culture supernatants (page 3717, Antibodies and reagents). Likewise, US 2008/008700 discloses the use of HAGG (heat-aggregated IgG) on page 8, [0093].

[0011] The present invention consequently relates to an in vitro method for determining the stability (such as the shelf stability or storage stability) of a composition, preferably of a pharmaceutical composition (equating with a medicament), which composition comprises or essentially consists of soluble human Fc gamma receptor IIA, IIB, IIIA and/or IIIB, said method comprising the steps of:

(a) contacting a surface comprising human Fc gamma receptor IIA, IIB, IIIA and/or IIIB with a set amount of aggregated human IgG;

(b) contacting said surface comprising human Fc gamma receptor IIA, IIB, IIIA and/or IIIB with a set amount of said composition of soluble human Fc gamma receptor IIA, IIB, IIIA and/or IIIB; and

(c) determining the amount of aggregated human IgG which is bound to said surface comprising said human Fc gamma receptor IIA, IIB, IIIA and/or IIIB,

(d) and comparing the amount of aggregated human IgG which is bound to said surface as determined in step (c) with a reference value and (thereby) determining the stability such as the shelf stability; stability over time; shelf life of said composition which comprises or essentially consists of soluble human Fc gamma receptor IIA, IIB, IIIA and/or IIIB.

[0012] Steps (a) and (b) of the methods of the invention can be carried out concomitantly or consecutively. It is also envisaged that the order of steps (b) and (a) is reversed.

[0013] It is envisaged that the methods of the invention comprise a washing step which is to be conducted prior to step (c) and preferably after step (a) and (b).

[0014] It is preferred that said soluble human Fc gamma receptor which is employed in the context of the present invention is a soluble Fc gamma IIB receptor. It is particularly preferred that said soluble human Fc gamma IIB receptor is SM101 (SEQ ID No. 1), including preparations of these receptors that can be obtained via expression of these receptors in host cells, such as for example mammalian cells or bacterial cells like E. coli.

[0015] Also disclosed is the use of the aggregated human IgG that is defined herein, in the methods and embodiments also disclosed.

“standard”) donors is preferred. The term "healthy" means an individual who meets the current (at the time of donation) standard eligibility criteria for donating blood, bearing in mind that such eligibility criteria are subject to continuous improvement and change.

[0024] In another embodiment, it is envisaged that said aggregated human IgG is an aggregated human IgG that is obtainable from said human protein by an isolation method comprising size exclusion chromatography. In a preferred embodiment, said size exclusion chromatography separates monomeric and/or dimeric IgG from said aggregated human IgG. Means and methods to isolate the aggregated human IgG that can be used in the context of the present invention are disclosed herein elsewhere (e.g. in the Examples). It is also envisaged that the aggregated human IgG of the present invention may not be heat aggregated human IgG like for example the one described in Engelhard, et al., (1990), Eur. J. Immunol. 20, 1367-1377, Bruhns et al. (2009), Blood J. 113(16), 3716-3725 or US 2008/008700. Indeed, heat aggregated IgG will differ from aggregated IgG as is obtained/is obtainable as described herein. Namely, heat aggregation is a result of the partial denaturation of proteins when temperatures above the melting temperature (Tm) are applied to a given protein. The melting temperature is a function of the protein and in case of a mixture of human IgGs a broad range of Tm will necessarily be observed. Thus the degree of aggregation will vary from one batch to the other depending on the mixture of IgG used. Moreover, a number of monomeric IgG will inevitably be part of the preparation of aggregated IgG by heat and and thus would not allow a preparation of aggregated IgG without varying amounts of monomeric IgG considered to be impurities. However, the methods for the preparation of aggregated IgG as described herein separate monomeric and/or dimeric IgG from aggregated IgG and, thus, the resulting aggregated IgG as is obtained/is obtainable by the methods described herein is different from heat-aggregated IgG from the prior art.

It is thus apparent that heat aggregated IgG is not comparable to the aggregated IgG preparations as disclosed by the present invention, which offer long-term stability and are perfectly suited for batch release testing or other routine testing.

[0025] It is envisaged that pooled human IgG preparations such as Intravenous immunoglobulin (IVIG) preparations, sometimes also called plasma immunoglobulin for intravenous administration, are derived from pooled plasma of hundreds of healthy donors and contain both immune and natural antibodies (NAbs) reflecting the cumulative antigen experience of the donor population. A pooled human IgG

set to be, for example, 100, with 100 being a relative value, since it merely reflects a certain amount of aggregated human IgG bound to a surface.

[0038] A reference value is regarded as acceptable if at least 50%, preferably 60% or 70%, more preferably 80% or 90% of the aggregated human IgG is not bound to a surface, but is bound by one or more soluble human Fc gamma receptors comprised by the composition whose stability is determined. Specifically, as explained above, the less aggregated human IgG is bound to a surface, the more is bound by one or more soluble Fc gamma receptors and vice versa. Accordingly, the higher the stability of a composition, the less aggregated human IgG will be bound by a surface, but by one or more soluble human Fc gamma receptors. Conversely, the lesser the stability of a composition, the more aggregated human IgG will be bound by a surface, and the less will be bound by one or more soluble human Fc gamma receptors

[0039] The present invention may also encompass a method for isolating aggregated human IgG from the mixed plasma of at least 500 (healthy or “standard”) donors by size exclusion chromatography.

