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(54) **Title**: NOVEL VACCINE COMPOSITION

(57) **Abstract**: The present invention relates to novel vaccine compositions, vials comprising such compositions, as well as to uses of and to methods for producing said compositions. In particular, the invention relates to vaccine compositions comprising certain excipient components and their use for the vaccination against influenza virus disease.

NOVEL VACCINE COMPOSITION

Technical field

The present invention relates to novel vaccine compositions, as well as processes for preparing such compositions and to uses of the compositions. In particular, the invention relates to influenza vaccine compositions that comprise of a excipient for stabilising the vaccine compositions.

Background of the invention

Flu vaccines are typically designed to protect against three different influenza viruses (trivalent influenza vaccine or TIV). These vaccines include two influenza A viruses with a B virus chosen either from the Yamagata or Victoria lineage, despite circulation of both Yamagata and Victoria viruses during most influenza seasons. The more recent quadrivalent influenza vaccine (QIV) is designed to protect against four different influenza viruses; two influenza A viruses and two influenza B viruses, one from each lineage. The inclusion of a second B virus to the vaccine gives a broader protection against circulating influenza viruses.

Concerns over the safety of thimerosal in vaccines have led to its removal from most vaccine formulations in recent years. Three single-dose presentations of thimerosal-free formulations of quadrivalent seasonal split virion influenza vaccines (QIV) have become available in the US market (FluLaval®, Fluarix® and Fluzone®).

The stabiliser systems used in the GlaxoSmithKline (GSK) FluLaval® Quadrivalent and Fluarix® Quadrivalent formulations consist of a combination of Tween® 80 and α-tocopheryl succinate or vitamin E succinate (VES) with or without Triton X-100® in the presence of phosphate buffered saline solution. In these formulations VES together with non-ionic surfactants, Tween® 80 and Triton X-100® are useful to form stabilising micelles (WO02/097072). Fluzone® Quadrivalent is formulated in sodium phosphate saline with Triton X-100®, formaldehyde and gelatin. The stabiliser performs the critical role of preventing the bioactive haemagglutinnin antigens (HA) from undergoing aggregation resulting in a significant decline in their activities over the designated shelf-life of 12 months when stored under refrigerated conditions.

Previously, WO02/097072 established the usefulness of VES in the stabilisation of HA. However, VES is a poorly soluble waxy solid at room temperature. To deal with this, existing vaccines manufacturing processes have used a large amount of Tween® 80 in order to dissolve the VES in the final vaccine formulation.

Such high amounts of stabilisers are not always acceptable under local regulatory provisions. There is a need therefore for provision of alternative methods for stabilising influenza vaccines.

Summary of the invention

Studies on stabilisation of HA in split-virion seasonal influenza vaccines by the inventors have demonstrated the need to include a stabiliser system in order to assure adequate HA content over their designated shelf-life. Stabilisation of the haemagglutinin antigen (HA) is believed to be brought about with the formation of complex micelles made up of proteins, lipids, VES, Tween® 80 and Triton X-100®. Stabilisation of HA is achieved through physical separation of HA within the complex micelles. This helps to maintain the HA Content at a reasonably constant level over a period of 12 months or more when stored under refrigerated conditions of 5±3 ^{0}C.

Surprisingly, the present inventor has found that it is possible to produce a vaccine that significantly reduces the amount of Tween® 80 present. This was achieved by substituting Tween® 80 in the formulation with Triton X-100® in order to solubilise adequate amount of vitamin E succinate (VES) which led to comparable or better stability profiles for haemagglutinnin antigens over the designated 1 year product shelf-life at refrigerated conditions. Stability data under accelerated (30±2 ^{0}C, 4 weeks) and real time (5±3 ^{0}C, 12 months) storage conditions were generated for monovalent vaccine bulks and filled syringes and demonstrated improved product stability for reduced stabiliser formulae for monovalent bulk and final QIV filled syringes over formulas using higher amounts of Tween® 80. Maintaining a comparable or better product stability profiles ensures vaccine efficacy and minimises production costs. Furthermore, an overall reduction in the amount of additives present in the vaccines may address any regulatory requirements (e.g. country/region specific) for lower amounts of stabilisers present in influenza vaccines.

Accordingly, the invention provides:

- a vaccine composition comprising a split influenza virus preparation or subunit influenza virus preparation and pharmaceutically acceptable excipient, said excipient comprising polyoxyethylene sorbitan monooleate (Tween® 80 or Polysorbate 80), alpha-tocopherol or a derivative thereof and t-octylphenoxypolyethoxyethanol (Triton X-100®), wherein the amount of polyoxyethylene sorbitan monooleate per human dose is ≤200µg and the amount of t-octylphenoxypolyethoxyethanol per human dose is ≥100µg.

– a multi-dose or single dose vial comprising the vaccine composition described above.

- a process for producing the above vaccine composition comprising the steps of: (i) preparing a split influenza virus preparation or subunit influenza preparation, (ii) optionally inactivating the split influenza virus preparation before or after the splitting step, (iii) purifying and filtering said preparation and (iv) adding a pharmaceutically acceptable excipient to the preparation, said excipient comprising the following stabilisers: polyoxyethylene sorbitan monooleate, alpha-tocopherol and t-octylphenoxypolyethoxyethanol, wherein the concentration of polyoxyethylene sorbitan monooleate is ≤200 µg/ml and the concentration of t-octylphenoxypolyethoxyethanol is from 300 to 700 µg/ml.

- the vaccine composition described above for use in medicine

- the vaccine composition described above for use in the treatment and/or prevention of disease caused by influenza.

- a method of inducing an immune response in a human subject, said method comprising administering to the subject the vaccine composition described above.

Brief description of the figures

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Figure 54: VES Content (µg/ml) for QIV Filled Syringes at 30±2 ºC

Detailed description

Provided herein is a vaccine composition comprising or consisting of a split influenza virus preparation or subunit influenza virus preparation and a pharmaceutically acceptable excipient said excipient comprising polyoxyethylene sorbitan monooleate (Tween® 80 or Polysorbate 80), alpha-tocopherol or a derivative thereof and t-octylphenoxypolyethoxyethanol (Triton X-100®), wherein the amount of polyoxyethylene sorbitan monooleate per human dose is ≤200 µg and the amount of t-octylphenoxypolyethoxyethanol per human dose is ≥100 µg.

Excipients and stabilisers

Many vaccines that are currently available require a preservative or stabiliser to prevent deterioration during processing and/or in the final vaccine. Such preservatives or stabilisers may be added for example in the final vaccine as part of a

pharmaceutically acceptable excipient. As described in WO02/097072, alternatives to the commonly used mercury based stabilisers such as thiomersal have been developed due to public concerns about the effects of mercury containing compounds.

As well as the function of thiomersal as a preservative to prevent microorganism growth, it also had a stabilisation effect on hemagglutinin (HA), particularly for the HA of B strain influenza, but also certain A strain HA such as H3. Stabilisers such as alpha-tocopherol have been successfully used however instead of thiomersal in influenza vaccines (WO02/097072).

The stabiliser systems used in the GlaxoSmithKline (GSK) FluLaval® Quadrivalent and Fluarix® Quadrivalent formulations consist of a combination of Tween® 80 and α-tocopheryl succinate or vitamin E succinate (VES) with or without Triton X-100® in the presence of phosphate buffered saline solution. In these formulations VES together with non-ionic surfactants, Tween® 80 and Triton X-100® are useful to form stabilising micelles (WO02/097072). Stabilisation of the haemagglutinin antigen (HA) is believed to be brought about with the formation of complex micelles made up of proteins, lipids, VES, Tween® 80, Triton X-100®. Stabilisation of HA is achieved through physical separation of HA within the complex micelles. This helps to maintain the HA content at a reasonably constant level over a period of 12 months or more when stored under refrigerated conditions of 5±3 ^{0}C. Importantly, stabilisation of HA antigens prevents the bioactive HA antigens from undergoing aggregation resulting in a significant decline in HA activity during storage.

The combination of stabilisers used in the vaccine composition of the present invention comprises Tween® 80, alpha-tocopherol or a derivative thereof and t-octylphenoxypolyethoxyethanol (Triton X-100®).

Polyoxyethylene sorbitan monooleate (Tween 80® or Polysorbate 80)

Despite high concentrations of Tween® 80 being used previously as part of certain stabilisation systems for split influenza vaccines, the present inventors have surprisingly found that as part of the stabilisation system of the invention, a lower concentration of Tween® 80 is sufficient to maintain stability of HA. Such higher concentrations of Tween® 80 were believed to be necessary for Tween® 80 to

solubilise alpha-tocopherol succinate (or VES) in order to maintain HA stability in split influenza vaccines. Such higher concentrations of Tween® 80 are not required to maintain HA stability in the vaccine compositions of the present invention.

Thus, in the vaccine composition of the invention, the amount of Tween® 80 per human dose is ≤200 µg, for example, ≤150 µg, ≤100 µg, ≤90 µg or ≤80 µg. In particular, the amount of Tween 80® per human dose (for example, an adult dose) may be from 10 to 200 µg, from 10 to 150 µg, from 20 to 100 µg, from 50 to 150 µg, from 50 to 100 µg, or from 50 to 80 µg, such as around 70 µg. Preferably, for an adult dose the amount per dose is from 20 to 100 µg.

Smaller doses are contemplated, for example for some paediatric human doses. Some paediatric doses may contain half the human adult dose amount of Tween® 80 as well as other vaccine ingredients such as other excipient or stabiliser components. In particular, the amount of Tween® 80 per paediatric human dose may be from 10 to 60 µg, from 10 to 50 µg, or from 20 to 45 µg, such as around 35 µg. Preferably, the paediatric human dose is from 10 to 50 µg.

t-octylphenoxypolyethoxyethanol

Synonyms for t-octylphenoxypolyethoxyethanol (Triton X-100®) include but are not limited to polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether, octyl phenol ethoxylate, polyoxyethylene octyl phenyl ether, 4-octylphenol polyethoxylate, Mono 30, TX-100, Octoxynol-9, octoxynol 10, X-100, and octylphenol ethylene oxide condensate.

In the vaccine composition of the invention, the amount of Triton X-100® per human dose is ≥100 µg, for example, ≥150 µg or ≥200 µg. In particular, the amount of Triton X-100® per human dose (for example, an adult dose) may be from 100 to 500 µg, from 100 to 400 µg, from 100 to 300 µg, from 110 to 500 µg, from 120 to 300 µg, from 190 to 500 µg, or from 190 to 300 µg, such as around 250 µg. Preferably, the adult human dose amount is from 120 to 300 µg.

Smaller doses are contemplated, for example for some paediatric human doses. Some paediatric doses may contain half the human adult dose amount of Triton X-100® as well as other vaccine ingredients such as other excipient or stabiliser components. In particular, the amount of Triton X-100® per paediatric

human dose may be from 100 to 175 µg, from 100 to 150 µg, from 100 to 140 µg, or from 115 to 135 µg, such as around 125 µg. Preferably, the paediatric dose amount is from 100 to 140 µg.

In one embodiment, the vaccine composition of the invention contains an amount of Tween® 80 of from 10 to 200 µg and an amount of Triton X-100® of from 100 to 500 µg per human dose. In another embodiment, the amount of Tween 80® is from 50 to 150 µg and the amount of Triton X-100® is from 190 to 500 µg per human dose.

In a further embodiment, the vaccine composition of the invention contains an amount of Tween® 80 of from 10 to 60 µg and an amount of Triton X-100® of from 100 to 175 µg per paediatric human dose. In another embodiment, the amount of Tween® 80 is from 50 to 150µg and the amount of Triton X-100® is from 190 to 500 µg per paediatric human dose.

Alpha tocopherol

Alpha tocopherol (α-tocopherol) and derivatives of α-tocopherol such as α-tocopherol succinate are included in the vaccine composition of the invention. Other preferred tocopherol derivatives for use in the invention include D-α tocopherol, D-δ tocopherol, D-γ tocopherol and DL-α-tocopherol. Preferred derivatives of tocopherols that may be used include acetates, succinates, phosphoric acid esters, formiates, propionates, butyrates, sulfates and gluconates. Alpha-tocopherol succinate is particularly preferred.

The α-tocopherol or derivative is present in an amount sufficient to stabilise the haemagglutinin (for example using the SRD method or any other method described herein for stability analysis). See for example WO2002/097072. Preferred concentrations for the α-tocopherol or derivative are between 1 μg/ml – 10 mg/ml, more preferably between 10 μg/ml – 500 μg/ml.

Pharmaceutically acceptable excipient

The influenza virus preparation in the vaccine composition of the invention is present along with a pharmaceutically acceptable excipient. This excipient comprises

Tween® 80, alpha-tocopherol or a derivative thereof and Triton X-100®. Typically, these and optionally any other standard excipient ingredients will be present in liquid form. For example, the stabiliser components, antigen and any adjuvant present may be diluted in a volume of aqueous buffer, mineral oil, water for injection or other suitable diluent well known to those skilled in the art, together with any additional excipient components, such as salts that are commonly used in such diluents in a vaccine context. Thus, in one aspect there is provided a vaccine composition of the invention in which each specified component is diluted in an aqueous buffer or water for injection.

The vaccine composition of the invention comprises Tween® 80 and Triton X-100® in specified human doses. By the term "human dose" is meant the amount of the specified ingredient in the vaccine composition which is delivered along with any other vaccine composition ingredients (other excipient components, antigen and any adjuvant) to a human subject, for example in a volume suitable for human use. Generally the dose volume is from 0.25 to 1.5 ml. In one embodiment, a human dose is about 0.5 ml. In a further embodiment, a human dose is higher than 0.5 ml, for example about 0.6, 0.7, 0.8, 0.9 or about 1 ml. In a further embodiment, a human dose is from 1 ml to 1.5 ml. In another embodiment, in particular when the vaccine composition is for the paediatric population, a human dose may be less than 0.6 ml, for example from 0.25 to 0.5 ml or exactly 0.1 ml, 0.2 ml, 0.25 ml, 0.3 ml or 0.4 ml. Preferably, the dose volume for an adult is from 0.4 to 1.2 ml and the dose volume for the paediatric population is from 0.2 to 0.6 ml. Slight adaptation of the dose volume will be made routinely depending on the HA concentration in the original bulk sample and depending also on the delivery route with smaller doses being given by the intranasal or intradermal route.

Typically, the stabiliser components, antigen and any adjuvant present will be diluted in a volume of aqueous buffer, water for injection or other suitable diluent well known to those skilled in the art, together with any additional excipient components, such as salts that are commonly used in such diluents in a vaccine context. Thus, in one aspect there is provided a vaccine composition of the invention in which each specified component is diluted in an aqueous buffer or water for injection.

Influenza virus preparation

Preferably the influenza virus preparation of the invention is either a split virus preparation, or a subunit antigen prepared from whole virus (e.g. particularly by a splitting process followed by purification of the surface antigen) or by expression of recombinant protein. Most preferred are split virus preparations.

The split or subunit influenza virus preparation according to the invention may be derived from the conventional embryonated egg method, or they may be derived from any of the new generation methods using tissue culture to grow the virus or express recombinant influenza virus surface antigens. Suitable cell substrates for growing the virus include for example dog kidney cells such as MDCK or cells from a clone of MDCK, MDCK-like cells, monkey kidney cells such as AGMK cells including Vero cells, suitable pig cell lines, or any other mammalian cell type suitable for the production of influenza virus for vaccine purposes. Suitable cell substrates also include human cells e.g. MRC-5 cells. Suitable cell substrates are not limited to cell lines; for example primary cells such as chicken embryo fibroblasts are also included.

Techniques for splitting and/or preparation of subunit antigens are well known in the art. Traditionally split flu was produced using a solvent/detergent treatment, such as tri-*n*-butyl phosphate, or diethylether in combination with Tween® (known as "Tween-ether" splitting) and this process is still used in some production facilities. Other splitting agents now employed include detergents or proteolytic enzymes or bile salts, for example sodium deoxycholate. Detergents that can be used as splitting agents include cationic detergents e.g. cetyl trimethyl ammonium bromide (CTAB), other ionic detergents e.g. laurylsulfate, taurodeoxycholate, or non-ionic detergents such as the ones described above including Triton X-100® (for example in a process described in Lina et. al., 2000, Biologicals 28, 95-103) and Triton N-101®, or combinations of any two or more detergents.

The preparation process for a split vaccine will include a number of different filtration and/or other separation steps such as ultracentrifugation, ultrafiltration, zonal centrifugation and chromatography (e.g. ion exchange) steps in a variety of combinations, and optionally an inactivation step e.g. with heat, formaldehyde or β-propiolactone or U.V. which may be carried out before or after splitting. The splitting

process may be carried out as a batch, continuous or semi-continuous process. After splitting, inactivation and filtration steps, the influenza virus preparation may be purified before using in the vaccine composition of the invention. For example, the split preparation may be prepared using the techniques described in WO2011/051235 or WO2002/097072.

The subunit influenza virus antigen preparation according to the invention may be derived from a source other than the live influenza virus, for example the haemagglutinin antigen may be produced recombinantly using techniques well known to those skilled in the art.

In one embodiment, the vaccine composition is monovalent, i.e. only comprises one specific type of influenza HA. For example, the vaccine composition may comprise a influenza HA associated with a pandemic or a strain noted and monitored by the World Health Organisation as a potentially pandemic strain of influenza (e.g. a H5, H7, H9, H8 or H10 type influenza strain, preferably an H5 strain). In alternative embodiments, the composition is multivalent, i.e. comprises multiple influenza virus antigens. For example, the composition may be bivalent, trivalent or quadrivalent, e.g. may contain two or three seasonal strains of influenza virus. Preferably, the vaccine composition is a trivalent or quadrivalent vaccine comprising seasonal strains of influenza virus (e.g. as recommended by the World Health Organisation each year for inclusion in a seasonal influenza vaccine). Such strains may include two A strains (e.g. H1N1 and H2N3) and one or two strains of B influenza virus (e.g. from Victoria and/or Yamagata lineages).

