP on peripheral blood mononuclear cells (PBMCs) is stable at room temperature for up to 4 d and the time from venesection to analysis should be within this period. Approximately 70% of *CD46* mutations are associated with decreased expression by FACS analysis. Most of these are heterozygous but there are a few rare compound heterozygous and homozygous individuals.

##### Recommendation

**In all patients presenting with clinical features compatible with a diagnosis of aHUS expression of CD46 on PBMCs should be assessed using FACS analysis in an appropriately accredited laboratory as the results guide prognosis and late transplantation options. (strong, moderate).**

##### 4.3 Genetic analysis

As mentioned previously normal levels or expression of factor H, factor I and MCP do not rule out the possibility of a normally expressed but functionally impaired mutant. Full genetic analysis is therefore recommended for all patients as the results guide prognosis and transplantation options.

In patients with low serum levels or expression of factor H, factor I and CD46 in association with nonsense mutations, frameshift mutations, splice site mutations or missense mutations affecting critical residues (such as the cysteine residues in factor H responsible for forming disulphide bonds) it is probable that the abnormalities predispose to the disease. The interpretation is more difficult when the change is novel, has not been examined functionally and occurs in a region where there is no known function.

Mutations have now been described in five complement genes and it is likely that further genes will be identified. At present the turn around time for screening *CFH*, *CFI* and *CD46* alone is several months. For clinicians to be able to make timely decisions, particularly about treatment, including transplantation, this needs to be improved. New sequencing platforms are being introduced which will substantially reduce this turnaround time. The Northern Molecular Genetics Service in Newcastle upon Tyne is the approved UK Genetics Testing Network laboratory for HUS (<http://www.ukgtn.org>). This laboratory has recently installed a Roche FLX System Genome Sequencer which allows ultra-rapid high-throughput sequencing. With a single read accuracy greater than 99.5% 100 million base pairs can be generated in an 8-h run. Platforms such as this will undoubtedly be the method of choice in the future for mutation screening in HUS patients.

### Recommendations

**Mutation screening of *CFH*, *CD46*, *CFI*, *CFB* and *C3* should be undertaken in all patients with aHUS. This includes all historical cases that are being considered for transplantation. This should be undertaken in appropriately accredited molecular diagnostic laboratories and include appropriate techniques to detect copy number variation and hybrid genes. If mutations are found in other genes in the research setting then these should be incorporated into the molecular diagnostic portfolio. (strong, moderate).**

#### 4.4 Autoantibody screening

Between 6% and 10% of aHUS patients have antibodies which bind to the C terminal region of factor H (Dragon-Durey *et al*, 2005; Jozsi *et al*, 2007). These can be detected with an enzyme-linked immunosorbent assay using human factor H-coated plates to capture anti-CFH antibodies.

### Recommendations

**Autoantibodies against factor H should be sought in all patients with aHUS. This should be undertaken in an appropriately accredited laboratory. (strong, moderate).**

#### 4.5 Rare causes of aHUS

As mentioned previously a thrombotic microangiopathy can also be seen in association with malignancy, drugs, HIV infection, pregnancy, SLE, antiphospholipid antibody syndrome and cblC disease.

### Recommendations

**The possibility of these rarer forms of aHUS should be considered at presentation and appropriate investigations undertaken. (strong, moderate).**

#### 4.6 Measurement and interpretation of ADAMTS13 activity

A clinical diagnosis of aHUS is made when the predominant presenting feature is acute renal failure, whereas a diagnosis of TTP is made when the predominant presenting feature is neurological. However, renal involvement is common in TTP, with proteinuria and microscopic haematuria being the most constant findings (Kennedy *et al*, 1980; Ruggenti & Remuzzi, 1990). Renal function is depressed in 40–80% of patients, although severe acute renal failure (which is the hallmark of aHUS) is rare (Ruggenti & Remuzzi, 1990). For this reason TTP has been closely intertwined with HUS, as shown by frequent use of the hybrid term HUS/TTP in the literature (Galbusera *et al*, 1999; Remuzzi *et al*, 2002a). Measurement of ADAMTS 13 activity can help to distinguish between these two

clinical diagnoses. In TTP two primary mechanisms for deficiency of the ADAMTS13 activity have been identified, namely a constitutive deficiency and the presence of a circulating acquired inhibitory antibody (Furlan *et al*, 1998; Tsai & Lian, 1998). In aHUS a reduction in the levels of protease activity can be seen, but rarely <5%, in the absence of either an inherited deficiency or anti-ADAMTS13 antibodies but both these two abnormalities can also present with a clinical phenotype of aHUS (Veyradier *et al*, 2001; Remuzzi *et al*, 2002a). It is therefore important that specific assays are undertaken to detect these two abnormalities in patients with a clinical diagnosis of aHUS in whom the presence of low levels of ADAMTS13 protease activity is detected.

