SUPPLEMENTARY MATERIAL

In-house ELISAs for the detection of free antiC1INHAbs and C1INH–antiC1INHAb complexes (H.U. Virgen del Rocío, Sevilla, Spain).

Ninety-six-well plates (Costar 3590; Life Technologies SA, Madrid, Spain) were coated with either 1 µg of C1INH (for free antiC1INHAbs detection) (Berinert®, CSL Behring, Marburg, Germany) or 1 µg of polyclonal anti-C1INH IgG (for C1INH–antiC1INHAb complexes detection) (CompTech, Tyler, TX, USA) in carbonate/bicarbonate buffer pH = 9·2 at 4ºC overnight and blocked with 3% gelatin

Tween-phosphate-buffered saline (PBS) for 1 h at 37ºC; 100 µl of 0·1% bovine serum albumin (BSA)–PBS-diluted duplicated serum samples from patients, healthy donors and a positive control were then seeded at convenient dilution factors (50, 200, 800, 1200) for 1 h at 37ºC.

Detection was performed with PBS-Tween-diluted horseradish peroxidase (HRP)-labeled antibodies to human IgG (VITRO; Jackson Immuno Research, Madrid, Spain) diluted 1/50 000, human IgM (Nordic MUBio, Cultek, Madrid, Spain) diluted 1/1000 or human IgA (Nordic MUBio) diluted 1/1000. Reactions were developed for 15 min with 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS), stopped with 0·1% azide and read at 405 nm.

For the detection of free anti-C1INH antibodies, positivity threshold was defined as threefold of the values obtained with two healthy donor sera at 1/50 dilutions.

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