A pharmaceutically acceptable excipient as described herein may be added to the influenza virus preparation to make up the vaccine composition of the invention. The influenza virus preparation along with the pharmaceutically acceptable excipient may be stored in bulk before transfer to vials ready for administration (e.g. in single-dose prefilled syringes or in single or multi-dose vials).

An adjuvant may be added before or after storage. For example, the adjuvant may be added to a vaccine composition of the invention just prior to administration by mixing the contents of adjuvant and vaccine composition in separate vials.

Thus, in one embodiment, the vaccine composition of the invention further comprises an adjuvant. Preferably, the adjuvant is an oil-in-water emulsion adjuvant.

Oil in water emulsion adjuvants, such as MF59 or AS03 are well known in the art and are described below.

Adjuvant

Thus, in one embodiment, the vaccine composition of the invention further comprises an adjuvant. Preferably, the adjuvant is an oil-in-water emulsion adjuvant. Oil in water emulsion adjuvants, such as MF59 or AS03 are well known in the art and are described below.

Tocols (e.g. Vitamin E) are also used in oil emulsions adjuvants (EP0382271B1; US5667784; WO95/17210). In an oil-in-water emulsion, the oil and emulsifier should be in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline or a citrate buffer. One example of a tocol-containing oil-in-water emulsion is AS03.

A preferred oil-in-water emulsion comprises a metabolisable oil, such as squalene, Tween® 80 and optionally alpha tocopherol. Additionally, the oil-in-water emulsion may contain Span® 85 and/or lecithin.

In one instance, the oil-in-water emulsion has one of the following compositions:

- From 0.5 to 11 mg squalene, from 0.05 to 5% polyoxythylene sorbitan monooleate (Tween 80® or Polysorbate 80) and optionally, from 2 to 12% alpha-tocopherol; or
- About 5% squalene, about 0,5% polyoxyethylene sorbitan monooleate (Tween® 80 or Polysorbate 80) and about 0.5% sorbitan trioleate (Span® 85). This adjuvant is called MF59.

An alternative adjuvant that may be used with the vaccine composition according to the present invention, comprises an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina (e.g. QS21) presented in the form of a liposome and a lipopolysaccharide (e.g. 3D-MPL), optionally further including a sterol (cholesterol). In one embodiment, the adjuvant comprises or consists of a saponin (e.g. QS21) presented in the form of a liposome, a lipid A derivative such as 3D-MPL and a sterol (e.g. cholesterol). The liposomes suitably contain a neutral lipid, for example, phosphatidylcholine, dioleoyl phosphatidylcholine (DOPC) or dilauryl phosphatidylcholine. The liposomes may also contain a charged

lipid which increases the stability of the liposome-QS21 structure for liposomes composed of saturated lipids. An example of such an adjuvant is AS01, which comprises 3D-MPL and QS21 in a quenched form with cholesterol, and can be made as described in WO96/33739. Either the AS01B or AS01E forms of this adjuvant may be used. The AS01 B adjuvant comprises liposomes, which in turn comprise dioleoyl phosphatidylcholine (DOPC), cholesterol and 3D-MPL (in an amount of approximately 1000 micrograms DOPC, 250 micrograms cholesterol and 50 micrograms 3D-MPL per vaccine dose), QS21 (50 micrograms/dose), phosphate saline buffer and water to a volume of 0.5 ml.

The AS01E adjuvant comprises the same ingredients than AS01 B but at a lower concentration in an amount of approximately 500 micrograms DOPC, 125 micrograms cholesterol, 25 micrograms 3D-MPL and 25 micrograms QS21, phosphate saline buffer and water to a volume of 0.5 ml.

Stabilisation

Stability of hemagglutinin can be measured by a variety of techniques and would be well known to those skilled in the art. For example, stability can be measured by the appearance of the antigen, such as the appearance of precipitation, or floccules that sediment out of solution. Appearance can be monitored over time under normal storage conditions (e.g. refrigerated at 0±3, 5±3, 10±2, 15±2 etc. ºC), or under accelerated stability testing conditions such as room temperature or higher temperatures (e.g. 20±2, 25±2, 30±2, 35±2, 37±2, 37.5±2, 38±2, 40±2, 45±2, 50±2 ºC). Samples may be stored for example in a constant temperature chamber or room. The appearance can be measured using techniques well known in the art, for example using a spectrophotometer to obtain a transmission reading, or upon visual inspection.

Another way of measuring stability is to measure the HA content over time either under normal storage conditions for a vaccine (e.g. refrigerated conditions) or accelerated conditions such as room temperature or higher temperatures (e.g. 20±2, 25±2, 30±2, 35±2, 37±2, 37.5±2, 38±2, 40±2, 45±2, 50±2 ºC). Preferably, the accelerated conditions are from 20 to 40 ºC. Techniques for measuring HA content, for example the commonly used SRD assay, would be well known to those skilled in the art in this field.

Stability may be measured over a period of time e.g. 1, 2, 3 or 4 weeks or 1, 2, 3, 4, 5, 6, or 12 months or more. For example, under accelerated conditions, the time period may be 1, 2, 3 or 4 weeks or more. Under normal storage conditions such as refrigerated conditions, the time period may be 6 or 12 months, or from about 6 months to about 12 months. Samples are withdrawn periodically at designated time points throughout the stability testing in accordance with established methods for stability testing in this way.

Thus, in one instance, stability is assessed after storage of the vaccine composition at from 20 to 40 degrees centigrade (e.g. 30±2 ºC) for 4 weeks or more. In another instance, stability is assessed after storage of the vaccine composition at from 2 to 10 degrees centigrade (e.g. 5±3 ºC) for about 12 months.

Further dosing and efficacy criteria

Typically, the vaccine composition according to the present invention is a standard 0.5 ml injectable dose in most cases, and contains 15 µg or less, of hemagglutinin antigen component from an/each influenza virus strain, as measured by single radial immunodiffusion (SRD) (J.M. Wood et al.: J. Biol. Stand. 5 (1977) 237-247; J. M. Wood et al., J. Biol. Stand. 9 (1981) 317-330).

In one embodiment, the vaccine composition according to the invention contains a low amount of HA antigen – e.g. any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 µg of HA per influenza virus strain (e.g. less than 10 µg of HA, or less than 7.5 µg of HA) or which does not exceed 15 µg of HA per strain. Said low amount of HA amount may be as low as practically feasible provided that it allows to formulate a vaccine which meets the international e.g. EU or FDA criteria for efficacy, as detailed below (see Table 1 and the specific parameters as set forth). A suitable low amount of HA is from 1 to 7.5 µg of HA per influenza virus strain, suitably from 3.5 to 5 µg, such as 3.75 or 3.8 µg of HA per influenza virus strain, typically about 5 µg of HA per influenza virus strain. Another suitable amount of HA is from 0.1 to 5 µg of HA per influenza virus strain, suitably from 1.0 to 2 µg of HA per influenza virus strain, such as 1.9 µg of HA per influenza virus strain.

The vaccine composition of the invention suitably meets certain international criteria for vaccines. Standards are applied internationally to measure the efficacy of influenza vaccines.

Serological variables are assessed according to criteria of the European Agency for the Evaluation of Medicinal Products for human use (CHMP/BWP/214/96, Committee for Proprietary Medicinal Products (CPMP). Note for harmonization of requirements for influenza vaccines, 1997. CHMP/BWP/214/96 circular N°96-0666:1-22) for clinical trials related to annual licensing procedures of influenza vaccines (Table below).

Table 1: CHMP criteria

	18 - 60 years	**> 60 years**
Seroconversion rate*	>40%	>30%

Conversion factor**	>2.5	>2.0
Protection rate***	>70%	>60%

** Seroconversion rate is defined as the proportion of subjects in each group having a protective post-vaccination titre ≥ 1:40. The seroconversion rate simply put is the % of subjects who have an HI titre before vaccination of <1:10 and ≥1:40 after vaccination. However, if the initial titre is ≥1:10 then there needs to be at least a fourfold increase in the amount of antibody after vaccination.*

*** Conversion factor is defined as the fold increase in serum HI geometric mean titres (GMTs) after vaccination, for each vaccine strain.*

**** Protection rate is defined as the proportion of subjects who were either seronegative prior to vaccination and have a (protective) post-vaccination HI titre of ≥ 1:40 or who were seropositive prior to vaccination and have a significant 4-fold increase in titre post-vaccination; it is normally accepted as indicating protection.*

The requirements are different for adult populations (18-60 years) and elderly populations (>60 years). For inter-pandemic influenza vaccines, at least one of the assessments (seroconversion factor, seroconversion rate, seroprotection rate) should meet the European requirements, for all strains of influenza included in the vaccine. The proportion of titres equal or greater than 1:40 is regarded most relevant because these titres are expected to be the best correlate of protection (Beyer et al. (1998) Clin Drug Invest 15:1).

The compositions for use according to the present invention suitably meet at least one such criteria for the influenza virus strain included in the composition (one criteria is enough to obtain approval), suitably at least two, or typically at least all three criteria for protection. Suitably the above response(s) is (are) obtained after one dose, or after two doses.

Methods of treatment

A method of inducing an immune response in a human subject, said method comprising administering to the subject a vaccine composition of any one of claims 1 to 6.

In a further aspect, the vaccine composition of the invention is for use in medicine, such as for use in the treatment, prevention of, or vaccination against, disease caused by influenza e.g. administered to a person (e.g. subject) at risk for influenza infection.

In one embodiment, the vaccine composition of the invention is for use in the prevention of or vaccination against, influenza disease caused by a different clade than the clade on which the influenza virus preparation was based. For example, a H5N1 clade 1 HA antigen could be used for protection against influenza caused by a non-clade 1 virus e.g. a H5N1 clade 2 virus.

In a further aspect, there is provided a method of treatment, prevention and/or vaccination against influenza disease, comprising the administration of a vaccine composition as described herein to a person in need thereof, e.g. to a person (e.g. subject) at risk for influenza infection, e.g. an elderly person (age 50 or over, particularly age 65 or over).

Routes of administration

The composition of the invention may be administered by any suitable delivery route, such as intradermal, mucosal (e.g. intranasal), oral, intramuscular or subcutaneous. Other delivery routes are well known in the art.

The intramuscular (IM) delivery route is particularly suitable for the vaccine composition. The composition according to the invention may be presented in a monodose container, or alternatively, a multidose container, particularly suitable for a pandemic vaccine. In this instance an antimicrobial preservative such a thiomersal may be present to prevent contamination during use. A thiomersal concentration of 5 µg/0.5 ml dose (i.e. 10 µg/ml) or 10 µg/0.5 ml dose (i.e. 20 µg/ml) is suitably present. A suitable IM delivery device could be used such as a needle-free liquid jet injection device, for example the Biojector 2000 (Bioject, Portland, OR). Alternatively a pen-injector device, such as is used for at-home delivery of epinephrine, could be used to allow self-administration of vaccine. The use of such delivery devices may be

particularly amenable to large scale immunization campaigns such as would be required during a pandemic.

Intradermal delivery is another suitable route. Any suitable device may be used for intradermal delivery, for example short needle devices. Such devices are well known in the art. Intradermal vaccines may also be administered by devices which limit the effective penetration length of a needle into the skin, such as those described in WO99/34850 and EP1092444, incorporated herein by reference, and functional equivalents thereof. Also suitable are jet injection devices which deliver liquid vaccines to the dermis via a liquid jet injector or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis. Also suitable, are ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis. Additionally, conventional syringes may be used in the classical mantoux method of intradermal administration.

Another suitable administration route is the subcutaneous route. Any suitable device may be used for subcutaneous delivery, for example classical needle. Suitably, a needle-free jet injector service is used. Such devices are well known in the art. Suitably said device is pre-filled with the liquid vaccine formulation.

Alternatively the vaccine is administered intranasally. Typically, the vaccine is administered locally to the nasopharyngeal area, suitably without being inhaled into the lungs. It is desirable to use an intranasal delivery device which delivers the vaccine formulation to the nasopharyngeal area, without or substantially without it entering the lungs.

Suitable devices for intranasal administration of the vaccines according to the invention are spray devices. Suitable commercially available nasal spray devices include Accuspray® (Becton Dickinson). Nebulisers produce a very fine spray which can be easily inhaled into the lungs and therefore does not efficiently reach the nasal mucosa. Nebulisers are therefore not preferred.

Suitable spray devices for intranasal use are devices for which the performance of the device is not dependent upon the pressure applied by the user. These devices are known as pressure threshold devices. Liquid is released from the nozzle only when a threshold pressure is applied. These devices make it easier to

achieve a spray with a regular droplet size. Pressure threshold devices suitable for use with the present invention are known in the art and are described for example in WO 91/13281 and EP 311 863 B and EP 516 636, incorporated herein by reference. Such devices are commercially available from Pfeiffer GmbH and are also described in Bommer, R. Pharmaceutical Technology Europe, Sept 1999.

Alternatively, the epidermal or transdermal vaccination route is also contemplated in the present invention.

Thus in a further aspect, there is provided a device as described above comprising or containing a vaccine composition of the invention.

The teaching of all references in the present application, including patent applications and granted patents, are herein fully incorporated by reference. Any patent application to which this application claims priority is incorporated by reference herein in its entirety in the manner described herein for publications and references.

For the avoidance of doubt the terms 'comprising', 'comprise' and 'comprises' herein is intended by the inventors to be optionally substitutable with the terms 'consisting of', 'consist of', and 'consists of', respectively, in every instance.

EXAMPLES

Example 1:

The current study is aimed at:

- determining the extent of VES solubilisation by Tween® 80 and Triton X-100® individually and in combination,
- optimising the combinations of VES/surfactants at laboratory-scale and realistic production-scale process operability trials,
- adapting the established influenza manufacturing processes for monovalent bulks, final quadrivalent bulks and syringe filling operations,
- verifying the stability profiles of monovalent and QIV filled syringes in reduces stabiliser formulations under accelerated (at 30±2^{0}C for 4 weeks) and real time conditions (at 5±3^{0}C for 12 months).

A set of preliminary formulae (**Table 2**) containing Tween® 80, Triton X-100® and VES is proposed.

Table 2: Proposed Formula for Monovalent Bulk and QIV Final Bulk and Filled Syringe

	Tween® 80 (µg/ml)	**Triton X-100® (µg/ml)**	**VES (µg/ml)**	**PBS (ml)**
Monovalent Bulk	140	500	Twice the amount for QIV	1.0
QIV Final bulk and Filled Syringe	70	250	q.s.	1.0

** q.s. = sufficient quantities*

Materials and Methods

Materials Used for Pre-Formulation Studies

The following materials are used in the pre-formulation study.

- α-tocopheryl succinate or vitamin E succinate, VES (B. 2012120574, BASF)
- Tween® 80 or Polysorbate 80 (B. 2013060415, Well Chemicals)
- Triton X-100® (B. L012017843320, Merck)
- Bovine serum albumin (B.0175, Amresco)
- Phosphate buffered saline solution, PBS (B.13126B, B.13142B, GSK Shenzhen)
- Ethanol 95% (B. 20130501, Guangzhou Chemicals)
- Stock solutions (2.654% VES in 95% ethanol, 1% Tween® 80, 2.5% Triton X-100® in PBS, GSK Shenzhen)
- Water for injection (GSK Shenzhen)

Materials Used for Manufacturing of Monovalent Bulks

The following materials are needed to manufacture monovalent bulks with various stabiliser concentrations.

In order to formulate a batch of quadrivalent Split-Virion Seasonal Influenza Vaccines (QIV), 4 individual lots of monovalent bulks, one bulk from each virus strain, are needed.

Five strains of working seeds (**Table 3**) are used for the manufacturing of monovalent bulks batches including two H1N1 strains (A/California/7/2009 NYMC X-179A, H1N1-179A and A/Christchurch/16/2010 NIB-74xp, H1N1-74xp), a H3N2 strain (A/Texas/50/2012 NYMC X-223A, H3N2) and two B strains with one from the Yamagata lineage (B/Massachusetts/2/2012 NYMC BX-51) and the other from the Victoria lineage (B/Brisbane/60/2008 NYMC BX-35). Monovalent bulk lots used to manufacture the monovalent bulks in different formulae A-G are given in **Table 4**.