[0040] It is also envisaged that the aggregated human IgG of the invention may be supplemented or replaced by the corresponding IgG preparations of non-human primates, such as cynomolgous, callithrix, macaqua, sanguis or the like.

[0041] The “soluble human FcγRIIA, FcγRIIB, FcγRIIIA or FcγRIIIB” of the invention are for example those described in EP 1 135 486 and EP 1 642 974 and EP 1 446 139. The “soluble human FcγRIIA, FcγRIIB, FcγRIIIA or FcγRIIIB” of the invention are in a preferred embodiment characterized by the absence of transmembrane domains and signal peptides. The claimed soluble FcγRs of the invention comprise or consist in a preferred embodiment of an amino acid sequence corresponding to that of SEQ ID NO.: 1 (SM101, recombinant human FcγRIIB), SEQ ID NO.: 3 (FcγRIIB), SEQ ID NO.: 5 (FcγRIIA), SEQ ID NO.: 7 (FcγRIIIA) or SEQ ID NO.: 9 (FcγRIIIB) encoded by the respective nucleic acid sequences SEQ ID NO.: 2, 4, 6, 8, and 10. The invention may also encompass soluble FcγRs that have at least 90%, preferably 95% identity to the proteins of SEQ ID Nos.: 1, 3, 5, 7, or 9. For the determination of sequence identity a comparison is made by aligning the sequences in a manner to provide the maximum correspondence of amino acids. . In a preferred embodiment of the invention, the soluble human Fc gamma receptor is FcγRIIB (SEQ ID NO.: 3). In a more preferred embodiment, the soluble human receptor is SM101 (SEQ ID NO.:1), which is a soluble FcγRIIB receptor. In a further preferred embodiment of the

a mammalian cell line expressing one or more human Fc gamma receptor IIA, IIB, IIIA and/or IIIB. These cells express in some embodiments the human Fc gamma receptor IIA, IIB, IIIA or IIIB in the absence of any other human Fc gamma receptor, i.e. these cells/cell lines only express one FcγR. It is particularly preferred that said surface comprising human Fc gamma receptor IIA, IIB, IIIA and/or IIIB, comprises in essence or even exclusively human FcγRIIB. In a further preferred embodiment, the subtype of the human Fc gamma receptor comprised by said surface and the subtype of the human Fc gamma receptor comprised by said composition of soluble human Fc gamma receptor is identical. When FcγRIIB is being tested, the cells preferably express only FcγRIIB, such as the Raji lymphoblastoid cell line (CCL-86, ATCC). For FcγRIIA, the erythroleukemic cell line K562 (CCL-243, ATCC) can be used, and for FcγRIIIA the NKL cell line (Robertson et al., Exp. Haematol. 1996, 24(3):406-15) can be used. Alternately, CHO cells transfected with the FcR(s) of interest can be used. Methods for the transfection of CHO cells to express a protein of interest are well known in the art, this method is described for example in Bruhns et al., Blood 2009, 113:3716-3725

[0045] It is also envisaged that the "surface comprising human Fc gamma receptor IIA, IIB, IIIA and/or IIIB" is or comprises a solid surface that can be coated with human Fc gamma receptor IIA, IIB, IIIA and/or IIIB. A "solid surface" thereby comprises either a particulate material (beads) which e.g. may be magnetically attractable or a flat surface such as an ELISA plate or a Biacore detection unit. The material of said surface may be any suitable solid material such as plastic, polymers, metal, glas etc. that can be employed in the respective methods. The coating of proteins like human Fc gamma receptor IIA, IIB, IIIA and/or IIIB to a solid surface (e.g. a flat surface or a particulate surface) is conducted by standard methods which are well known to the skilled person. The coating of a solid surface may be conducted with "human FcγRIIA, FcγRIIB, FcγRIIIA or FcγRIIIB" and/or with "soluble human FcγRIIA, FcγRIIB, FcγRIIIA or FcγRIIIB" of the invention, as there is no need of biological expression on the cell surface (which would require a membrane anchor).

[0046] In a preferred embodiment, the invention relies on the use of aggregated human IgG in an in vitro method for determining the binding activity of soluble human Fc gamma receptors. The first step in the method comprises contacting a mammalian cell from a cell line expressing only one of the human Fc gamma receptors IIA, IIB, IIIA or IIIB (preferably in the absence of any other

Fcγreceptor) with a set amount of aggregated human IgG and a set amount of the corresponding soluble human Fc gamma receptor IIA, IIB IIIA or IIIB. It is preferred that the mammalian cells chosen for the first step of the method express only the Fc gamma receptor of interest and if possible no other Fc gamma receptors, as the presence of further receptors that bind to IgG could interfere with the activity of the soluble FcγR being tested.

