Journal of Scientific and Innovative Research 2015; 4(6): 221-223
Available online at: www.jsirjournal.com

Letter to Editor

ISSN 2320-4818
JSIR 2015; 4(6): 221-223

Received: 24-11-2015
Accepted: 23-12-2015

Kheir Eddine Kerboua
Unit of Immunology, Military University Hospital of Oran, Oran, Algeria

Correspondence:
Dr. Kheir Eddine Kerboua, Pharm D
Unit of Immunology, Military University Hospital of Oran, Oran, Algeria
Email: k.k.eddine[at]gmail.com

fH-aHUS hemolytic agarose plate: A descendents of Sanchez-corral assay for a rapid diagnosis of hereditary and acquired factor H-related Atypical Hemolytic Uremic Syndrome

Kheir Eddine Kerboua

INTRODUCTION

Hemolytic uremic syndrome (HUS) is not only known by its approved drug Eculizumab, the most expensive medicine up to now (An annual drug cost US $573,720 per patient), but also by its catastrophic outcomes (70% of patients progress to death or renal failure in less than 1 year after the onset). In the absence of repetitive infections, this ultra-rare form of thrombotic microangiopathy (TMA) is qualified by the "atypical" form of HUS (aHUS). The factor H related- aHUS (fH-aHUS) belongs of this latter and represents more than a half of the aHUS. It is caused by genetic or acquired autoimmune defects of the alternative complement pathway regulation by the factor H.

Stat of the art:

Making the correct diagnosis for fH-aHUS is critical because these disorders require different treatment. At least for now, complement consumption and low serum C3levels are not specific for aHUS. Moreover, relatively few patients have low serum fH levels and genetic testing is slow and uninformative in up to 50% of cases. Unfortunately, only one method has been described to detect the functional defect of fH in aHUS and it lacks in reliability because it is based on C3b binding capacity of fH while almost of fH-aHUS are a consequence of cell surface binding defect of fH (C-terminal portion defect) [1].

To overwhelm these obstacles, in 2004, Sanchez-corral proposed an elegant miraculous quantitative hemolytic assay that detects, within a few hours of presentation, all the fH abnormalities identified up to now in aHUS pathogenesis [2]. However, this assay cannot distinguish between genetic abnormalities and acquired autoantibodies, and needs a specialized laboratory and an extemporaneous preparation of buffers and reagents. Curiously, Yoko Yoshida *et al* reported that this assay may show no apparent hemolysis (<25%) for patients having anti-fH autoantibody without deletion in CFHR1 and CFHR3 proteins, which may be its talent d'Achile [3]. Although the usefulness of a qualitative hemolytic assay using sheep RBCs has been postulated by Yoko Yoshida *et al*, Sanchez-corral assay drew attention of several researchers over the world and the last months of the current year has known a widespread of use and an intensive effort for its optimization (Fig. 1 A). Despite of Lubka Rumenina described a hemolytic assay for diagnosis of aHUS, no discrepancies were noted with the original one, except the blood specimens that were EDTA plasmas [4]. Moreover, in Japan, sheep RBCs at a final concentration of 2.5×10^6 cells/µl and citrated plasma were chosen for the assay, and 100% hemolysis was defined as the absorbance at optimal density (OD) 414 nm obtained with 20 µl of normal citrated plasma spiked with a novel monoclonal antibody (mAb O72) at 200 µg/ml [3]. Recently, Gavriilaki *et al* published in *Blood* a Flow cytometry-based assay using human cells like endothelial cell line 'Ea.hy926 cells' and human myeloid TF-1 cells that reduce the production of formazan following exposure to aHUS plasma [5].

The challenge:

Sanchez-corral assay is highly effective and less subjective, but it was challenging for simple laboratories like ours in a low incomes country that cannot equip al their hospital laboratories to perform this assay.

The patent:

To overcome this roadblock, we have proceeded to render this method more practical to each laboratory with a purpose to be stored and transported for several weeks. This includes a hemolytic agarose plates for screening for fH-aHUS; a special adaptation of the hemolytic plates to prevent complement activation via the classical pathway (CP) and facilitate complement activation via the alternative pathway (AP) in agarose gels. This version of the hemolytic assay presents valuable advantages over the Sanchez-corral one as resumed in Table 1.

The procedure is essentially the same as for the AP gels except the concentration of AP buffer and the sheep erythrocyte density used to make the gels. A value of 100% of plasma CFH function was defined by the pooled normal plasmas, prepared from a total of 100 healthy individuals (50 males and 50 females) (Fig 1. C). However, although it can be used to estimate levels of fH function they will not give accurate quantitative results. Interpretation of fH-aHUS plates is difficult. The range of size of fH-HUS hemolytic rings is wide and small rings do not necessarily indicate fH deficiency. One of the main uses of the fH-aHUS test in screening is that it allows the differentiation of terminal component deficiency from classical pathway deficiency, and small rings suggest low C3 levels. Otherwise, to address this problem we incubated 20 µl of normal plasma mixed with patient plasma at different final volumes at room temperature for 30 min. Subsequently, these mixtures were diluted to 100 µl with fH-CFTD buffer. The diluted samples were incubated in fH-aHUS plates and assayed as described above (Fig. 1 B & C).