Table 3: Details of Reference and Working Seeds

Description (Seed Strain)	**Reference Seed** (Batch)	**Working Seed** (Batch)	**Monovalent Bulk Produced** (Batch)
A/California/7/2009 NYMC X-179A (H1N1-179A)	2012020059	P/A/CL/P3/1 2026	2013FS02 2014FS01
A/Christchurch/16/2010 NIB-74xp (H1N1-74xp)	2013050326	P/A/CC/P2/1 3018	2014FS02
A/Texas/50/2012 NYMC X-223A (H3N2)	2013040152	P/A/TX/P3/1 3011	2013FS01
B/Brisbane/60/2008 NYMC BX-35 (B-Victoria)	2013040149	P/B/BX/P3/1 3026	2013FS04
B/Massachusetts/2/2012 NYMC BX-51B (B-Yamagata)	2013040151	P/B/MC/P3/ 13012	2013FS03

Table 4: Monovalent Bulk Batch used for the Manufacture of Monovalent Bulks in Various Formulae

Monovalent Bulk Batch Prior to Stabilizer Addition	Batch number	Batches of Formulated Monovalent Bulk Produced
A/Texas (H3N2) Monovalent Bulk	B.2013FS01	B.2013FS01A, B, C, D and E
A/California (H1N1-179A) Monovalent Bulk	B.2013FS02	B.2013FS02A, B, C, D and E
B/Massachusetts (B-Yamagata) Monovalent Bulk	B.2013FS03	B.2013FS03A, B, C, D and E
B/Brisbane (B-Victoria) Monovalent Bulk	B.2013FS04	B.2013FS04A, B, C, D and E
A/California (H1N1-179A) Monovalent Bulk	B.2014FS01	B.2014FS01A, B, C, D, D1 and E
A/Christchurch (H1N1-74xp) Monovalent Bulk	B.2014FS02	B.2014FS02A, B, C, D and E

- A total of 6 lots of 0-day eggs are sourced from Da Hua Nong Farm, Guangdong Province, P.R. China. Each lot consists of 70,000 eggs.
- Triton X-100® (B. lo12017843320) is supplied by Merck.
- VES (B.2013050344) is sourced from Badische Anilin-und-Soda-Fabrik
- Tween® 80 (B.2013060415) is purchased from Well Chemical, Nanjing.
- Other items include 2 L glass bottles supplied by Fisher (Item code: FB800-2000), 500 ml glass bottles supplied by Kimble Chase (Item code: 89000-930).
- Other ancillary materials needed during production are recorded in detail (Laboratory Books - Primary 02, 08, 09, 10 and 11 and Batch Manufacturing Records for batches 2013FS01, 2013FS02, 2013FS04, 2014FS01 and 2014FS02).

Manufacturing of Final Bulk and Filled Syringes

In order to formulate a batch of quadrivalent (QIV) Split Virion Seasonal Influenza Vaccines, one monovalent bulk from each of the 4 virus strains, is needed. The following materials are used in the manufacturing final bulks and finished products with various stabiliser concentrations (**Tables 5** - **7**). Other sterile materials used during production include 2 L glass bottles, flexible silicon tubing, magnetic stirrer beads, stainless-steel scissors and stainless-steel forceps. These are recorded in manufacturing records (Laboratory Book 07).

Table 5: Monovalents Bulks (MVB) Used for Manufacturing of Final bulks

Description	Batch No.	HA [µg/ml]	VES [µg/ml]	Tween® 80 [µg/ml]	Triton X-100® [µg/ml]
A/Texas (H3N2) MVB in Formula A	2013FS01-A	292	-	-	-
A/Texas (H3N2) MVB in Formula B	2013FS01-B	431	624	1,130	-
A/Texas (H3N2) MVB in Formula C	2013FS01-C	457	360	140	500
A/Texas (H3N2) MVB in Formula D	2013FS01-D	455	412	140	500
A/Texas (H3N2) MVB in Formula E	2013FS01-E	450	459	140	500
A/Calif. (H1N1-179A) MVB in Formula A	2013FS02-A	113	-	-	-
A/Calif. (H1N1-179A) MVB in Formula B	2013FS02-B	133	626	1,130	-
A/Calif. (H1N1-179A) MVB in Formula C	2013FS02-C	132	366	140	500
A/Calif. (H1N1-179A) MVB in Formula D	2013FS02-D	135	416	140	500
A/Calif. (H1N1-179A) MVB in Formula E	2013FS02-E	132	469	140	500
B/Mass. (B-Yamagata) MVB in Formula	2013FS03-A	332	-	-	-
B/Mass. (B-Yamagata) MVB in Formula	2013FS03-B	406	623	1130	-
B/Mass. (B-Yamagata) MVB in Formula	2013FS03-C	424	367	140	500
B/Mass. (B-Yamagata) MVB in Formula	2013FS03-D	426	428	140	500
B/Mass. (B-Yamagata) MVB in Formula	2013FS03-E	415	471	140	500
B/Brisbane (B-Victoria) MVB in Formula	2013FS04-A	442	-	-	-
B/Brisbane (B-Victoria) MVB in Formula	2013FS04-B	496	616	1130	-
B/Brisbane (B-Victoria) MVB in Formula	2013FS04-C	502	365	140	500
B/Brisbane (B-Victoria) MVB in Formula	2013FS04-D	498	415	140	500
B/Brisbane (B-Victoria) MVB in Formula	2013FS04-E	507	468	140	500
A/Christ. (H1N1-74xp) MVB in Formula	2014FS02-B	331	637	1,130	-
A/Christ. (H1N1-74xp) MVB in Formula	2014FS02-C	338	366	140	500
A/Christ. (H1N1-74xp) MVB in Formula	2014FS02-D	337	422	140	500

Table 6: Buffer Solutions Used for Manufacturing of Final bulks

Buffer Solution Description	Material	Batch No.
5.0% (w/v) Tween® 80 in Water for	30025	B. RD-B-20131215
0.2% (w/v) Tween® 80 in Water for	RD-S-02	B. RD-B-20131213
2.6% VES in 95% Ethanol	30042	B. 13080B
1.0% VES in 95% Ethanol	RD-S-06	B.RD-B-20131212
Standard PBS solution	30017	B.13088B
1.0% Triton X-100® in PBS Solution	RD-S-04	B.RD-B-20131211

Table 7: Disposable Sterile Materials Used for Manufacturing of Final bulks and Filled Syringes

Material Description	Code No.	Batch No.
Disposal pipette, 1 ml Disposal pipette, 10 ml Disposal pipette, 50 ml	40052 40054 40055	B.2058254 B.2051476 B.8020172
Sterile plastic bottle, 125 ml Sterile plastic bottle, 250 ml	40040 40050	B.1095261 B.1096349
Ceramic Coated Tip Prefilled syringes (Becton Dickenson)	20024	B.2013080529 B.2013080527 B.2013080530
FM457 Stopper (Becton Dickenson)	20026	B.2013080556

Example 2: Preliminary studies on VES solution

Dilution of VES Stock Solution with PBS

In this study, a PBS solution is gradually added into a 250 ml conical flask containing 30 ml solution of VES Stock Solution containing 2.654% VES solution in 95% ethanol with constant agitation. The solution is observed while the PBS solution is added and allowed to stand for several hours.

Dilution of VES Stock Solution with 80 µg/ml Tween® 80 in PBS

This study is conducted by gradual addition of a solution of Tween® 80 with a concentration of 80 µg/ml in PBS into a 250 ml conical flask containing 30 ml solution of 2.654% VES Stock Solution (in 95% ethanol) with constant agitation. The mixture is observed while Tween® 80 solution is added. The final solution is allowed to stand for several hours.

Results and Discussion

Dilution of VES Stock Solution with PBS

The solution develops an opaque white appearance after addition of 9 ml of PBS. The amount of precipitate present increases after further addition of PBS. Aggregates are observed after the solution is left standing for a few hours.

Dilution of VES Stock Solution with 80 µg/ml Tween® 80 in PBS

The solution develops an opaque white appearance after addition of 7.3 ml of 80 µg/ml Tween® 80 in PBS. The amount of precipitates increased after a total of addition of 8.0 ml of PBS. When the solution is left to stand for a few hours, the insoluble precipitates remain and some greasy materials are seen at the bottom of the flask.

Example 3:

Effects of Concentrations of Tween® 80 and VES on % Transmittance

A combination of specified quantities of VES Stock Solution containing 2.654% VES solution in 95% ethanol, 1% Tween® 80 solution in PBS and PBS solution, with a combined total volume of 10ml, is mixed within a 14 ml BD tube with agitation. The concentration for VES in this study is between 50 – 200 µg/ml while the Tween® 80 concentration is studied at between 30 - 150 µg/ml. Details of the study are given in **Table 24**. The transmittance of the solutions is measured at 580 nm against a blank PBS solution.

Results and Discussion

Effects of Concentrations of Tween® 80 and VES on % Transmittance

The % transmittance for the various solutions at 580 nm are recorded in **Table 21**. The %T results recorded range from 57.19% - 95.61%. The %T for the QIV product is 86.45%. Solutions with VES:Tween® 80 at 100:60, 100:90, 150:90, 150:120, 150:60, 200:30, 200:60, 200:90, 200:120, 200:150. A non-linear relationship exists between the ratios of the VES/Tween® 80 but this is influenced by both the concentrations of the VES and Tween® 80.

Table 21: Transmittance of the Mixtures of VES and Tween® 80

Sample Identity	2.6% VES Solution in Ethanol (ml)	Tween® 80 1% Solution (ml)	PBS (ml)	Concentration Ratio	VES : Tween® 80 Ratio	%T
1	0.020	0.030	9.950	50:30	1.667	91.96
2	0.020	0.060	9.920	50:60	0.833	91.69
3	0.020	0.090	9.890	50:90	0.556	94.28
4	0.020	0.120	9.860	50:120	0.417	94.18
5	0.020	0.150	9.830	50:150	0.333	95.18
6	0.040	0.030	9.930	100:30	3.333	95.61
7	0.040	0.060	9.900	100:60	1.667	79.67
8	0.040	0.090	9.870	100:90	1.111	83.32
9	0.040	0.120	9.840	100:120	0.833	86.48
10	0.040	0.150	9.810	100:150	0.667	88.84
11	0.060	0.030	9.910	150:30	5.000	89.81
12	0.060	0.060	9.880	150:60	2.500	83.39
13	0.060	0.090	9.850	150:90	1.667	79.92
14	0.060	0.120	9.820	150:120	1.250	84.55

15	0.060	0.150	9.790	150:150	1.000	86.10
16	0.080	0.030	9.890	200:30	6.667	78.75
17	0.080	0.060	9.860	200:60	3.333	76.53
18	0.080	0.090	9.830	200:90	2.222	57.19
19	0.080	0.120	9.800	200:120	1.667	80.95
20	0.080	0.150	9.770	200:150	1.333	80.51
QIV B.20130907	N/A	N/A	N/A	533:1360	0.392	86.45

Example 4: Solubilisation of VES in Tween® 80 Solution

In this study, specified quantities of VES Stock Solution are added to diluted Tween® 80 solutions with constant agitation. The concentration for VES in this study is increased to 50 - 400 µg/ml while the Tween® 80 concentration is studied at between 90 - 150 µg/ml. Details of the study are given in **Table 25**. The appearance of the mixtures is recorded together with their transmittance, measured at 580 nm against a blank PBS solution.

Results and Discussion

Solubilisation of VES in Tween® 80 Solution

The %T for different combinations of Tween® 80 and VES solution are given in **Table 22**. At Tween® 80 concentration of 90 µg/ml, the concentration of VES has to be below 150 µg/ml in order to achieve a solution clarity equivalent or better than the QIV product whereas for Tween® 80 concentration of 150 µg/ml, the concentration of VES has to be below 200 µg/ml in order to achieve a solution clarity equivalent or better than the QIV product.

Table 22: Solubilisation of VES in Tween® 80 Solution

Sample Identity	2.6% VES in Ethanol (ml)	1% Tween® 80 in PBS (ml)	PBS Added (ml)	Concentration Ratio	VES: Tween® 80 Ratio	%T
1	0.020	0.090	9.890	50:90	0.556	95.85
2	0.040	0.090	9.870	100:90	1.111	90.68
3	0.060	0.090	9.850	150:90	1.667	85.80
4	0.080	0.090	9.830	200:90	2.222	84.73
5	0.100	0.090	9.810	250:90	2.778	54.51
6	0.120	0.090	9.790	300:90	3.333	60.91
7	0.140	0.090	9.770	350:90	3.889	55.38
8	0.160	0.090	9.750	400:90	4.444	55.51
9	0.020	0.150	9.830	50:150	0.333	97.33
10	0.040	0.150	9.810	100:150	0.667	93.09
11	0.060	0.150	9.790	150:150	1.000	91.60
12	0.080	0.150	9.770	200:150	1.333	77.30
13	0.100	0.150	9.750	250:150	1.667	66.57
14	0.120	0.150	9.730	300:150	2.000	66.58
15	0.140	0.150	9.710	350:150	2.333	51.58
16	0.160	0.150	9.690	400:150	2.667	52.08
QIV B.20130911	N/A	N/A	N/A	533:1365	0.390	86.44

Example 5: Solubilisation of VES in Triton X-100® Solution

In this study, specified quantities of VES Stock Solution are added to a diluted Triton X-100® Solution with constant agitation. Details of the study are given in **Table 26**. The appearance of the mixture is recorded together with their transmittance (%T) measured at 580 nm against a blank PBS solution.

Results and Discussion

Solubilisation of VES in Triton X-100® Solution

The %T for different combinations of Triton X-100® and VES solution are given in **Table 23**. At increasing concentration of Triton X-100® from 100 to 600 µg/ml, there is a corresponding increasing in the concentration of VES solution that can be solubilised achieving a %T result of at least the same or greater than that of the QIV reference sample. At a Triton X-100® concentration of 500 µg/ml, a VES concentration less than 400 µg/ml will result in a %T close to that of the reference sample.

Table 23: Solubilisation of VES in Triton X-100® Solution

Sample Identity	2.6%VES (ml)	2.5%Triton X-100® (ml)	PBS Added (ml)	Concentration Ratio	VES : Triton X-100® Ratio	%T
1-1	0.020	0.040	9.940	50:100	0.500	98.64
1-2	0.040	0.040	9.960	100:100	1.000	77.34
1-3	0.060	0.040	9.910	150:100	1.500	66.36
1-4	0.080	0.040	9.880	200:100	2.000	73.57
1-5	0.100	0.040	9.860	250:100	2.500	74.42
1-6	0.120	0.040	9.840	300:100	3.000	62.08
1-7	0.140	0.040	9.820	350:100	3.500	50.82
1-8	0.160	0.040	9.800	400:100	4.000	39.71

Sample Identity	2.6%VES (ml)	2.5%Triton X-100® (ml)	PBS Added (ml)	Concentration Ratio	VES : Triton X-100® Ratio	%T
1-9	0.180	0.040	9.880	450:100	4.500	32.52
1-10	0.200	0.040	9.760	500:100	5.000	26.85
1-11	0.220	0.040	9.740	550:100	5.500	20.20
1-12	0.240	0.040	9.720	600:100	6.000	8.14
2-1	0.020	0.080	9.900	50:200	0.250	99.78
2-2	0.040	0.080	9.880	100:200	0.500	97.97
2-3	0.060	0.080	9.860	150:200	0.750	92.39
2-4	0.080	0.080	9.840	200:200	1.000	41.15
2-5	0.100	0.080	9.820	250:200	1.250	59.20
2-6	0.120	0.080	9.800	300:200	1.500	57.34
2-7	0.140	0.080	9.780	350:200	1.750	38.51
2-8	0.160	0.080	9.760	400:200	2.000	31.95
2-9	0.180	0.080	9.740	450:200	2.250	29.71
2-10	0.200	0.080	9.720	500:200	2.500	35.35
2-11	0.220	0.080	9.700	550:200	2.750	25.74
2-12	0.240	0.080	9.680	600:200	3.000	34.12
3-1	0.020	0.120	9.860	50:300	0.167	99.44
3-2	0.040	0.120	9.840	100:300	0.333	99.94
3-3	0.060	0.120	9.820	150:300	0.500	99.54
3-4	0.080	0.120	9.800	200:300	0.667	97.20
3-5	0.100	0.120	9.780	250:300	0.833	82.44
3-6	0.120	0.120	9.760	300:300	1.000	25.66
3-7	0.140	0.120	9.740	350:300	1.167	24.60
3-8	0.160	0.120	9.720	400:300	1.333	29.38

Sample Identity	2.6%VES (ml)	2.5%Triton X-100® (ml)	PBS Added (ml)	Concentration Ratio	VES : Triton X-100® Ratio	%T
3-9	0.180	0.120	9.700	450:300	1.500	22.23
3-10	0.200	0.120	9.680	500:300	1.667	25.00
3-11	0.220	0.120	9.660	550:300	1.833	22.81
3-12	0.240	0.120	9.640	600:300	2.000	30.48
4-1	0.020	0.160	9.820	50:400	0.125	99.79
4-2	0.040	0.160	9.800	100:400	0.250	100.04
4-3	0.060	0.160	9.780	150:400	0.375	99.67
4-4	0.080	0.160	9.760	200:400	0.500	99.77
4-5	0.100	0.160	9.740	250:400	0.625	95.86
4-6	0.120	0.160	9.720	300:400	0.750	85.87
4-7	0.140	0.160	9.700	350:400	0.875	68.80
4-8	0.160	0.160	9.680	400:400	1.000	18.56
4-9	0.180	0.160	9.660	450:400	1.125	19.24
4-10	0.200	0.160	9.640	500:400	1.250	18.66
4-11	0.220	0.160	9.620	550:400	1.375	12.50
4-12	0.240	0.160	9.600	600:400	1.500	39.81
5-1	0.020	0.200	9.880	50:500	0.100	99.59
5-2	0.040	0.200	9.760	100:500	0.200	99.89
5-3	0.060	0.200	9.740	150:500	0.300	99.61
5-4	0.080	0.200	9.720	200:500	0.400	99.88
5-5	0.100	0.200	9.700	250:500	0.500	99.37
5-6	0.120	0.200	9.680	300:500	0.600	94.36
5-7	0.140	0.200	9.660	350:500	0.700	91.09
5-8	0.160	0.200	9.640	400:500	0.800	68.64

Sample Identity	2.6%VES (ml)	2.5%Triton X-100® (ml)	PBS Added (ml)	Concentration Ratio	VES : Triton X-100® Ratio	%T
5-9	0.180	0.200	9.620	450:500	0.900	29.02
5-10	0.200	0.200	9.600	500:500	1.000	13.68
5-11	0.220	0.200	9.580	550:500	1.100	12.64
5-12	0.240	0.200	9.560	600:500	1.200	20.39
6-1	0.020	0.240	9.740	50:600	0.083	99.72
6-2	0.040	0.240	9.720	100:600	0.167	99.55
6-3	0.060	0.240	9.700	150:600	0.250	99.60
6-4	0.080	0.240	9.680	200:600	0.333	99.62
6-5	0.100	0.240	9.660	250:600	0.417	98.99
6-6	0.120	0.240	9.640	300:600	0.500	99.30
6-7	0.140	0.240	9.620	350:600	0.583	97.97
6-8	0.160	0.240	9.600	400:600	0.667	92.09
6-9	0.180	0.240	9.580	450:600	0.750	78.41
6-10	0.200	0.240	9.560	500:600	0.833	70.36
6-11	0.220	0.240	9.540	550:600	0.917	27.56
6-12	0.240	0.240	9.520	600:600	1.000	14.11
QIV B.20130915	N/A	N/A	N/A	533:1365	0.390	87.52

Example 6: Solubilisation of VES in a Mixture of Tween® 80 and Triton X-100® Solutions (MVB)

In this study, specified volumes of VES Stock Solution are added to a diluted Tween® 80 Solution (with a concentration of 140 µg/ml) and Triton X-100® Solution (with a concentration of 500 µg/ml), adjusted to a final volume of 10.0 ml with constant agitation. Details of the study are given in **Table 27**. Sample M3' is based on a Triton X-100®:Tween® 80:VES ratio of 500:140:500. This test is performed to assess this combination of stabilisers on the appearance of the solution. The appearance of the mixture is recorded together with their transmittance (%T) measured at 580 nm against a blank PBS solution. Measurement of %T is repeated after 2 days.