[0047]. "Determining the amount of aggregated human IgG which is bound to said surface" refers to the measurement or quantification of the amount of aggregated human IgG bound to the surface, i.e. what is preferably measured in the context of the present invention is the amount of aggregated human IgG which is bound to the surface comprising human Fc gamma receptor IIA, IIB, IIIA and/or IIIB. Said bound aggregated human IgG is for example detected by means of an indirect label (e.g. an anti-human IgG binding domain) or by means of a direct label (i.e. the aggregated human IgG is labeled directly - explained herein elsewhere). For example, a low level of labeled secondary antibody bound to the aggregated human IgG indicates a high level of binding of the soluble FcγR to the aggregated human IgG and vice versa.

It is alternatively or additionally likewise possible to analyze, measure or quantify the amount of aggregated human IgG that was removed by washing (before step (c) of the methods of the invention) – said aggregated human IgG is bound to the soluble FcγR of interest (which was comprised in the composition) and is washed out together with it.

[0048] The "contacting step" of the methods of the invention may involve bringing the surface, the receptor and the aggregated human IgG into physical contact with each other, so that they can bind to each other. This step may involve an incubation step, so that the reagents have time to react.

[0049] The term "set amount" simply indicates that the amount of the respective reagents should be fixed, so that the results can be estimated or even quantified. For example, and as can be seen from the examples below, a standard amount of aggregated human IgG was employed with a serial dilution of the soluble receptor, so that the amount at which the soluble receptor inhibits aggregated human IgG binding to the surface can be determined.

[0050] The washing step, may involve the removal of unbound aggregated human IgG and unbound soluble human FcγR by "washing" prior to step (c). It should be noted that this step refers to the removal of reagents that arenot bound to the surface used in the method. The washing step can be

performed with any standard buffer, such as the FACS buffer used in the examples below.

[0051] When applied to a serial dilution of the soluble FcγR of the invention, the "detection" of the label allows the determination of the IC_{50} of the soluble FcγR. The "half maximal inhibitory concentration (IC_{50})" is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance is needed to inhibit a given biological process by half. In other words, it is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC_{50}). It is commonly used as a measure of antagonist drug potency in pharmacological research. According to the FDA, IC_{50} represents the concentration of a drug that is required for 50% inhibition *in vitro*. Thus in the present context, the amount of aggregated IgG bound to the mammalian cells compared to the amount of aggregated IgG used in the method allows the determination of the amount of the FcγR needed to inhibit 50% of the aggregated IgG binding to the cell. Likewise, the IC_{95} could be measured, which is the amount necessary to inhibit 95% of the IgG binding to the cells. Any other evaluation technique for the data can be used by the person skilled in the art, for example the four parameter logistic method (4PL) according to the European Pharmacopoeia 5.0; 2005; Chapter 5.3 ("Statistical analysis of results of biological assays and tests").

[0052] In a particularly preferred embodiment of the in vitro method of the invention, the soluble human receptor is FcγRIIB (SEQ ID NO.: 3), even more preferably SM101 (SEQ ID NO.: 1), the cells are Raji cells, the aggregated human IgG is isolated from a human IgG preparation e.g. Beriglobin® and the secondary antibody has a fluorescence label being excitable with a laser beam and emitting light at a set wave length being detectable by means of a FACS apparatus.

[0053] Also disclosed is a kit comprising a surface of the invention and/or the aggregated human IgG of the invention. Said kit may further comprise means which are necessary to conduct the methods of the invention (e.g. a label, buffers, reference values, controls, standards etc.).

[0054] The invention may also relate to aggregated human IgG as disclosed herein.

[0055] In the experiments described below, it was found using the in vitro method of the invention that SM101 (soluble human FcγRIIB, SEQ ID NO.: 1) competes with cell-bound FcγRIIB on Raji cells for the binding to aggregated IgG, and that increasing concentrations of SM101 added to constant amounts of cells and

6. The method of any one of the preceding claims, wherein said aggregated human IgG is an aggregated human IgG that is obtainable from human protein by an isolation method comprising size exclusion chromatography.

7. The method of claim 6, wherein said size exclusion chromatography separates monomeric and/or dimeric IgG from said aggregated IgG.

8. The method of any one of the preceding claims, wherein said aggregated human IgG is labeled.

9. The method of claim 8, wherein said label comprises a secondary labeled human anti-IgG antibody.

10. The method of any one of the preceding claims, wherein said soluble human Fc gamma receptors is a soluble Fc gamma IIB receptor.

11. The method of claim 10, wherein said soluble human Fc gamma IIB receptor is SM101 (SEQ ID No. 1).

12. The method of any one of claims 3 to 11, wherein said mammalian cell is a Raji cell as deposited under ATCC No. CCL-86.

13. The method of any one of the preceding claims, wherein the subtype of the human Fc gamma receptor comprised by said surface and the subtype of the human Fc gamma receptor comprised by said preparation of soluble human Fc gamma receptor, is identical.