Table 1: fH-aHUS hemolytic agarose plate features in comparison with the referential hemolytic assay

	The referential assay	**fH-aHUS hemolytic agarose plate**
Number of samples	<5 per day	>10 per day
Activators	Sheep red cells	Sheep red cells + Agarose
Assessment	Obtical density by Spectroscopy	Direct diameter measurement
Laboratory Steps	+++	less
Time consuming	+++	less
Storage	No	15 days
Transport	No	Yes (at 4C)
Cost	+++	Less
Technical linked error	+++	Less
Human linked error		Less
Other		➢ Much adapted as screening tool ➢ Don't need a deep immunotechnology handling ➢ Storage of utilized plates for *intra* and *inter*-laboratory comparisons

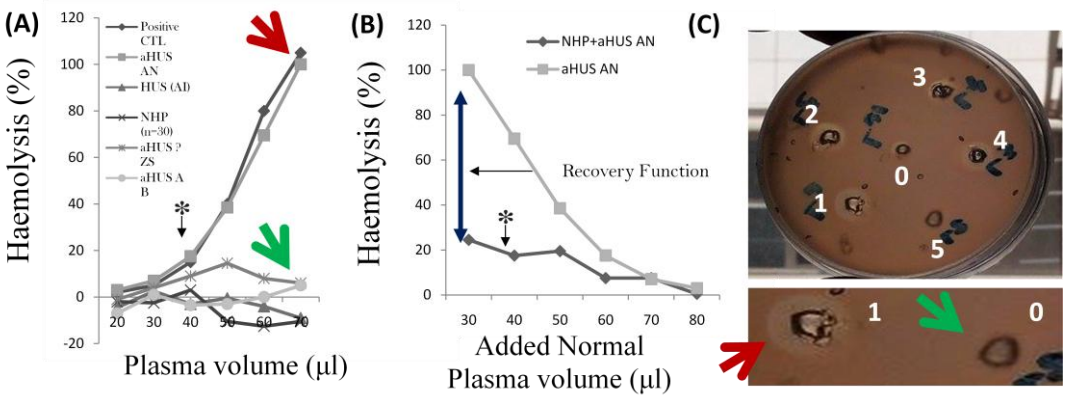
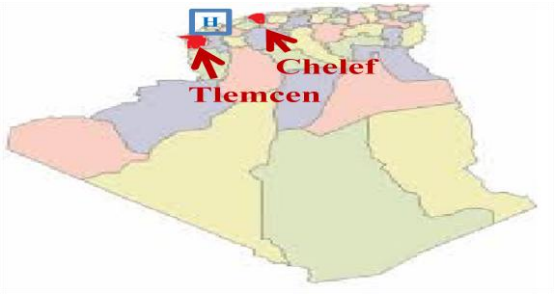


Figure 1: Lysis of sheep erythrocytes by plasma from aHUS AN patient and pooled normal plasmas. (A) Lysis as a function of plasma added. Plasma samples from 20 to 70% were used. Lysis is represented as percentage of the control of total lysis. (B) Lysis of sheep RBCs that was corrected after by the addition of normal plasma in a dose-dependent manner. Different dilutions of aHUS NA plasma with normal human plasma show that the normal function of the plasma can be restored immediately by adding only 30% of NHP. The lysis observed is represented as percentage of the lysis in the absence of added factor H.(C) fH-aHUS screening by the hemolytic agarose plates : well 0: the pooled normal plasmas, well 1:plasma of aHUS NA, wells from 2 to : haemolysis corrections of aHUS NA by different volumes of the pooled normal plasmas. *Red arrow shows patient plasma with fH functional deficiency, Green arrow show the pooled normal plasmas.* ✻*: The threshold fixed by Lubka Roumenina (20% of NHP lysis)[4]*

Supporting Information:



S. Figure: Geographical distribution of patients with atypical hemolytic uremic syndrome (aHUS) in Algeria shown in red. : The Military University Hospital of Oran.

CONCLUSION & PERSPECTIVES

The aHUS is an extremely rare disease, and so far only ten patients have been diagnosed across Algeria, a country of 50 million inhabitants. Three patients with MCP mutation were found only in the Tlemcen prefectures. The reminding ones are fH-aHUS and are essentially localized in the Chelef prefectures. Interestingly, patients carrying these mutations were not found in other areas of West Algeria, which may indicate a reflection of 'founder effect'. (S. Fig). Once its optimization for commercial scale was performed, thanks to fH-aHUS plates, we are expecting to find merely 10–15 new patients every year, and therefore this promising approach may facilitate a prospective analysis.

REFERENCES

1. Volokhina EB, Westra D, van der Velden TJ, van de Kar NC, Mollnes TE, van den Heuvel LP. Complement activation patterns in atypical hemolytic uremic syndrome during acute phase and in remission. Clin Exp Immunol. 2014. doi: 10.1111/cei.12426.
2. Sanchez-Corral P, Gonzalez-Rubio C, Rodriguez de Cordoba S, Lopez-Trascasa M. Functional analysis in serum from atypical Hemolytic Uremic Syndrome patients reveals impaired protection of host cells associated with mutations in factor H. Mol Immunol. 2004; 41: 81–84.
3. Yoko Yoshida, Toshiyuki Miyata, Masanori Matsumoto, Hiroko Shirotani-Ikejima,Yumiko Uchida, Yoshifumi Ohyama, Tetsuro Kokubo, Yoshihiro Fujimura. A Novel Quantitative Hemolytic Assay Coupled with Restriction Fragment Length Polymorphisms Analysis Enabled Early Diagnosis of Atypical Hemolytic Uremic Syndrome and Identified Unique Predisposing Mutations in Japan. PLoS ONE. 2015. 10(5): e0124655.
4. Roumenina LT, Roquigny R, Blanc C, Poulain N, Ngo S, Dragon-Durey MA. Functional evaluation of factor H genetic and acquired abnormalities: application for atypical hemolytic uremic syndrome (aHUS). Methods Mol Biol. 2014; 1100: 237–247.
5. Gavriilaki E, Yuan X, Ye Z. Modified Ham test for atypical hemolytic uremic syndrome. Blood. 2015; 125(23):3637-3646.