Results and Discussion

Solubilisation of VES in a mixture of Tween® 80 and Triton X-100® Solutions (Mixing Order 1)

The results of preliminary studies on VES solubilisation of VES in Tween® 80 and Triton X-100® are given in **Table 24**. Samples M1, F1 and F2 have higher transmittance than that of the comparator while the %T for M2 and F3 are similar. The %T remains essentially unchanged with storage after 2 days. In the presence of 500 µg/ml of Triton X-100® and 140 µg/ml Tween® 80, a concentration of VES at 530 µg/ml will have a %T of $\leq$ that of the reference. Therefore, the VES concentration has to be set between 440 and 530 µg/ml. A repeat of M3 (M3') using a lower amount of VES of 500 µg/ml gives rise to a %T of $\geq$ 80%.

Table 24: Solubilisation of VES in a Mixture of Tween® 80 and Triton X-100® Solutions (MVB)

Sample Identity	2.5%Triton X-100® (ml)	1% Tween® 80 (ml)	2.5% VES (ml)	PBS Added (ml)	Triton X-100®:Tween® 80:VES	T% t=1day	T% t=2 days
M1	0.192	0.128	0.151	9.529	480:128:377.5	96.06	95.20
M2	0.196	0.134	0.176	9.494	490:134:440	81.87	85.21
M3	0.200	0.140	0.212	9.448	500:140:530	63.63	66.01
M3'	0.200	0.140	0.200	9.460	500:140:500	82.76	N/A
M4	0.200	0.140	0.264	9.396	500:140:660	23.21	22.50
F1	0.096	0.064	0.076	9.764	240:64:190	98.53	97.62
F2	0.096	0.064	0.076	9.914	240:64:190	99.20	98.48
F3	0.098	0.067	0.088	9.747	245:67:220	97.17	95.85
F4	0.098	0.067	0.088	9.897	245:67:220	95.57	95.35
F5	0.100	0.070	0.100	9.730	250:70:250	84.94	84.52
F6	0.100	0.070	0.100	9.880	250:70:250	84.83	84.59
F7	0.100	0.070	0.160	9.670	250:70:400	16.61	16.40
Product B.20130916	N/A	N/A	N/A	N/A	About 0:1365:533	86.68	N/A
Product B.20130915	N/A	N/A	N/A	N/A	N/A	N/A	86.19

Example 7: Solubilisation of VES in a Mixture of Tween® 80 and Triton X-100® (QIV Formulation)

In this study, specified volumes of VES Stock Solution are added to a mixture of Tween® 80 Solution (70 µg/ml) and Triton X-100® Solution (250 µg/ml) at various concentration ratios, with constant agitation and then adjusted to a final volume of 10.0 ml. Details of the study are given in **Table 28**. The appearance of

the mixture is recorded together with their transmittance (%T) measured at 580 nm against a blank PBS solution.

Results and Discussion

Solubilisation of VES in a Mixture of Tween 80 and Triton X-100 (QIV Formulation)

The results of this solubilisation study (**Table 25**) showed that all mixtures are above that of the product comparator except for F13 and F14 which are cloudy. Samples F12 are slightly lower %T than the product comparators and has similar appearance.

Table 25: Solubilisation of VES in Tween® 80 (140 µg/ml)/Triton X-100® (500 µg/ml) Mixing Order 2.

Sample Identity	2.5%Triton X-100® (ml)	1% Tween® 80 (ml)	2.5%VES (ml)	PBS Added (ml)	Triton X-100®:Tween® 80:VES	T%
F8	0.100	0.070	0.040	9.790	250:70:100	100.57
F9	0.100	0.070	0.060	9.770	250:70:150	98.48
F10	0.100	0.070	0.080	9.750	250:70:200	97.95
F11	0.100	0.070	0.100	9.730	250:70:250	89.54
F12	0.100	0.070	0.120	9.710	250:70:300	82.70
F13	0.100	0.070	0.140	9.690	250:70:350	23.90
F14	0.100	0.070	0.160	9.670	250:70:400	8.76
Product B.20130915	N/A	N/A	N/A	N/A	0:1365:533	86.19

Results of Solubilisation of VES with Tween® 80 and Triton X-100®

Pre-formulation studies established a non-linear relationship between the concentration and solution clarity as measured with % Transmittance (%T). Transmittance of mixtures of VES with surfactants is influenced by both the VES/Surfactant ratios and the concentrations of the surfactant. A certain amount of

the stabiliser is needed to stabilise the HA in a flu vaccine [6]. However, it is important to determine the amount of VES that can be solubilised while ensuring that the clarity of the flu vaccine is unaffected by the stabilisers.

At low VES concentrations (50 µg/ml), Tween® 80 at concentrations of 30-150 µg/ml are able to readily solubilise the VES present. Further increase in VES to 200 µg/ml gradually increases the difficulties in solubilising the VES eventually results in incomplete solubilisation, as indicated by the presence of white particulate matter, when the VES concentration reached 200 µg/ml. Refer to **Figure 5**.

Triton X-100® has a poorer solubilising capacity than Tween® 80. At VES concentrations between 50 and 600 µg/ml, the solublisation results are satisfactory when the VES:Triton X-100® ratio is equal or below 0.75. At VES:Triton X-100® ratios higher than 0.75, incomplete solubilisation is seen. Refer to **Figure 6**.

In the absence of bovine serum albumin, simulated monovalent formulae with concentration ratios for Tween® 80:Triton X-100®:VES of 450:140:500 and simulated QIV formulation with concentration ratios for Tween® 80: Triton X-100®:VES of 70:250:250 µg/ml are able meet the solution clarity requirements (%T > 80%) and these mixtures are able to be stored for 1 day at 5±3 ºC without obvious changes to solution appearance or measured %T. Further increase in VES to 500 µg/ml for the simulated monovalent bulk solutions results in acceptable %T (> 80%) confirming the stabiliser combination of 70:250:250 µg/ml is feasible for finished product. In the presence of Tween® 80 and Triton X-100® at 70 and 250 µg/ml respectively, any further increase in VES concentration results in %T lower than acceptable limit.

In order to prepare a relatively clear solubilised solution of VES in a combination of Tween® 80 and Triton X-100® in PBS, it is necessary to add the VES solution in a drop-wise manner with continuous agitation.

Based on the maximum VES/Surfactant ratios needed to achieve sufficient clarity (meaning the clarity of vaccine solution is no worse than the FluLaval® QIV formulation that has a high surfactant and VES content). Maximum ratios for VES/Tween® 80 and VES/Triton X-100® are 1.1 and 0.75 respectively. From these, maximum VES concentrations possible are derived for lab studies.

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Table 28: Preliminary Stabiliser Formulation for QIV Final Bulk

.	**Target Surfactant concentration** (µg/ml)	**Highest VES Solubilisation Ratio**	**VES Solubilised by Surfactant** (µg/ml)
Tween® 80	70	1.1	88.0
Triton X-100®	250	0.75	187.5
Total VES			275.5

Table 29: Preliminary Stabiliser Formulation for Monovalent Bulk

	Target Surfactant concentration Chosen (µg/ml)	**Highest VES Solubilisation Ratio**	**VES Solubilised by Surfactant (µg/ml)**
Tween® 80	140*	1.1	176.0
Triton X-100®	500*	0.75	375.0
Total VES			551.0*

**Based on 2x concentration in QIV final bulk.*

Further production-scale process feasibility studies (**Tables 24** to **26**) identified difficulties to solubilise more than 500 µg/ml VES in a stabiliser mixture of 140 µg/ml Tween® 80 and 500 µg/ml Triton X-100® without reducing %T below 80%. These studies lead to adjustment of the maximum VES content that can be

solubilised 450 µg/ml while keeping the Tween® 80 (140 µg/ml) and Triton X-100® (500 µg/ml) content at the same level. Further, fine-tuning of the processing method leads to the best results for %T when the VES concentration is set at 450 µg/ml. This can be further diluted to prepare final bulk formulations. The proposed formulations for monovalent bulks and QIV final bulks for process trials are given in **Tables 30** & **31** respectively.

Table 30: Final Formulae Proposed for Monovalent Bulk Formulation

Formula	**VES** (µg/ml)	**Tween® 80** (µg/ml)	**Triton X-100®** (µg/ml)	**Comment**
A	0	0	0	Negative Control (Stabiliser Free)
B	600	1,130	0	Positive Control (FluLaval® QIV Formulation)
C	350	140	500	Low VES Concentration
D	400	140	500	Medium VES Concentration
E	450	140	500	High VES Concentration

Table 31: Final Formulae Proposed for Final Bulk QIV Formulation

Formula	VES (µg/ml)	Tween® 80 (µg/ml)	Triton X-100® (µg/ml)	Comment
A	0	0	0	Negative Control (Stabiliser Free)
B	533	1,366	0	Positive Control (FluLaval® QIV Formulation)
C	180	70	250	Low VES Concentration
D	200	70	250	Medium VES Concentration
E	225	70	250	High VES Concentration

Conclusions

Solubilisation of VES in Tween® 80 and Triton X-100® Solutions

Solubilisation studies demonstrate that Tween® 80 has a higher solubilising property when compared with Triton X-100® with a solubilising ratio between VES and surfactant of 1.1 and 0.75 respectively. VES present in concentration higher than this ratio will result in cloudiness in the solution.

Example 8: Effects of Rate of Addition of VES Solution to Tween® 80 Solution

Using the stabiliser concentrations for the original MVB (**Table 29**), a study is performed to evaluate the impact of adding the VES in one shot or at a gradual rate with intermittent mixing on the final solution transmittance (%T). The results are compared with the reference standard – monovalent bulk (B.12049B) and finished product (B.20130911).

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Results and Discussion

Effects of Rate of Addition of VES Solution to Tween 80 Solution

The appearance and %T for different mixtures following drop-wise or a single-shot addition of the VES are recorded in **Table 26**. The appearance of the mixtures following one-shot addition of VES into Tween 80 solution is consistently more cloudy and higher %T than those following gradual addition of VES.

Table 26: Effects of Rate of Addition of Ethanolic VES Solution to Tween 80 on Transmittance

Sample Identity	1%Tween® 80 (ml)	2.5%VES (ml)	Rate of Addition	Added PBS (ml)	Concentra-tion ratio	VES:Tween® 80	% T
12049B-B	1.113	0.228	One shot	8.659	570:1,113	0.512	42.58
12049B-B'	1.113	0.228	Drop-wise	8.659	570:1,113	0.512	88.13
Monovalent Bulk B.12049B*	N/A	N/A	N/A	10.0	570:1,113	0.512	72.95
Product B.20130911 *	N/A	N/A	N/A	10.0	533:1,365	0.390	87.88

** Both B.12049B (monovalent bulk) and B.20130911 (QIV product) are used as benchmark references for appearance and %T, as their appearance is deemed satisfactory.*

Example 9: Effects of Bovine Serum Albumin on the Stabiliser Mixture

Using the blank stabiliser mixture for the MVB (VES:Tween® 80 of 533:1,360), a study is performed to evaluate any potential change to the % Transmittance of the solution with addition of bovine serum albumin (BSA). A concentration of 450 µg/ml of BSA is chosen to simulate total protein concentration at

4.5 times the amount of HA in a worst case scenario for total protein concentration. The %T of the stabiliser mixture containing added BSA is compared with that of a standard monovalent bulk solution and a blank stabiliser mixture. Refer to details in **Table 30**.

Results and Discussion

Effects of Bovine Serum Albumin on the Stabiliser Mixture

The appearance and %T for mixtures of VES/Tween® 80 following addition of Bovine Serum Albumin (BSA) are recorded in **Table 27**. The %T for BSA-containing and BSA-free stabiliser mixture are 81.1% and 72.91% respectively. There is some apparent increase to the %T with BSA.

Table 27: Effects of Bovine Serum Albumin on Transmittance of Stabiliser Mixture

Sample Identity	1% Tween® 80 (ml)	2.5% VES (ml)	Rate of Addition	Added PBS (ml)	Bovine Serum Albumin Added (µg/ml)	Concentra-tion ratio	VES: Tween® 80	% T
B.12049B-B'	1.113	0.228	Drop-wise	8.659	0	540:1,365	0.396	79.21
B.12049B-B'-BSA	1.113	0.228	Drop-wise	8.659	450	540:1,365	0.396	81.10

Example 10: Stabiliser System used for Manufacturing of Monovalent Bulks

Pre-formulation development study described above establishes a series of formulae with reduced Tween® 80 content in the presence of Triton X-100® and VES. Positive (Formula B, FluLaval® monovalent bulk) and negative controls (Formula A, stabiliser-free) are included in this study. Monovalent bulks of each virus

strain are manufactured in each of the formulae below (**Table 8**) against established specifications (**Table 9**).

Table 8 Target Stabiliser Concentrations for Monovalent Bulks

Formula	VES (µg/ml)	Tween® 80 (µg/ml)	Triton X-100® (µg/ml)	Comment
A	0	0	0	Negative Control (Stabiliser-free)
B	600	1,130	0	Positive Control (based on FluLaval® monovalent stabiliser system)
C	350	140	500	Low VES content
D, D1	400	140	500	Medium VES content
E	450	140	500	High VES

Table 9: Release Specifications for Monovalent Bulks

Formula	Test								
	HA Content				Total Protein (by Lowry) (µg/ml)	VES Content (µg/ml)	Tween® 80 Content (µg/ml)	Triton X-100® Content (µg/ml)	Sterility
	H1N1 A/California-179A; A/Christchurch-74xp (µg/ml)	H3N2 A/Texas (µg/ml)	B/Massachusetts (µg/ml)	B/Brisbane (µg/ml)					
A	≥120	≥120	≥120	≥120	N/A	N/A	N/A	N/A	No growth
B	≥120	≥120	≥120	≥120	N/A	480-720	904-1,356	N/A	No growth
C	≥120	≥120	≥120	≥120	400-600	280-420	112-168	400-600	No growth
D, D1	≥120	≥120	≥120	≥120	400-600	320-480	112-168	400-600	No growth
E	≥120	≥120	≥120	≥120	400-600	360-540	112-168	400-600	No growth

Manufacturing of Monovalent Bulks in Various Formulations

The process used for the manufacture of monovalent bulks is given in **Figure 1**.

Buffer Preparation

Solutions containing 1% Tween® 80 (B. RD-B-20131208), 5% Tween® 80 (B. RD-B-20131215) and 2.5% Triton X-100® (B. RD-B-20131210) are prepared using phosphate-buffered saline (PBS) solution (B. RD-B-20131203). The 2.6% VES

(B. 13127B) is prepared using 95% ethanol. Comprehensive buffer preparation records are included in Laboratory Book-Secondary 02.

Monovalent Bulk Preparation

Working seeds are prepared in 2012 and 2013. Refer to manufacturing batch records for the following seed passages.

- P/A/TX/P3/13011 (A/Texas/50/2012 NYMC X-223A (H3N2));
- P/A/CL/P3/12026 (A/California/7/2009 NYMC X-179A (H1N1-179A));
- P/B/MC/P3/13012 (B/Massachusetts/2/2012 NYMC BX-51B (B-YMG));
- P/B/BX/P3/13026 (B/Brisbane/60/2008 NYMC BX-35 (B-VIC));
- P/A/CC/P2/13018 (A/Christchurch/16/2010 NIB-74xp (H1N1-74xp)).

The method employed for the production of monovalent bulks used in this formulation study is based on the process for monovalent bulk production as described in manufacturing instructions: FSOP0600B03, FSOP0600B04, FSOP0600B05, FSOP0600B06, FSOP0600B07 and FSOP0600B09. These instructions are based on those developed for GSK FluLaval® Quadrivalent. For the purpose of this formulation study, the addition of stabiliser post-homogenization is omitted. Mixtures of stabilisers are added to a 1 L volume of the various pre-sterile filtered bulks in 2 L bottles in accordance with pre-set formulation components requirements. Refer to **Figure 1** for schematic process flow chart.