### Recommendations

**Measurement of ADAMTS13 activity should be undertaken in all patients with a clinical diagnosis of aHUS. If ADAMTS13 activity is found to be lower than 10% specific assays to detect inherited deficiency or anti-ADAMTS13 antibodies should be undertaken. (weak, low).**

## 5. Management of aHUS

### 5.1 Plasma exchange/plasma infusion

Plasma exchange and/or plasma infusions have become empirical, first line management of a patient presenting with the features of aHUS (reviewed in (Barz *et al*, 2002; Loirat *et al*, 2008; von Baeyer, 2002)). However, the information for which therapeutic strategy is most appropriate is limited. Plasma exchange is commonly undertaken daily using 1–2 plasma volumes per session in adults and 50–100 ml/kg in children. Typically, plasma exchange is undertaken daily initially, the duration and frequency of treatment is then determined by the clinical response. The theoretic advantage of plasma exchange over infusion has been emphasised in childhood cases in whom the risk of rapid progression to end-stage renal failure is high (Ariceta *et al*, 2009). Plasma infusion is undertaken daily initially, but may be dose limited because of impaired renal function and hypertension. If it appears successful with reversal of the microangiopathic anaemia the dose and frequency may be reduced later to weekly or biweekly intervals. However, individual patients respond differently and some require daily plasma infusions for long periods. Apart from this and general supportive measures there is currently no specific therapy for aHUS. Genotype-phenotype correlations have helped to explain some of the variation seen in patient response. For instance patients with abnormalities in soluble regulators such as factor H respond better to plasma exchange than patients with abnormalities in the transmembrane regulator CD46 (Caprioli *et al*, 2006).

The clinical outcome for patients who present with aHUS is poor. There is an initial 25% mortality and 50% of survivors do not recover renal function (Caprioli *et al*, 2006).

### Recommendations

All patients presenting with aHUS should be offered a trial of plasma exchange and/or plasma infusions. (weak, low).

### 5.2 Kidney transplantation

Renal transplantation is associated with an overall *c.* 50% rate of HUS recurrence in the allograft. The outcome varies according to the underlying genetic abnormality. Renal transplantation in patients known to have either a *CFH* or *CFI* mutation has a very poor outcome with 80% of patients losing their graft to recurrent disease within 2 years (Bresin *et al.*, 2006). In contrast, transplantation in patients with defects in only the transmembrane regulator CD46 usually have a good outcome. In this case the defective protein is replaced with the transplant and the normal situation is restored provided that endothelial microchimerism does not subsequently occur (Fremeaux-Bacchi *et al.*, 2007). Information about the outcome of transplantation in patients with *C3* and *CFB* mutations is becoming available and suggests that the outcome of renal transplantation is also compromised by a significant risk of recurrence. There is, however, insufficient data at present to make specific recommendations for this group of patients. Likewise information about the outcome of renal transplantation alone in those patients known to have anti factor H autoantibodies is limited but does suggest again that there is a significant risk of recurrence. However, there is evidence that use of plasma exchange in combination with Rituximab can enable a satisfactory outcome (Kwon *et al.*, 2008). Thus, there is a need to define the primary genetic defect in patients with aHUS, as this information is informative for therapy and disease progression. Live related renal transplantation alone is associated with the same risk of recurrence as in the aforementioned groups. There is also a risk of the donor developing the disease if he/she should carry the same mutation as the recipient or other risk factors (Donne *et al.*, 2002).

### Recommendations

Renal transplantation alone is not recommended in patients known to have a *CFH* or *CFI* mutation. Patients carrying an *CD46* mutation, but no additional mutation in factor *CFH*, *CFI*, *CFB* and *C3* or an anti-factor H autoantibody can be informed that the risk of recurrence post-transplantation is low. Patients known to have a *C3* or *CFB* mutation should be informed that current evidence suggests that there is a significant risk of disease recurrence post-transplantation. Patients known to have an anti-factor H autoantibody should be treated to minimise the antibody titre before proceeding to renal transplantation. Living related renal transplantation alone should be avoided in aHUS. (strong, moderate).