Addition of Stabilizers

Stabilizer mixtures are prepared according to **Figure 2** below. For each stabiliser mixture, twice the amount of needed volumes are prepared in 500 ml glass bottles, in accordance with the required quantities listed in **Table 10**. This is followed by the addition of VES stock solution into the same bottle. The stabilizer mixtures are continuously stirred in the bottle using a magnetic stirrer. Stabiliser mixtures in required volumes as defined in **Table 11** are added into the 1 L monovalent bulks respectively. See **Figure 2** for detailed processing steps.

Table 10: Stabilizer Mixture Compositions

Stabiliser Mixture	VES 2.6% Stock Solutions (ml)	Tween® 80 1% Stock Solutions (ml)	Tween® 80 5% Stock Solutions (ml)	Triton X-100® 2.5% Stock Solutions (ml)	Volume of Final Stabiliser Mixture (ml)
SM-B	60.0	0.0	60.0	0.0	120.0
SM-C	27.6	29.4	0.0	42.0	99.0
SM-D	31.6	29.4	0.0	42.0	103.0
SM-E	35.8	29.6	0.0	42.2	107.6

Table 11: Volume of Stabilizer Mixture Added to 1L Sterile Filtered Monovalent Bulk

Formulated MVB	Monovalent bulk (ml)	Stabiliser Mixture	Volume of Stabilizer Mixture added to Monovalent Bulk (ml)	Final Formulated Monovalent Bulk (ml)
MVB in Formula A	1,000.0	N/A	N/A	1,000.0
MVB in Formula B	1,000.0	SM-B	47.4	1,047.4
MVB in Formula C	1,000.0	SM-C	49.5	1,049.5
MVB in Formula D	1,000.0	SM-D	51.5	1,051.5
MVB in Formula E	1,000.0	SM-E	53.8	1,053.8

Sampling

After the addition of stabilizers to the monovalent bulk, it is continuously stirred for 15 minute before a sample of 310 ml is obtained for release testing. The samples are tested against limits included in **Table 9**.

Stability Testing Programs for Monovalent Bulks

A total of 31 batches of monovalent bulks are manufactured from the 5 different viral strains (H1N1/California, H1N1/Christchurch, H3N2/Texas, B/Massachusetts and B/Brisbane) in accordance with the different formulae. The contents for HA, VES, Tween® 80 and Triton X-100® in each monovalent bulk are listed in **Table 32**.

Each batch of monovalent bulk is filled into a plastic bottle. They are placed under 30±2 ^{0}C for up to 4 weeks (accelerated conditions) and at 5±3 ^{0}C (real time conditions) for up to 12 months within constant temperature stability chambers or validated stability rooms. Details are included in **Tables 12**. Samples are withdrawn periodically at the designated time points throughout the stability testing program for the following tests: Product Appearance, HA Content, VES Content and sterility in accordance with established stability testing programs. Stability monitoring of Tween® 80 performed during earlier stability studies on trivalent split-virion seasonal influenza products has confirmed that it is stable in the PBS formulation. The contents of Triton X-100® in the MVB are reported as "quantified by input". Monovalent bulks stored under real time conditions of 5±3 ^{0}C are required to meet the End-of Life Specifications (**Table 13**).

Table 12: Stability Testing Programs for MVBs

	Stability Testing Program 1	**Stability Testing Program 2**
Viral Strains	A/California H1N1-179A, A/Texas H3N2, B/Massachusetts (YMG) and B/Brisbane (VIC) strains.	A/California H1N1-179A, A/Christchurch H1N1 -74xp,

Batches	2013FS01-A, B, C, D, E (A/Texas H3N2); 2013FS02-A, B, C, D, E (A/California H1N1-179A H1N1); 2013FS03-A, B, C, D, E (B/Massachusetts-Yamagata); 2013FS04-A, B, C, D, E (B/Brisbane-Victoria).	2014FS01-A, B, C, D, E, D-1 (A/California H1N1-179A); 2014FS02-A, B, C, D, E (A/Christchurch H1N1-74xp)
Temperature Conditions/Time Points/Tests	5 ± 3 ^{0}C at 0, 1, 2, 3, 6, 9 and 12 months. HA Content for 4 strains and VES Contents are only tested at 0, 1, 2, 3, 6, 9, and 12 months. All tests are performed at 0 and 12 months. 30 ± 2 ^{0}C at 0, 1, 2, 3 and 4 weeks. All tests except sterility are performed for all test points.	5 ± 3 ^{0}C at 0, 1, 2, 3, 6, 9 and 12 months. Only HA Content for A/California H1N1-179A or A/Christchurch H1N1-74xp strain, VES Content are tested at 0, 1, 2, 3 and 12 months; All tests are performed at 0 and 12 months. 30 ± 2 ^{0}C at 0, 1, 2, 3 and 4 weeks. All tests except sterility are performed for all test points.
Date of commencement	2013FS01-A, B, C, D, E (A/Texas H3N2) - 02.Jan.2014 2013FS02-A, B, C, D, E (A/California H1N1-179A) – 05.Jan.2014 2013FS03-A, B, C, D, E (B/Massachusetts-Yamagata) – 08.Jan.2014 2013FS04-A, B, C, D, E (B/Brisbane-Victoria) –	2014FS01 (A/California H1N1-179A) – 12.Mar.2014 2014FS02 (A/Christchurch H1N1-74xp) – 19.Mar.2014

	15.Jan.2014	
Comment	Provides full stability data for MVBs in different formulae.	Provides full stability data HA Content for all formulae for A/California H1N1-179A and A/Christchurch H1N1-74xp strains.

Table 13: End-of-Life Specifications for Monovalent Bulks

Formula	Test					
	HA Content				VES Content (µg/ml)	Sterility (No growth)
	H1N1 A/California-179A; A/Christchurch-74xp (µg/ml)	H3N2 A/Texas (µg/ml)	B/Massachusetts (µg/ml)	B/Brisbane (µg/ml)		
A	≥120	≥120	≥120	≥120	N/A	No growth
B	≥120	≥120	≥120	≥120	480-720	No growth
C	≥120	≥120	≥120	≥120	280-420	No growth
D, D1	≥120	≥120	≥120	≥120	320-480	No growth
E	≥120	≥120	≥120	≥120	360-540	No growth

Results and Discussion - *Monovalent Bulks in Different Formulae*

Manufacturing of Monovalent Bulks

The batch sizes for eggs are typical for normal production scale. The monovalent bulk manufacturing process for formulation study is identical with existing flu vaccine monovalent manufacturing process except that the stabilizer addition is shifted from after homogenization to after sterile filtration.

A total of 31 lots of monovalent bulks are manufactured in different stabiliser formulae as defined in **Table 14**. Two H1N1 seeds, A/California/7/2009 NYMC X-179A and A/Christchurch/16/2010 NIB-74xp, are used to prepare monovalent bulks in different formulae. Refer to **Table 32** for detailed information on manufacturing dates, stabiliser addition times and the quantities of each batch of monovalent bulk

produced. Stabiliser addition times for stabiliser addition are different for each monovalent bulk manufactured as these lots are produced in a sequential manner. Time lapse from the end of sterile filtered bulks to addition of stabilisers are monitored. Typically, it is between 110 and 255 minutes. One batch with Formula B (2013FS01-B) has stabilisers added after 410 minutes due to some difficulties encountered during the preparation of the stabilisers as it is the first formula manufactured. The impact of varying time lapses between end of sterile filtration and stabiliser addition is unknown but it is expected to have some negative influence on the stability of the HA antigen resulting in possibly in a slightly lowered final HA content results in the purified bulks. For future full-scale manufacturing process for monovalent bulks, there will be no major delays for stabilizers addition. The stabilizers will be added directly into the sterile filtered monovalent bulks immediately after homogenization as for the stabiliser addition in the current QIV manufacturing process.

Routine IPC (in-process control) checks are performed during the manufacturing process of the monovalent bulks. Refer to **Table 33** for IPC data for upstream process (Incubation rates, Egg Surface Bioburden (bacterial, mould and yeasts) at Pre-Incubation, Egg Reject Rates at Candling steps, Bioburden after UV step and Total Protein Content after Inactivation step). All IPC results for incubation rates, bioburden limits and egg reject rate are within acceptable limits. The Total Proteins Content varies from 2,600 to 10,000 µg/ml for after inactivation with batches 2014FS01 (range of between 2,646 – 2,873 µg/ml) and 2014FS02 (range of between 2,906 – 3,224 µg/ml) having lower than the 3,000 µg/ml limit set.

IPC data for downstream processes (**Table 33**) include Sucrose Content at the Isopycnic Centrifugation step, Sucrose Content at the Sucrose Removal step, Transmittance at the Removal of the Splitting Agent, sodium deoxycholate (DOC) and Bioburden after Homogenization step. All the results for downstream IPC tests are within limits and are generally comparable with normal IPC results. These data show that the manufacturing of all batches of monovalent bulks (prior to stabiliser addition) are typical.

Table 32: Monovalent Bulk Manufactured

Batch No.	Manufacture Date	Delay in Stabilizer Addition (min)	Volume (ml)	Working Seed
2013FS01-A	24 Dec 2013	0	690.0	H3N2 A/Texas/50/2012 NYMC X-223A (H3N2)
2013FS01-B		410	737.4	
2013FS01-C		122	739.5	
2013FS01-D		133	741.5	
2013FS01-E		171	743.8	
2013FS02-A	28 Dec 2013	0	690.0	H1N1 A/California/7/2009 NYMC X-179A (H1N1-179A)
2013FS02-B		117	737.4	
2013FS02-C		146	739.5	
2013FS02-D		157	741.5	
2013FS02-E		171	743.8	
2013FS03-A	01 Jan 2014	0	690.0	B B/Massachusetts/2/2012 NYMC BX-51B (B-YMG)
2013FS03-B		219	737.4	
2013FS03-C		229	739.5	
2013FS03-D		241	741.5	
2013FS03-E		255	743.8	
2013FS04-A	04 Jan 2014	0	690.0	B B/Brisbane/60/2008 NYMC BX-35 (B-VIC)
2013FS04-B		213	737.4	
2013FS04-C		219	739.5	
2013FS04-D		227	741.5	
2013FS04-E		235	743.8	
2014FS01-A	27 Feb 2014	0	690.0	H1N1 A/California/7/2009 NYMC X-179A (H1N1-179A)
2014FS01-B		110	737.4	
2014FS01-C		146	739.5	
2014FS01-D		157	616.5	

2014FS01-E		170	743.8	
2014FS01-D-1		129	616.5	
2014FS02-A	10 Mar 2014	0	690.0	H1N1 A/Christchurch/16/2010 NIB-74xp (H1N1-74xp)
2014FS02-B		202	737.4	
2014FS02-C		216	739.5	
2014FS02-D		228	741.5	
2014FS02-E		242	743.8	

Table 33: IPC results (with control limits) for monovalent bulks prior to stabilizer addition

Batch No.	Upstream Process						Downstream Process			
	Pre-Incubation (Incubation rate ≥80%)	**Egg shell *(Bacteria≤10^{5}CFU/egg)***	**Egg Surface *(Mould and Yeast ≤10^{3}CFU/egg)***	**Candling (Egg reject rate< 20%)**	**After UV *(Bioburden <10^{5}CFU/ml)***	**After Inactivation *(Total protein 3000-10000 µg/ml)***	**Isopycnic Centrifugation (Sucrose content 36.5% - 50.4%)**	**Sucrose Removal (Sucrose content <2%)**	**DOC Removal (Transmittance ≥90 %)**	**After homogenization *(Bioburden <10 CFU/ml)***
2013 FS01	95 %	1,300	4	6%	<10	3,928	38.1%-49.2%	1%	100%	<1
					<10	4,028				
					<10	4,154				
2013 FS02	95%	11,000	20	7%	<10	3,618	37.8%-50.1%	1%	103%	1
					<10	4,028				
					<10	3,995				
2013 FS03	95%	660	140	4%	<10	3,417	39.3%-49.3%	1%	98%	<1
					<10	3,492				
					<10	3,384				
2013 FS04	96%	2,600	20	4%	20	3,509	39.4%-49.4%	1%	102%	<1
					30	3,501				
					<10	3,325				
2014 FS01	95%	8,000	20	3%	<10	2,646	38.3%-49.3%	1%	106%	<1
					<10	2,873				
					<10	2,772				
2014 FS01	95%	1,000	13	3%	<10	2,906	36.6%-49.6%	1%	98%	<1
					<10	3,057				
					<10	3,224				

Monovalent Bulk Batch Analysis Data (with Specification Limits)

A total of 31 batches of monovalent bulks are manufactured from each of the 5 viral strains. Each monovalent bulk is stabilised with a different combination of stabilisers. Formula A does not contain any stabiliser. Formula B contains the same amount of Tween® 80 and VES as in the FluLaval® Quadrivalent formula.

After addition of the stabilizer mixtures to each monovalent bulk, several samples are taken for monovalent bulk release testing. Batch quantities of monovalent bulks of various formulae produced are between 616 and 771 ml. Variability between batch quantity produced is due to different volumes of stabiliser mixtures added to a fixed quantity of monovalent bulk (1 L) prior to addition of stabilisers. Refer to the **Table 34** for all test data reported against respective specifications for each lot of monovalent bulk. Tween® 80 concentrations in these monovalent bulks are quantified by inputs.

Due to presence of interference with the Chinese Pharmacopoeia (Ch.P.) analytical test methods for Tween® 80 in the presence of Triton X-100®, data for Tween® 80 and Triton X-100® are provided as "quantified by inputs" or actual quantities added to the formulae. Since the limits for these stabilisers are 20% around the target stabiliser concentrations, these targets are expected to be achievable given the small-scale trial operations with more accurate controls. Degradation of two surface active agents Tween® 80 and Triton X-100® is not expected to be significant. All the VES Contents for those formulae containing this stabiliser are measured by HPLC against a specification limit of target ± 20%.

Except for the HA content of 2 batches of A/California H1N1-179A containing no stabiliser (Formula A: 2014FS01-A and 2013FS02-A) where their HA contents are below specification of 120 µg/ml, all lots of monovalent bulks containing different stabilizer combinations show that they are all within acceptable ranges for Appearance, VES Content, HA Content, Triton X-100® Content and Total Protein Content. Sterility tests conducted on all these batches show absence of microbial contaminations.

The batch analysis data for HA Content in monovalent bulks produced from A/Christchurch/16/2010 NIB-74xp (A/Christchurch H1N1-74xp) are all above 300 µg/ml when compared with another A/California H1N1-179A strain that gives typically

very low contents for HA antigen. Monovalent bulks that are low in HA content are not considered practical for routine production. Further, monovalent bulk lots for A/California H1N1-179A are associated with the formation of precipitate sediments under accelerated conditions. A repeated manufacturing campaign for A/California H1N1-179A viral strain also gives rise to low HA Content. From these results, it can be seen that the low HA Content is consistently seen with the A/California H1N1-179A viral strains but not for the A/Christchurch H1N1-74xp strain. This difference is likely to be due to the nature of different A/H1N1 strains. The A/Christchurch H1N1-74xp viral strain is therefore recommended for future manufacture of A/H1N1 monovalent bulks. It is recommended that further studies be conducted to evaluate the reason for the difference between the H1N1 seeds.

HA Contents for all the negative control batches (monovalent bulks with Formula A) are typically 15-30% lower than the positive control batches (monovalent bulks with Formula B). Based on these comparisons, there is clear evidence that stabilizers are needed for monovalent bulks.

HA Content for monovalent bulks manufactured from A/Texas H3N2, B/Massachusetts YMG and B/Brisbane VIC strains with reduced stabilisers are better than negative control (Formula A) and are comparable to positive controls (Formula B). The HA Contents achieved for these strains are comparable to past experience with monovalent bulks manufactured between 2011 and 2013. These data demonstrate that despite having reduced stabiliser concentrations, comparable HA stabilisation effects of HA antigen in monovalent bulks can be achieved.

All 31 lots are subject to stability testing in accordance with approved stability testing protocols and they are also used for the formulation of QIV Final Bulks and then filled into pre-filled syringes.

Table 34: Monovalent Bulk Batch Analysis Data (with Specification Limits)

Batch No.	Test Items									
	HA Content					Total Protein (by Lowry) (µg/ml)	Tween 80 Content* (µg/ml)	VES Content (µg/ml)	Triton X-100 Content (µg/ml)	Sterility (No growth)
	H1N1-California-179A (µg/ml)	H1N1-Christchurch-74XP (µg/ml)	H3N2 A/Texas (µg/ml)	B/Massachu-setts (YMG) (µg/ml)	B/Bris-bane (VIC) (µg/ml)					
2013FS01-A	N/A	N/A	293	N/A	N/A	1,091	N/A	N/A	N/A	Complies
2013FS01-B	N/A	N/A	431	N/A	N/A	1,172	1,130	624	N/A	Complies
2013FS01-C	N/A	N/A	457	N/A	N/A	1,155	140	360	510	Complies
2013FS01-D	N/A	N/A	455	N/A	N/A	1,184	140	412	503	Complies
2013FS01-E	N/A	N/A	450	N/A	N/A	1,195	140	459	489	Complies
2013FS02-A	113	N/A	N/A	N/A	N/A	169	N/A	N/A	N/A	Complies
2013FS02-B	133	N/A	N/A	N/A	N/A	233	1,130	626	N/A	Complies
2013FS02-C	132	N/A	N/A	N/A	N/A	234	140	366	556	Complies
2013FS02-D	135	N/A	N/A	N/A	N/A	232	140	416	552	Complies

2013FS02-E	132	N/A	N/A	N/A	N/A	231	140	469	546	Complies
2013FS03-A	N/A	N/A	N/A	332	N/A	1,364	N/A	N/A	N/A	Complies
2013FS03-B	N/A	N/A	N/A	406	N/A	1,483	1,130	623	N/A	Complies
2013FS03-C	N/A	N/A	N/A	424	N/A	1,536	140	367	552	Complies
2013FS03-D	N/A	N/A	N/A	426	N/A	1,508	140	428	566	Complies
2013FS03-E	N/A	N/A	N/A	415	N/A	1,510	140	471	525	Complies
2013FS04-A	N/A	N/A	N/A	N/A	442	1,611	N/A	N/A	N/A	Complies
2013FS04-B	N/A	N/A	N/A	N/A	496	1,689	1,130	616	N/A	Complies
2013FS04-C	N/A	N/A	N/A	N/A	502	1,726	140	365	543	Complies
2013FS04-D	N/A	N/A	N/A	N/A	498	1,739	140	415	522	Complies
2013FS04-E	N/A	N/A	N/A	N/A	507	1,733	140	468	512	Complies
2014FS01-A	102	N/A	N/A	N/A	N/A	239	N/A	N/A	N/A	Complies
2014FS01-B	120	N/A	N/A	N/A	N/A	297	1,130	653	N/A	Complies
2014FS01-C	123	N/A	N/A	N/A	N/A	294	140	372	500*	Complies
2014FS01-D	122	N/A	N/A	N/A	N/A	292	140	428	500*	Complies

2014FS01-E	122	N/A	N/A	N/A	N/A	291	140	433	500*	Complies
2014FS01-D-1	122	N/A	N/A	N/A	N/A	291	140	484	500*	Complies
2014FS02-A	N/A	282	N/A	N/A	N/A	752	N/A	N/A	N/A	Complies
2014FS02-B	N/A	331	N/A	N/A	N/A	830	1,130	637	N/A	Complies
2014FS02-C	N/A	338	N/A	N/A	N/A	832	140	366	500*	Complies
2014FS02-D	N/A	337	N/A	N/A	N/A	823	140	422	500*	Complies
2014FS02-E	N/A	335	N/A	N/A	N/A	826	140	481	500*	Complies

** Quantified by input"*

Conclusions

Proposed Stabiliser System for Monovalent Bulks and QIV Final Bulks

Separate studies at realistic process scale on solubilisation of VES in both Tween® 80 and Triton X-100® lead to a series of proposed formulations for monovalent bulks with the highest possible VES content of 450 µg/ml in the presence of 140 µg/ml Tween® 80 and 500 µg/ml Triton X-100® without causing any visible increase in cloudiness in the product. Medium and Low VES content levels are set based on 20% from the High VES content and 20% from the Medium VES content respectively. Accordingly, a 1:2 dilution of these formulae is proposed for final QIV bulk. Thus, low, medium and high VES formulae are proposed for manufacturing process trials for both monovalent and final bulks.

Monovalent Bulks Manufactured

A total of 31 batches of monovalent bulks from 5 virus strains (A/California H1N1-179A, A/Christchurch H1N1-74xp, A/Texas H3N2, B/Massachusetts - YMG and

B/Brisbane-VIC) have been successful manufactured using existing manufacturing processes for flu vaccines monovalent bulks with minor adaptations for the addition of stabiliser mixtures.

All monovalent bulks manufactured are within specifications for HA content, except for monovalent batches for A/California H1N1-179A strain, total protein, stabiliser contents and sterility. Monovalent bulks manufactured from A/California H1N1-179A have HA Contents that are much lower than others (close to 120 µg/ml). All monovalent bulks pass sterility tests at release.

Batch analysis data for monovalent bulks confirm that stabilizers are useful in ensuring stability of HA as HA Contents for negative controls batches (Formula A) are lower than all the other batches with added stabilizers. Therefore, stabilizers are necessary excipients for the stabilisation of monovalent bulks. HA Contents for all formulae with reduced amount of Tween® 80 (140 µg/ml) present in the presence of VES (350-450 µg/ml) and Triton X-100® (500 µg/ml) are comparable with the FluLaval® Quadrivalent formula (Formula B, positive control) batches where the amount of Tween® 80 present is 1,130 µg/ml (with 600 µg/ml VES).

The monovalent bulks manufactured with reduced Tween® 80 contents are suitable for in QIV formulation and conduct of long term and accelerated stability studies.

Results and Discussion - *Stability Testing Program for Monovalent Bulks in Various Formulae*

Stability Data for MVBs at 5±3 ^{0}C and 30±2 ^{0}C

Stability data for Appearance of all MVBs at 5±3 ^{0}C and 30±2 ^{0}C are included in **Tables 38** and **39** respectively. Stability data for HA Content of MBVs (B. 2013FS01-04, 2014FS01 & 2014FS02) at 5±3 ^{0}C and 30±2 ^{0}C are included in **Tables 40** to **51** and **Figures 7** to **18**. Stability data for VES Content of MVBs (B. 2013FS01-X to 04-X, 2014FS01-X and 2014FS02-X) at 5±3 ^{0}C and 30±2 ^{0}C are included in **Tables 52** to **63** and **Figures 19** to **30.** Data for Sterility test on all MVBs at 5±3 ^{0}C are included in **Table 64**. Sterility test is not performed for 30±2 ^{0}C.

Table 38 : Appearance for MVBs at 5±3 ^{0}C

Appearance for MVBs at 5±3 ^{0}C				**Spec: Colourless Light Opalescent Liquid**			
Batch No.	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS01-A (A/Texas H3N2)	Complies	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment
2013FS01-B (A/Texas H3N2)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS01-C (A/Texas H3N2)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS01-D (A/Texas H3N2)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS01-E (A/Texas H3N2)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS02-A (A/California H1N1-179A)	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment
2013FS02-B (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Complies	Complies	Complies

Table 38 (Continued) : Appearance for MVBs at 5±3 ^{0}C

Appearance for MVBs at 5±3 ^{0}C				**Spec: Colourless Light Opalescent Liquid**			
Batch No.	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS02-C (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS02-D (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS02-E (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS03-A (B/Massachusetts YMG)	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment
2013FS03-B (B/Massachusetts YMG)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS03-C (B/Massachusetts YMG)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS03-D (B/Massachusetts YMG)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS03-E (B/Massachusetts YMG)	Complies	Complies	Complies	Complies	Complies	Complies	Complies

2013FS04-A (B/Brisbane VIC)	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment
2013FS04-B (B/Brisbane VIC)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS04-C (B/Brisbane VIC)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS04-D (B/Brisbane VIC)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2013FS04-E (B/Brisbane VIC)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2014FS01-A (A/California H1N1-179A)	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment
2014FS01-B (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2014FS01-C (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Complies	Complies	Complies

2014FS01-D (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2014FS01-D1 (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2014FS01-E (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2014FS02-A (A/Christchurch H1N1-74xp)	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated	Small amount of flocculated
2014FS02-B (A/Christchurch H1N1-74xp)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2014FS02-C (A/Christchurch H1N1-74xp)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2014FS02-D (A/Christchurch H1N1-74xp)	Complies	Complies	Complies	Complies	Complies	Complies	Complies
2014FS02-E (A/Christchurch H1N1-74xp)	Complies	Complies	Complies	Complies	Complies	Complies	Complies

Table 39: Appearance for MVBs at 30±2 °C

Appearance for MVBs at 30±2 °C Liquid			**Spec: Colourless Light Opalescent**		
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2013FS01-A (A/Texas H3N2)	Complies	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment
2013FS01-B (A/Texas H3N2)	Complies	Complies	Complies	Complies	Complies
2013FS01-C (A/Texas H3N2)	Complies	Complies	Complies	Complies	Complies
2013FS01-D (A/Texas H3N2)	Complies	Complies	Complies	Complies	Complies
2013FS01-E (A/Texas H3N2)	Complies	Complies	Complies	Complies	Complies
2013FS02-A (A/California H1N1-179A)	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Cancelled
2013FS02-B (A/California H1N1-179A)	Complies	Complies	Complies	Complies	Cancelled
2013FS02-C (A/California H1N1-179A)	Complies	Large amount of flocculated sediment	Large amount of flocculated sediment	Large amount of flocculated sediment	Cancelled
2013FS02-D (A/California H1N1-179A)	Complies	Large amount of flocculated sediment	Large amount of flocculated sediment	Large amount of flocculated sediment	Cancelled
2013FS02-E (A/California H1N1-179A)	Complies	Large amount of flocculated sediment	Large amount of flocculated sediment	Large amount of flocculated sediment	Cancelled

2013FS03-A (B/Massachusetts YMG)	Complies	Complies	Complies	Complies	Cancelled
2013FS03-B (B/Massachusetts YMG)	Complies	Complies	Complies	Complies	Complies
2013FS03-C (B/Massachusetts YMG)	Complies	Complies	Complies	Complies	Complies
2013FS03-D (B/Massachusetts YMG)	Complies	Complies	Complies	Complies	Complies
2013FS03-E (B/Massachusetts YMG)	Complies	Complies	Complies	Complies	Complies
2013FS04-A (B/Brisbane VIC)	Complies	Complies	Small amount of flocculated sediment	Cancelled	Cancelled
2013FS04-B (B/Brisbane VIC)	Complies	Complies	Complies	Complies	Complies
2013FS04-C (B/Brisbane VIC)	Complies	Complies	Complies	Complies	Complies
2013FS04-D (B/Brisbane VIC)	Complies	Complies	Complies	Complies	Complies
2013FS04-E (B/Brisbane VIC)	Complies	Complies	Complies	Complies	Complies
2014FS01-A (A/California H1N1-179A)	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment
2014FS01-B	Complies	Complies	Complies	Complies	Complies

(A/California H1N1-179A)					
2014FS01-C (A/California H1N1-179A)	Complies	Small amount of aggregates	Large amount of aggregates	Cancelled	Cancelled
2014FS01-D (A/California H1N1-179A)	Complies	Small amount of aggregates	Large amount of aggregates	Cancelled	Cancelled
2014FS01-D-1 (A/California H1N1-179A)	Complies	Small amount of aggregates	Large amount of aggregates	Cancelled	Cancelled
2014FS01-E (A/California H1N1-179A)	Complies	Small amount of aggregates	Large amount of aggregates	Cancelled	Cancelled
2014FS02-A (A/California H1N1-74xp)	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Cancelled	Cancelled
2014FS02-B (A/Christchurch H1N1-74xp)	Complies	Complies	Complies	Cancelled	Cancelled
2014FS02-C (A/Christchurch H1N1-74xp)	Complies	Complies	Small amount of flocculated sediment	Cancelled	Cancelled
2014FS02-D (A/Christchurch H1N1-74xp)	Complies	Complies	Small amount of flocculated sediment	Cancelled	Cancelled
2014FS02-E (A/Christchurch H1N1-74xp)	Complies	Complies	Small amount of flocculated sediment	Cancelled	Cancelled

Table 40 : HA Content (A/Texas H3N2) in MVBs (µg/ml) at 5±3 °C

HA Content (A/Texas H3N2) at 5±3 °C					**Spec: ≥120 µg/ml**		
Batch No.	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS01-A	293	352	321	348	358	320	292
2013FS01-B	431	417	412	405	438	385	379
2013FS01-C	457	414	407	411	442	385	373
2013FS01-D	455	411	413	413	444	384	369
2013FS01-E	450	402	403	419	439	383	370

Table 41 : HA Content (A/Texas H3N2) in MVBs (µg/ml) at 30±2 °C

HA Content (A/Texas H3N2) at 30±2 °C					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2013FS01-A	293	351	299	258	243
2013FS01-B	431	410	407	360	390
2013FS01-C	457	403	402	357	375
2013FS01-D	455	404	405	362	375
2013FS01-E	450	410	402	356	373

Table 42 : HA Content (A/California H1N1-179A) in MVBs (µg/ml) at 5±3 °C

HA Content (H1N1-179A) at 5±3 °C					**Spec: ≥120 µg/ml**		
Batch No.	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS02-A	113	105	95	100	103	88	85
2013FS02-B	133	127	115	122	122	110	114
2013FS02-C	132	127	117	122	123	109	112
2013FS02-D	135	127	116	124	123	109	113
2013FS02-E	132	126	117	124	123	110	113

Table 43 : HA Content (A/California H1N1-179A) in MVBs (µg/ml) at 5±3 ºC

HA Content (A/California H1N1-179A) at 5±3 ºC Spec: ≥120 µg/ml							
Batch No.	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2014FS01-A	102	95	91	90	90	82	84
2014FS01-B	120	120	115	115	119	110	112
2014FS01-C	123	122	116	116	123	113	114
2014FS01-D	122	120	116	116	122	112	114
2014FS01-D-1	122	120	116	118	122	110	114
2014FS01-E	122	119	115	119	122	112	115

Table 44 : HA Content (A/California H1N1-179A) in MVBs (µg/ml) at 30±2 ºC

HA Content (A/California H1N1-179A) at 30±2 ºC					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2013FS02-A	113	82	76	66	57
2013FS02-B	133	92	84	65	52
2013FS02-C	132	87	77	58	44
2013FS02-D	135	84	71	55	41
2013FS02-E	132	82	68	51	36

Table 45 : HA Content (A/California H1N1-179A) in MVBs (µg/ml) at 30±2 ºC

HA Content (A/California H1N1-179A) at 30±2 ºC					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2014FS01-A	102	77	72	65	55
2014FS01-B	120	98	84	70	56
2014FS01-C	123	98	83	66	50
2014FS01-D	122	97	83	63	46
2014FS01-D-1	122	96	83	63	47
2014FS01-E	122	95	80	62	45

Table 46 : HA Content (A/Christchurch H1N1-74xp) in MVBs (µg/ml) at 5±3 ºC

HA Content (A/Christchurch H1N1-74xp) at 5±3 ºC µg/ml							**Spec: ≥120**
Batch No.	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2014FS02-A	282	298	266	274	256	254	256
2014FS02-B	331	329	333	331	331	331	329
2014FS02-C	338	334	333	333	333	337	340
2014FS02-D	337	337	330	335	335	336	338
2014FS02-E	335	342	327	330	333	335	340

Table 47 : HA Content (A/Christchurch H1N1-74xp) in MVBs (µg/ml) at 30±2 ºC

HA Content (A/Christchurch H1N1-74xp) at 30±2 ºC					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2014FS02-A	282	272	252	229	219
2014FS02-B	331	318	315	298	301
2014FS02-C	338	324	314	299	298
2014FS02-D	337	322	317	297	300
2014FS02-E	335	321	317	294	301

Table 48 : HA Content (B/Massachusetts - YMG) in MVBs (µg/ml) at 5±3 ºC

HA Content (B/Massachusetts -YMG) at 5±3 ºC µg/ml							**Spec: ≥120**
Batch No.	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS03-A	332	344	314	324	279	242	253
2013FS03-B	406	389	357	354	306	251	252
2013FS03-C	424	400	376	366	339	280	282
2013FS03-D	426	411	374	368	337	281	284
2013FS03-E	415	399	375	366	338	274	288

Table 49 : HA Content (B/Massachusetts -YMG) in MVBs (µg/ml) at 30±2 ºC

HA Content (B/Massachusetts -YMG) at 30±2 ºC					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2013FS03-A	332	237	263	226	191
2013FS03-B	406	289	329	269	240
2013FS03-C	424	297	323	271	254
2013FS03-D	426	291	317	276	255
2013FS03-E	415	288	309	273	248

Table 50 : HA Content (B/Brisbane-VIC) in MVBs (µg/ml) at 5±3 ºC

HA Content (B/Brisbane -VIC) at 5±3 ºC					Spec: ≥120 µg/ml		
Batch No.	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS04-A	442	344	400	369	340	319	342
2013FS04-B	496	389	456	410	392	306	328
2013FS04-C	502	398	474	428	420	329	360
2013FS04-D	498	410	472	432	416	338	370
2013FS04-E	507	399	474	427	416	337	375

Table 51 : HA Content (B/Brisbane -VIC) in MVBs (µg/ml) at 30±2 ºC

HA Content (B/Brisbane -VIC) at 30±2 ºC					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2013FS04-A	442	325	278	282	238
2013FS04-B	496	411	317	313	263
2013FS04-C	502	422	331	332	288
2013FS04-D	498	414	333	323	285
2013FS04-E	507	408	319	318	282

Table 52 : VES Content in MVBs (µg/ml) at 5±3 ºC (A/Texas H3N2)

VES Content at 5±3 ºC (A/Texas H3N2) Spec: Target ± 20%								
Batch No.	**Spec**	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS01-A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2013FS01-B	480-720	624	621	628	623	593	594	595
2013FS01-C	280-420	360	355	364	364	342	342	343
2013FS01-D	320-480	412	415	423	415	394	397	392
2013FS01-E	360-540	459	464	465	463	444	445	436

Table 53 : VES Content in MVBs (µg/ml) at 30±2 ºC (A/Texas H3N2)

VES Content (A/Texas H3N2) at 30±2 ºC					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2013FS01-A	N/A	N/A	N/A	N/A	N/A
2013FS01-B	624	612	603	565	568
2013FS01-C	360	351	339	329	328
2013FS01-D	412	411	395	378	378
2013FS01-E	459	457	439	420	422