### 5.3 Live and combined liver/kidney transplantation

Factor H is primarily produced by the liver, and liver or combined liver/kidney transplantation is therefore a logical treatment for patients known to have a *CFH* mutation. Combined liver/kidney transplantation has been undertaken for those patients with a *CFH* mutation, and one child with moderate renal damage has been treated with an isolated liver graft. This appears logical given that both factor H and factor I are produced predominantly by the liver. Whilst the initial experience with this was not favourable (Remuzzi *et al.*, 2002b, 2005; Cheong *et al.*, 2004) more recent studies have documented a good outcome (Saland *et al.*, 2006; Jalanko *et al.*, 2008). This may be influenced by the use of prophylactic plasma exchange immediately prior to surgery and plasma infusion intraoperatively. In patients who have stable renal function but disease relapses despite plasma therapy an isolated orthotopic liver transplant may be considered.

### Recommendations

In aHUS patients with a known mutation in either *CFH* or *CFI* consideration should be given either an isolated liver or a combined liver/kidney transplant as part of an internationally coordinated clinical trial. Within the UK an advisory panel should be established to consider all patients prior to listing. Within the UK, liver transplantation alone or in combination with a kidney transplant should only be undertaken in a limited number of centres with appropriate expertise. (weak, low).

## 6. Arranging investigations for aHUS patients in the UK

Details of European laboratories that are able to undertake the aforementioned investigations are provided by Ariceta *et al.* (2009). Within the UK the UK Genetics Testing Network (<http://www.ukgtn.nhs.uk/gtn/Home>) approved laboratory is the Northern Molecular Genetics Service in Newcastle upon

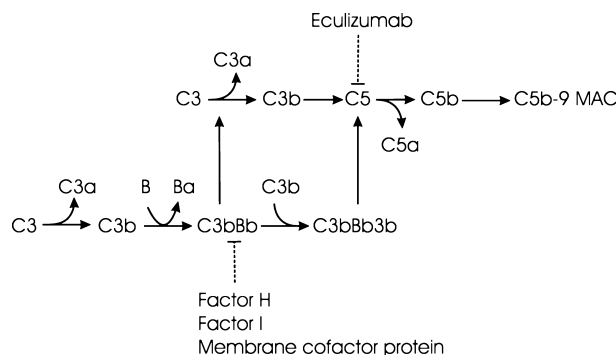


Fig 1. The alternative complement pathway – the site of action of factor H, factor I, CD46 (membrane cofactor protein) and eculizumab are shown.

Tyne. The request form for this service is given in Appendix I. This gives details of sample requirements and costs. The von Willebrand Factor-cleaving protease (ADAMTS13) assay is available from the haematology department at University College, London. A copy of the request form is given in Appendix II, and again gives details of sample handling.

## 7. Clinical trials in aHUS in the UK

Clinical trials of complement inhibitors in patients with aHUS have recently commenced in the UK. The first agent to be assessed is eculizumab. Anecdotal case reports have suggested a potential role for this agent in the management of aHUS (Gruppo & Rother, 2009; Nurnberger *et al*, 2009). Eculizumab is a high affinity humanised monoclonal antibody that binds to and blocks the cleavage of C5. It has been shown to be a safe and effective treatment to reduce haemolysis in paroxysmal nocturnal haemoglobinuria (Parker, 2009). The site of action of eculizumab in relation to the alternative complement pathway is shown in Fig 1. Activation of the alternative pathway is initiated by a variety of cellular surfaces including both host cells and pathogens. Host cells are protected by regulators including factor H, CD46 and factor I. Factor H and CD46 act as cofactors for the serine protease factor I that cleaves C3b; factor H also increases the rate of decay of the C3 convertase. As mentioned previously, aHUS is associated with inactivating mutations in *CFH*, *CFI* and *CD46*, and activating mutations in *C3* and *CFB*. As can be seen in Fig 1 the action of eculizumab is distal to all five of these. This would suggest that the pathogenesis of aHUS is dependent on generation of the powerful anaphylatoxin C5a and/or the membrane attack complex. The outcome of trials with agents such as eculizumab is eagerly awaited and will undoubtedly inform future versions of these guidelines.

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Appendix I



CPA Accredited Laboratory

**Complement genotyping request form**

**Patient information:**

Surname.....  
 Forename.....  
 Sex..... Date of Birth (dd/mm/yy).....  
 Address.....  
 .....ZIP or Postcode.....  
 Hospital.....  
 Hospital number.....  
 Other identifier (e.g. NHS number, social security number)  
 .....