Table 54 : VES Content in MVBs (µg/ml) at 5±3 ºC (A/California H1N1-179A)

VES Content at 5±3 ºC (A/California H1N1-179A) Spec: Target ± 20%								
Batch No.	**Spec**	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS02-A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2013FS02-B	480-720	626	635	632	622	614	601	593
2013FS02-C	280-420	366	369	369	361	353	351	347
2013FS02-D	320-480	416	422	424	410	405	399	394
2013FS02-E	360-540	469	478	472	461	456	449	440

Table 55 : VES Content in MVBs (µg/ml) at 5±3 ºC (A/California H1N1-179A)

VES Content at 5±3 ºC (A/California H1N1-179A) Spec: Target ± 20%								
Batch No.	**Spec**	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2014FS01-A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2014FS01-B	480-720	653	631	613	625	619	612	593
2014FS01-C	280-420	372	370	360	363	363	361	351
2014FS01-D	320-480	428	423	412	415	415	411	398
2014FS01-D-1	320-480	433	427	418	418	419	415	406
2014FS01-E	360-540	484	466	468	464	466	461	449

Table 56 : VES Content in MVBs (µg/ml) at 30±2 ºC (A/California H1N1-179A)

VES Content at 30±2 ºC (A/California H1N1-179A)					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2013FS02-A	N/A	N/A	N/A	N/A	N/A
2013FS02-B	626	612	585	599	564
2013FS02-C	366	357	340	344	332
2013FS02-D	416	406	384	396	376
2013FS02-E	469	455	431	446	418

Table 57 : VES Content in MVBs (µg/ml) at 30±2 ºC (A/California H1N1-179A)

VES Content at 30±2 ºC (A/California H1N1-179A)					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2014FS01-A	N/A	N/A	N/A	N/A	N/A
2014FS01-B	653	618	609	590	568
2014FS01-C	372	361	358	344	333
2014FS01-D	428	410	410	391	380
2014FS01-D-1	433	416	410	394	385
2014FS01-E	484	460	456	442	427

Table 58 : VES Content in MVBs (µg/ml) at 5±3 ºC (A/Christchurch H1N1-74xp)

VES Content at 5±3 ºC (A/Christchurch H1N1-74xp) **Spec: Target ± 20%**								
Batch No.	**Spec**	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2014FS02-A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2014FS02-B	480-720	637	632	625	618	611	592	604
2014FS02-C	280-420	366	365	359	354	348	340	343
2014FS02-D	320-480	422	418	411	408	403	389	394
2014FS02-E	360-540	481	473	465	463	459	447	446

Table 59 : VES Content in MVBs (µg/ml) at 30±2 ºC (A/Christchurch H1N1-74xp)

VES Content at 30±2 ºC (A/Christchurch H1N1-74xp)					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2014FS02-A	N/A	N/A	N/A	N/A	N/A
2014FS02-B	637	620	600	580	566
2014FS02-C	366	359	346	333	326
2014FS02-D	422	411	397	384	375
2014FS02-E	481	468	457	441	428

Table 60 : VES Content in MVB (µg/ml) at 5±3 °C (B/Massachusetts-YMG)

VES Content at 5±3 °C (B/Massachusetts -YMG)								**Spec: Target ± 20%**
Batch No.	**Spec**	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS03-A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2013FS03-B	480-720	623	633	623	609	612	598	585
2013FS03-C	280-420	367	374	364	353	349	344	340
2013FS03-D	320-480	428	435	427	415	414	406	399
2013FS03-E	360-540	471	475	478	461	456	444	437

Table 61 : VES Content in MVBs (µg/ml) at 30±2 °C(B/Massachusetts -YMG)

VES Content at 30±2 °C (B/Massachusetts -YMG)					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2013FS03-A	N/A	N/A	N/A	N/A	N/A
2013FS03-B	623	610	571	568	546
2013FS03-C	367	351	330	326	320
2013FS03-D	428	418	375	385	385
2013FS03-E	471	470	429	435	418

Table 62 : VES Content in MVBs (µg/ml) at 5±3 °C (B/Brisbane-VIC)

VES Content at 5±3 °C (B/Brisbane-VIC)								**Spec: Target ± 20%**
Batch No.	**Spec**	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**	**12M**
2013FS04-A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2013FS04-B	480-720	616	644	622	609	600	589	576
2013FS04-C	280-420	365	365	363	352	346	339	336
2013FS04-D	320-480	415	438	418	401	401	391	382
2013FS04-E	360-540	468	495	470	460	451	438	432

Table 63 : VES Content in MVBs (µg/ml) at 30±2 ºC (B/Brisbane-VIC)

VES Content at 30±2 ºC (B/Brisbane-VIC)					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
2013FS04-A	N/A	N/A	N/A	N/A	N/A
2013FS04-B	616	584	587	582	545
2013FS04-C	365	341	340	334	328
2013FS04-D	415	389	392	385	377
2013FS04-E	468	440	439	431	419

Table 64: Sterility Results for MVBs at 5±3 ºC

Batch No.	Seed	Sterility at 5±3 ºC						Spec: No growth	
		0M	1M	2M	3M	6M	9M	12M	18
2013FS01-A	A/Texas H3N2	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS01-B	A/Texas H3N2	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS01-C	A/Texas H3N2	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS01-D	A/Texas H3N2	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS01-E	A/Texas H3N2	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS02-A	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS02-B	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS02-C	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS02-D	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS02-E	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS03-A	B/Mass. - YMG	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS03-B	B/Mass. - YMG	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS03-C	B/Mass. - YMG	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS03-D	B/Mass. - YMG	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS03-E	B/Mass. - YMG	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS04-A	B/Brisbane - VIC	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS04-B	B/Brisbane - VIC	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS04-C	B/Brisbane - VIC	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS04-D	B/Brisbane - VIC	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2013FS04-E	B/Brisbane - VIC	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS01-A	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS01-B	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS01-C	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS01-D	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS01-E	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS01-D-	A/Calif. H1N1-179A	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS02-A	A/Christ. H1N1-	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS02-B	A/Christ. H1N1-	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS02-C	A/Christ. H1N1-	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS02-D	A/Christ. H1N1-	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
2014FS02-E	A/Christ. H1N1-	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A

Stability Data for MVB at 5±3 ºC and 30±2 ºC

Appearance

The appearance of all batches of MVBs remained unchanged for 12 months when stored at 5±3 ^{0}C except for MVB in Formula A. MVBs in Formula A for from both H1N1 strains (179A and 74xp) and the two B-strains have small amount of flocculated sediments after 1M while the flocculated sediments are only visible after 2 months for MVB in Formula A for A/Texas H3N2. Under accelerated conditions (30±2 ^{0}C), the appearance of all batches of MVB is acceptable initially. The appearance of MVB from A/Texas H3N2, B/Massachusetts-YMG and B/Brisbane-VIC in Formulae B, C, D and E remains unchanged after 4 weeks. MVB from A/Christchurch H1N1-74xp and A/California H1N1-179A strains develop flocculated appearance after 1 and 2 weeks respectively. These floccules appear like protein precipitates. These data indicate that stabilisers are needed for MVBs in order to ensure stable appearance throughout 12 months of storage at 5±3 ^{0}C.

HA Content (A/Texas H3N2)

The HA Contents for A/Texas H3N2 strain for all batches remain well within the proposed specifications over 12 months. Despite presence of some analytical variability with the HA Content results, Formula A has significantly (about 35%) lower HA Content than Formulae B to E. Formulae B to E have very similar HA stability profiles. HA Content stability data clearly support the proposed 12 month shelf-life for these MVBs when stored at 5±3 ºC. At elevated temperature of 30±2 ^{0}C, HA Contents for A/Texas H3N2 strain for Formulae B to E decline gradually over the four weeks period when stored at 30±2 ºC. Although starting at a lower HA Content, a similar trend is observed for Formula A.

HA Contents for various A/Texas H3N2 MVBs appear to be very stable at 5±3 ^{0}C over 12 months. Formula A has a lower starting point at release. All Formulae are within specifications of > 120 µg/ml. At the elevated temperature of 30±2 ^{0}C, HA is less stable. The results show that A/Texas H3N2 requires inclusion of stabilisers for its stabilisation.

HA Content –H1N1

HA Content (A/California H1N1-179A)

HA Contents for A/California H1N1-179A strain for all formulae are close to the specification limit of ≥120 µg/ml from the outset and remain close to or below specification limit during the 12 month period when stored at 5 ± 2 ºC. HA Content for Formula A is markedly lower than the rest of the Formulae. It is below the specification limit at release and remains below the limit throughout the 12 months storage period. A decline of about 10% is seen during the first month. From the second month onwards the decrease is much more gradual until the 12 month time point. For overall trend point of view, the proposed 12 months shelf-life when stored refrigerated cannot be supported since the starting concentration is very close to specification limit. Linear decline of HA Content is seen for all formulae at the elevated temperature 30±2 ^{0}C over the 4 weeks. These results demonstrate A/California H1N1-179A is very temperature sensitive and it needs to be stored at 2-8 ^{0}C.

HA Content (A/Christchurch H1N1-74xp)

HA Contents for A/Christchurch H1N1-74xp achieve much higher concentrations at release for all Formulae and they remain stable over 12 months. Formula A although starts off at a significantly lower point, its overall stability profile remain similar. HA Content stability data clearly support the proposed 12 month shelf-life at 5±3 ^{0}C for A/Christchurch H1N1-74xp MVBs.

The HA Contents for A/Christchurch H1N1-74xp Formulae B to E are relatively stable (loss of about 10 %) after 4 weeks. Formula A has a lower HA Content at release and declines much faster than those formulae containing stabilisers. The higher thermal stability is noted for the HA from the A/Christchurch H1N1-74xp strain when compared with the A/California H1N1-179A strain.

HA Content – H1N1

All A/California H1N1-179A Formulae have HA Content at close to or below 120 µg/ml at the start of the stability studies. At 30 ^{0}C the A/California H1N1-179A

HA degrades at a fast rate at 30 ^{0}C. HA Content from A/Christchurch H1N1-74xp strain are generally higher than the A/California H1N1-179A strain and are much more stable at elevated temperature of 30±2 ^{0}C. However, this difference is not as obvious at the 5±3 ^{0}C where the HA Content for A/California H1N1-179A is stable over 12 months after initial loss following 1 month of storage.

HA Content (B/Massachusetts YMG)

The HA Contents for B/Massachusetts strain from the Yamagata lineage for all Formulae remain within the proposed specifications over 12 months at 5±2 ^{0}C despite a decreasing trend. The HA Content decreases by over 30% in 12 months. Formula A has a lower starting point but displays the same trend. HA stability data clearly support the proposed 12 month shelf-life at 5±3 ^{0}C for B/Massachusetts YMG MVB. An overall decrease of 40% is seen after 4 weeks at 30±2 ^{0}C. Formula A has a much lower HA Content at release than all the MVBs with stabilisers but displays a stable stability profile.

The HA Content results for the B/Massachusetts (Yamagata Lineage) for all Formula B to E are all within specifications over 12 months at 5±3 ºC despite some linear reduction. Without any stabiliser present, HA Content decreases markedly during manufacturing and prior to testing. All stabiliser combinations containing VES, Tween® 80 and Triton X-100® are useful to stabilise the HA.

HA Content (B/Brisbane-VIC)

The HA Contents for B/Brisbane strain from the Victoria lineage for Formulae B to E display slight decreasing trends but remain within the propose specifications over 12 months at 5±3 ºC. Formula A has a lower HA Content at release but displays the same stability trend. HA stability data clearly support the proposed 12 month shelf-life at 5±3 ^{0}C for B-VIC MVB. The HA Contents for B/Brisbane strain from the Victoria lineage for Formulae B to E decrease almost linearly from 500 to 280 µg/ml after 4 weeks at 30±2 ^{0}C. Formula A starts off at a 10% lower HA Content at 450 µg/ml than all the MVB with stabilisers but maintains a similar profile.

All the HA Contents for B/Brisbane (Victoria lineage) are within specifications. A gentle decline in HA Content is observed following a faster decline over the first month of storage at 5±3 ^{0}C. At 30±2 ^{0}C, the HA contents are declining faster. The results show that in MVBs HA Content for B/Brisbane-VIC required an addition of a combination of stabilisers in order to meet the proposed shelf-life.

VES Content

VES Contents for all Formulae B to E for all strains remain within the limits of ± 20% of targets over 12 months whens stored at 5±3 ^{0}C. VES Contents for Formulae B to E for all strains decline about 10% after 4 weeks at 30±2 ^{0}C. This decline in VES is linked to the increased rates of hydrolysis at high temperatures.

All the VES results are within acceptable limits of ± 20 % around the targets. A slight declining trend is visible over the 12 months at 5±3 ^{0}C. For the elevated condition of 30±2 ^{0}C, VES trend is more obvious than Formula B where the VES concentration is the highest. This is to be expected as degradation of VES follows first order kinetics as seen in previous studies. Overall, the limits of ± 20% is not expected to be exceeded over the proposed shelf-life of the MVB of 12 months at 5±3 ^{0}C.

Sterility

Sterility is performed at the beginning of the stability testing program for all Formulae. All batches pass sterility tests at the start and at the 12-month time point in the program. Sterility tests are not repeated for all batches at the end of 4 weeks at 30±2 ^{0}C. Sterility test results for all batches of MVBs are satisfactory at initial and final test points of 12 months.

Conclusion

Stability Studies on Monovalent Bulks

Available 12 months real time (5±3 ºC) and 4 weeks accelerated (30±2 ºC) stability data showed that MVBs formulated without any stabiliser result in the formation of flocculated sediments, and unstable HA resulting in lower starting concentrations. There is some decrease in the VES Content in the MVBs with Formula B (FluLaval® formula) but all are within the ± 20% acceptable limits over 12 months. This study showed that stabilisers are needed in order to maintain a high concentration of HA in MVB and to prevent aggregations of HA antigens. Overall, batches containing a combination of stabilisers including VES at concentrations between 350 and 450 µg/ml, Tween® 80 at a concentration of 140 µg/ml and Triton X-100® at a concentration of 500 µg/ml, provide stabilisation effects to the HA antigens in the MVBs over a storage of 12 months at 5±3 ºC.

Those formulae with reduced stabiliser (Formulae C, D and E) achieve the same or better stabilisation effects of HA when compared with the FluLaval® MVB formula (Formula B) that has a very high concentration of VES at 600 µg/ml and Tween® 80 at 1,130 µg/ml. These results confirm that reduced stabiliser concentration of VES and Tween® 80 while adding Triton X-100® at a concentration of 500 µg/ml in the MVBs results in no loss of stability for HA Content. Available data support the proposed shelf-life of 12 months for the in Formulae C, D and E.

Example 11: Manufacturing of QIV Final Bulks and Filled Syringes in Various Formulations

Stabiliser System used for Manufacturing of QIV Final Bulks

Pre-formulation development study described above established a series of formulae with reduced Tween® 80 content in the presence of Triton X-100® and VES that are identified as potential stabilizers. Positive (Formula B, FluLaval® Quadrivalent) and negative controls (Formula A, stabiliser-free) are included in this study. Final QIV bulks are manufactured based on each of the 7 formulae below (**Table 14**). A total of 9 lots of QIV filled syringes are manufactured with different

formulae in lots of 1 L and filled aseptically into 0.5 ml ceramic-coated tip (CCT) prefilled syringes within a sterile filling production area. Samples are taken from each lot for testing against established release specifications (Refer to **Table 15**).

Table 14: Target Stabiliser Concentrations for Final Quadrivalent Bulks and Filled Syringes

Formula	**VES** *(µg/ml)*	**Tween ® 80** *(µg/ml)*	**Triton X-100®** *(µg/ml)*	**Comment**
A	0	0	0	Negative control (Stabliser-Free). *1 lot of QIV formulated with one lot of H1N1(179A)*
B	533	1,366	0	Positive control (based on FluLaval® Quadrivalent). *1 lot of QIV formulated with each lot of H1N1 (179A & 74xp)*
C	180	70	250	Low VES Content. *1 lot of QIV formulated with one lot of H1N1 (179A)*
D	200	70	250	Medium VES Content. *1 lot of QIV formulated with each lot of H1N1 (179A and -74xp)*
E	225	70	250	High VES. *1 lot of QIV formulated with one lot of H1N1 (-179A)*
M	130	70	250	Lower VES Content. *1 lot of QIV formulated with one lot of H1N1 (-74xp)*
N	130	50	190	Lower VES content/ Tween® 80 / Triton X-100®. *1 lot of QIV formulated with one lot of H1N1 (-74xp)*

Representative samples taken from each the bulk and each lot of filled syringes are obtained for release testing to the specifications contained in **Table 15**. Abnormal toxicity test, in accordance with Chinese Pharmacopoeia (Ch.P.), is a vaccine product safety test involving slow administration by IM injection of doses of 0.5 (25 ml/kg) and 5.0ml (16.7 ml/kg) of the test samples into the mice (body weight of approximately 20g each) and guinea pigs (body weight of approximately 300g each) respectively after equilibration to room temperature. The doses tested in mice and guinea pigs represent 2,167 to 3,250 times the intended human dose calculated from an average body weight of 65 kg in China respectively. The gains in the body weight of treated animals are determined for 1 week and compared with the weight gain for groups of untreated animals. Absence of abnormal toxicity is indicated by comparable weight gains in both the treated and untreated animal groups.