**Referring Clinician (person to whom result will be sent):**

Name.....  
 Address.....  
 .....ZIP or Postcode.....  
 e-mail address.....

**To whom should the invoice be addressed (e.g. referring clinician, healthcare provider)?**

**Clinical Information:**

(where available please include the levels of any complement components measured such as C3, C4, Factor I, Factor B, Factor H or if any genetic testing for aHUS has been carried out elsewhere. Please also include a pedigree if appropriate)

**Disease:**

- Atypical haemolytic uraemic syndrome
- MPGN (membranoproliferative glomerulonephritis)   
*please state which type (I, II or III)*
- D+HUS (diarrhoeal associated haemolytic uraemic syndrome)
- TMApt (post transplant thrombotic microangiopathy)
- HELLP (haemolysis, elevated liver enzymes, low platelets)
- Macular degeneration

**Tests required:**

**Please note that payment must be received prior to testing (except UK and Ireland referrals). Please contact [Hazel.Forrest@nuth.nhs.uk](mailto:Hazel.Forrest@nuth.nhs.uk) for more information on payment.**

**Cost Tests required**

(£) (please tick)

**Molecular Genetic analysis**

- All exons of *CFH*, *CFI*, *CD46* 1200
- Test for known mutation 150

**Immunology analysis**

- Serum complement screen 90   
(C3, C4, factor H, factor I)\*
- CD46 expression by FACS\* 35
- Factor H auto-antibodies   
(on a research basis only at present)\*\*

*\*Serum screen and FACS analysis carried out at Immunology, RVI, Newcastle upon Tyne.*

*\*\*factor H auto-antibody analysis carried out at the Institute of Cellular Medicine, Newcastle University.*

**Samples required:**

One 10 ml EDTA and one 5 ml clotted sample should be taken and **each labelled with surname, forename and date of birth**. The samples should be sent by courier (outside UK) or first class post (within UK) in a secure container (at ambient temperature) to:

Dr Lisa Strain  
Northern Molecular Genetics Service  
Institute of Human Genetics  
The International Centre for Life  
Central Parkway  
Newcastle upon Tyne  
NE1 3BZ  
United Kingdom

**Date of sample (dd/mm/yyyy):**.....

**Related links:**

aHUS mutation database: <http://www.fh-hus.org/>  
Genetests: <http://www.genetests.org/> Lab ID 777  
UK Genetic Testing Network: <http://www.ukgtn.org/gtn/>

Appendix II



**VWF Cleaving Protease (ADAMTS13) Assay request form**

Patient Name .....

Hospital Number..... Date of Sample Collection .....

Date of Birth..... Consultant ordering the assay.....

Date of last plasma infusion or exchange .....

Assay required for Diagnosis/Clinical Management **YES / NO**

NHS Patient **OR** PRIVATE Patient **(please circle)**

This patient is registered in the Rituximab Study **YES / NO**

The patient is part of the Pneumococcal HUS Study **YES / NO**

The patient is part of the Kings Sickle Study **YES / NO**

**Full address for Report:**  
.....  
.....  
.....

**Institution's Order No.:**  
**Name & Address for Billing** (if different to above):

We will assay ADAMTS13 by the collagen binding method or a peptide substrate assay, as available (and an inhibitor test if the activity is low).  
If additional types of ADAMTS13 assay are required (for which additional charges will be made), please telephone and discuss.

**SAMPLE REQUIREMENTS:**

- **Collect 5 ml citrated blood and ship immediately, to arrive within 4hr, OR:**
- Centrifuge at 2000 g for 15 min and (avoiding the buffy coat) pipette into 2–3x 0.5 ml aliquots, freeze at –70°C and ship **on dry ice (in a suitably vented container)**.

Contact the Haemostasis Research Unit to arrange delivery. The sample must arrive between 09.00–16.00 Monday–Friday (avoid public holidays!) with a copy of this form. The Department is closed outside these times.

**Delivery Address:** Haemostasis Research Unit, Haematology Dept, University College London, 1st floor, 51 Chenies Mews, London WC1E 6HX  
**Please telephone Dr Ian Mackie or other HRU staff (020 7679 6416/6421/6423) before sending sample to ensure that it is processed. (E-mail: i.Mackie@ucl.ac.uk)**