In order to avoid unnecessary use of lab animals to detect any adventitious toxic contaminants or any unexpected toxic components, testing for abnormal toxicity is kept to a minimum. Testing is only performed on two lots, where the stabiliser concentrations are the highest (Batches RD-Fill-2014-B-0101 and RD-Fill-2014-E-0105).

Table 15: Release Specifications for Quadrivalent Final Bulks and Filled Syringes

Formula	Appearance	pH	Osmolality (mOsmol/kgH2O)	H1N1-179A [µg/ml]	H1N1-74XP [µg/ml]	H3N2 [µg/ml]	B-YMG [µg/ml]	B-VIC [µg/ml]	VES [µg/ml]	Tween® 80 [µg/ml]	Triton X-100® [µg/ml]	Abnormal Toxicity	Sterility
A	Colourless, light opalescent liquid	6.5 - 8.0	To be recorded	26-40	N/A	26-40	27-41	27-41	N/A	N/A	N/A	N/A	No growth
B, B2	Colourless, light opalescent liquid	6.5 - 8.0	To be recorded	26-40	26-40	26-40	27-41	27-41	426-640	1092 - 1,639	N/A	No significant weight loss	No growth
C	Colourless, light opalescent liquid	6.5 - 8.0	To be recorded	26-40	N/A	26-40	27-41	27-41	144-216	< 80	< 300	N/A	No growth
D, D2	Colourless, light opalescent liquid	6.5 - 8.0	To be recorded	26-40	26-40	26-40	27-41	27-41	160-240	< 80	< 300	N/A	No growth
E	Colourless, light opalescent liquid	6.5 - 8.0	To be recorded	26-40	N/A	26-40	27-41	27-41	180-270	< 80	< 300	No significant weight loss	No growth
M, M2	Colourless, light opalescent liquid	6.5 - 8.0	To be recorded	26-40	26-40	26-40	27-41	27-41	104-156	< 80	< 300	N/A	No growth
N	Colourless, light opalescent liquid	6.5 - 8.0	To be recorded	26-40	26-40	26-40	27-41	27-41	104-156	40 - 60	152-228	N/A	No growth

Buffer Preparation

All the buffer solutions are prepared using WFI (Water for injection) except for the VES buffer solution is used as the solvent vehicle. The buffer preparations are conducted under Grade C environment following the standard operating procedure for Buffer preparation. All buffer solutions are filtered into sterile bags, sterile Nalgene® bottles or sterile glass bottles under the Unidirectional Airflow (UDF) bench. The 2.6% VES/95% Ethanol solution uses 95% ethanol solution as solvent vehicle. For detailed instructions on buffer preparation and comprehensive records and, refer to Secondary Laboratory Book 03.

Formulation

Laboratory experiments are performed to determine the critical process parameters such as stabiliser concentrations, order of stabilizers addition, stirring speed and stirring durations.

The final bulk preparation is performed in batch sizes of 1 L each within 2 L sterile glass bottles instead of the normal 10 L sterile plastic bags. The use of bottles instead of plastic bags does not have any impact on the formulations. This formulation process is similar to the existing influenza vaccine formulation process (FSOP0600B84) with more the use of a sterile bag and a close system (pump, tubing, filter) for product and solution transfer. This process provides a better formulation accuracy and provides a better sterile assurance as it consisted of a sterile bags.

The manufacturing of final bulks is described below in the **Figure 3**. All the formulations are performed under laminar airflow (LAF) within a Grade B background to ensure formulation sterility (Refer to Laboratory Book 07). Required volumes of monovalent bulks and stabilizers are calculated according to FSOP0600A25V00.

The formulation of all the final bulks for the series A-E or B2, M and N campaigns are completed within the same day with all the samples tested on the following day. In this way, variation in test results for the initiation of stability testing is minimised.

The only test item performed at in-process control (IPC) for the final bulks is HA content. If HA content of any strain exceeds ±10 % of the target, adjustment of formulation is normally required.

Due to the presence of cross-interference between the absorbance of Tween® 80 and Triton X-100® at the measured wavelength in the current Chinese Pharmacopoeia (Ch.P.) test method for Tween® 80 is unsuitable for use in the study. Therefore, the concentrations of these stabilisers in the final bulks and finished products are reported as "quantified by input".

Table 16: Manufacture of Final Bulks and Filled Syringes

Description	Formulation Batch No. (RD-Form-2014-)	Manu. Date	Description	Filling Batch No. (RD-Fill-2014-)	Manu. Date
Final QIV Bulk in Formula A	**A**-0109	20.Jan.2014	QIV Filled Syringes in Formula A	**A**-0106	23.Jan.2014
Final QIV Bulk in Formula B	**B**-0101	17.Jan.2014	QIV Filled Syringes in Formula B	**B**-0101	20.Jan.2014
Final QIV Bulk in Formula C	**C**-0102	17.Jan.2014	QIV Filled Syringes in Formula C	**C**-0102	20.Jan.2014
Final QIV Bulk in Formula D	**D**-0103	17.Jan.2014	QIV Filled Syringes in Formula D	**D**-0103	20.Jan.2014
Final QIV Bulk in Formula E	**E**-0105	17.Jan.2014	QIV Filled Syringes in Formula E	**E**-0105	20.Jan.2014
Final QIV Bulk in Formula B	**B2**-0511	05.May.2014	QIV Filled Syringes in Formula B	**B2**-0511	07.May.2014
Final QIV Bulk in Formula M	**M**-0512	05.May.2014	QIV Filled Syringes in Formula M	**M**-0512	07.May.2014
Final QIV Bulk in Formula N	**N**-0513	05.May.2014	QIV Finished Product in Formula N	**N**-0513	07.May.2014
Final QIV Bulk in Formula D2	**D2**-0514	26.May.2014	QIV Finished Product in Formula D	**D2**-0514	28.May.2014

Note: Due to low yield of HA in the A/California/7/2009NYMC X-179A monovalent bulk and its poor stability under accelerated condition, a repeat of H1N1-179A monovalent bulk is conducted in parallel with a second H1N1 strain (A/Christchurch/16/2010 NIB-74xp). Final QIV bulks are manufactured using H1N1-74xp strain.

Required volumes of MVBs needed for each QIV final bulk are determined from the HA contents shown in **Table 32**. Target volumes of stabilizer buffers needed, as required for each formula, are calculated according to manufacturing instructions: FSOP0600A25. Both target and actual volumes dispensed are recorded (**Tables 17** and **18**).

Table 17: Target and Actual Volumes of MVBs Added to the Final Bulks

Final Bulk Batch No. (RD-Form-2014-)	H1N1 (A/California/7/2009N YMC X-179A) for Formula A,B,C,D,E			H3N2 (A/Texas/50/2012 NYMC X-223A)			B-YMG (B/Massachusetts/2/2012 NYMC BX-51B)			B-VIC (B/Brisbane/60/2008 NYMC BX-35)		
	H1N1 (A/Christchurch/16/2010 NIB-74xp) for Formula B2,M,N,D2											
	MVBs Lot No.	Required Volume (ml)	Actual Volume (ml)	MVBs Lot No.	Required Volume (ml)	Actual Volume (ml)	MVBs Lot No.	Required Volume (ml)	Actual Volume (ml)	MVBs Lot No.	Required Volume (ml)	Actual Volume (ml)
A-0109*	2013FS02-A	292.0+ 64.0	292.0+ 65.0	2013FS01-A	101.2	101.2	2013FS03-A	108.4+ 22.0	108.4+ 22.0	2013FS04-A	81.4+ 21.0	81.4+ 21.0
B-0101	2013FS02-B	248.1	248.1	2013FS01-B	76.6	76.6	2013FS03-B	88.7	88.7	2013FS04-B	72.6	72.6
C-0102	2013F	250.0	250.0	2013FS	72.2	72.2	2013FS0	84.9	84.9	2013FS04	71.7	71.7

	S02-C		01-C		3-C		-C		
D-0103	2013F S02-D	244.4	244.4	2013FS 01-D	72.5	72.5	2013FS0 3-D	84.5	84.5	2013FS04 -D	72.3	72.3
E-0105	2013F S02-E	250.0	250.0	2013FS 01-E	73.3	73.3	2013FS0 3-E	86.7	86.7	2013FS04 -E	71.0	71.0
B2-0511	2014F S02-B	100.3	100.3	2013FS 01-B	81.5	81.5	2013FS0 3-B	101.7	101.7	2013FS04 -B	87.8	87.8
M-0512	2014F S02-C	98.8	98.8	2013FS 01-C	80.3	80.3	2013FS0 3-C	98.4	98.4	2013FS04 -C	84.1	84.1
N-0513	2014F S02-C	98.8	98.8	2013FS 01-C	80.3	80.3	2013FS0 3-C	98.4	98.4	2013FS04 -C	84.1	84.1
D2-0514	2014F S02-D	99.9	99.9	2013FS 01-D	79.9	79.9	2013FS0 3-D	97.8	97.8	2013FS04 -D	83.3	83.3

*Note: * The HA content of final bulk batch (RD-Form-2014-**A**-0109 is corrected as it exceeds IPC range of 10% target.*

Table 18: Required and Actual Volume of Stabilizers Added in Final Bulk

Final Bulk Batch No. (RD-Form-2014-)	2.6%w/v VES in Ethanol		1.0%w/v VES in Ethanol		5.0%w/v Tween® 80 in WFI		0.2%w/v Tween® 80 in WFI		1.0% Triton X-100® in PBS	
	Required Volume (ml)	Actual Volume (ml)	Required Volume (ml)	Actual Volume (ml)	Required Volume (ml)	Actual Volume (ml)	Required Volume (ml)	Actual Volume (ml)	Required Volume (ml)	Actual Volume (ml)
A-0109	-	-	-	-	-	-	-	-	-	-
B-0101	8.8	8.8	-	-	16.3	16.3	-	-	-	-
C-0102	-	-	0.8	0.8	-	-	1.5	1.5	1.0	1.0
D-0103	-	-	0.6	0.6	-	-	1.8	1.8	1.3	1.3
E-0105	-	-	0.3	0.3	-	-	1.3	1.3	1.0	1.0
B2-0511	11.4	11.4	-	-	18.9	18.9	-	-	-	-
M-0512	-	-	-	-	-	-	9.7	9.7	5.9	5.9
N-0513	-	-	-	-	-	-	-	-	-	-
D2-0514	-	-	5.1	5.1	-	-	9.7	9.7	6.0	6.0

Filling

The formulated final bulks are filled into ceramic coated tip (CCT) glass syringes using the existing Corimar filling machine under laminar airflow unit (LAF) in Grade A area. The filling process used for this study is similar to that used for the existing QIV (Master Manufacturing Instructions FSOP0600B69). Each final bulk is filled into filled syringes (PFS) in lots of approximately 1,000 each. The different batches of final bulks are filled on the same day according to the process flow in **Figure 4**.

Results and Discussion

QIV Final Bulks and Filled Syringes in Different Formulae

Final Bulk Formulation

Nine batches of QIV final bulk formulations are manufactured in accordance with **Tables 17** and **18**. One of these batches (A-0109) has HA contents adjusted since they are outside of the 10% IPC limits. Batch analysis results for all 9 batches are included in **Table 35**.

Table 35: Batch Analysis Data for Quadrivalent Final Bulks

Batch No. (RD-Form-2014-)	**HA Content**					**VES Content** (µg/ml) *[Spec: target± 20%]*
	H1N1-179A [26-40 µg/ml]	**H1N1-74XP** *[26-40 µg/ml]*	**H3N2** *[26-40 µg/ml]*	**B-YMG** *[27-41 µg/ml]*	**B-VIC** *[27-41 µg/ml]*	
A-0109*	31	-	32	36	34	N/A
B-0101	32	-	35	32	30	543 [*426-640*]
C-0102	33	-	34	32	30	185 [*144-216*]
D-0103	32	-	34	31	30	207 [*160-240*]
E-0105	33	-	34	31	29	234 [*180-270*]
B2-0511	-	35	34	31	31	520 [*426-640*]
M-0512	-	34	34	33	32	126 [*104-156*]
N-0513	-	34	34	33	32	127 [*104-156*]
D2-0514	-	33	32	31	33	Not tested [*160-240*]

** HA content of this batch is adjusted.*

Due to presence of interference with the Chinese Pharmacopoeia (Ch.P.) analytical test methods for Tween® 80 in the presence of Triton X-100®, data for Tween® 80 and Triton X-100® are provided as "quantified by inputs" or actual quantities added to the formulae. Since the limits for these stabilisers are 20% around the target stabiliser concentrations, these targets are expected to be achievable given the small-scale trial operations with more accurate controls. Degradation of two surface active agents Tween® 80 and Triton X-100® is not expected to be significant. All the VES Contents for those formulae containing this stabiliser are measured by HPLC against a specification limit of target ± 20%. All 9 batches of QIV final bulks meet specifications and are acceptable for filling into CCT pre-filled syringes.

Filling of QIV Final Bulks into Syringes

A total of 9 batches of filled syringes are manufactured in PFS syringes in accordance with existing routine filling procedures (FSOP0600B69). All IPC results for final products are within control limits. All syringes are stored in nests within plastic tub, with protection from light. Batch analysis data for these batches are given in **Table 36**.

Table 36: Batch Analysis Data at Release for QIV Final Bulk

Batch Number (RD-Fill-2014-)	**VES Specification (µg/ml)**	**VES Content (µg/ml)** *[target±20%]*	**HA Content (80-120% of Target HA)**			
			H1N1 [26-40 µg/ml]	**H3N2** *[26-40 µg/ml]*	**B-YMG** *[27-41 µg/ml]*	**B-VIC** *[27-41 µg/ml]*
A-0106	-	-	31	33	36	35
B-0101	426-640	521	34	34	34	32
C-0102	144-216	162	33	32	34	32
D-0103	160-240	195	33	33	34	34
E-0105	180-270	217	34	32	35	31
B2-0511	426-640	516	34	35	30	29
M-0512	104-156	125	34	35	33	32
N-0513	104-156	126	34	34	33	32
D2-0514	160-240	196	32	35	32	32

11.3 Batch Analysis Data for Filled Syringes

Each lot of naked filled syringes (non-blister packed nor filled into carton) is tested against proposed release specifications (Refer to **Table 37**). All formulations are able to be successfully formulated to within targets of 26 - 40 µg/ml and 27 - 41 µg/ml for the two A strains (H1N1 & H3N2) and the two B strains (B-YMG & B-VIC) respectively. These lots are filled into pre-filled syringes. Due to interference with the Chinese Pharmacopoeia (Ch.P.) test

method for Tween® 80, the contents of Tween® 80 are given as "quantified by input". Release data for the finished products containing different stabilizer combinations show that they are all within acceptable ranges for Appearance, pH, Osmolality, VES Content, HA Content and Total Protein Content. Sterility tests conducted on all these batches show absence of microbial contaminations.

Table 37: Batch Analysis Data at Release for QIV Filled Syringes

Batch Number (RD-Fill-2014-)	**Appearance** *[Colourless Light Opalescent Liquid]*	**pH** *[6.5-8.0]*	**Osmolality (mOsmol/kgH_2O)** *[To be Recorded]*	**VES Content (µg/ml)** *[Spec: target± 20%]*	**Tween® 80** ***[µg/ml]***	**Triton X-100®** ***[µg/ml]***	**HA Content (80-120% of Target HA)**				**Abnormal Toxicity** *[No significant weight loss]*	**Sterility** *[No micro-biological growth]*
							H1N1 *[26-40µg/ml]*	**H3N2** *[26-40µg/ml]*	**B-YMG** *[27-41µg/ml]*	**B-VIC** *[27-41µg/ml]*		
A – 0106	Complies	7.2	430	N/A	0	0	31	33	36	35	Not tested	No growth
B – 0101	Complies	7.0	760	521 *[426-640]*	1,366*	0	34	34	34	32	Complies	No growth
C – 0102	Complies	7.1	516	162 *[144-216]*	70*	250*	33	32	34	32	Not tested	No growth
D – 0103	Complies	7.1	547	195 *[160-240]*	70*	250*	33	33	34	34	Not tested	No growth
E – 0105	Complies	7.1	561	217 *[180-270]*	70*	250*	34	32	35	31	Complies	No growth
B2 – 0511	Complies	7.0	Not tested	516 *[426*	1,366*	250*	34	35	30	29	Not teste	No growt

				- *640]*							d	h
M – 0512	Complies	7.2	Not tested	125 *[104 - 156]*	70*	250 *	34	35	33	32	Not tested	No growth
N – 0513	Complies	7.2	Not tested	126 *[104 - 156]*	50*	190 *	34	34	33	32	Not tested	No growth
D2 – 0514	Complies	7.1	Not tested	196 *[160 - 240]*	70*	250 *	32	35	32	32	Not tested	No growth

** quantified by input*

Conclusions

QIV Final Bulks and QIV Filled Syringes Manufactured

Nine batches of final bulks and filled syringes have been successfully manufactured following the set procedure without any deviation. All IPC results for final bulks and final products are within the control limits. All formulated QIV final bulks and filled syringes met all pre-established release limits. Data from release testing show that QIV Final Bulk formulations with reduced stabilisers can be manufactured using existing manufacturing process with minor adaptations. Batch analysis data for final bulks and filled syringes confirm no significant differences in HA Content between these batches. Stabilisers used do not impact product sterility. In addition, test results from two batches selected to represent worst cases in terms of content of stabilisers are tested for abnormal toxicity in mice and guinea pigs. Both batches do not show any sign of toxicity. Hence, samples from each batch of filled syringes are satisfactory for use in the real time and accelerated stability studies.

Example 12: Stability Studies for QIV Filled Syringes

QIV Filled Syringes Batches

Opaque plastic tubs of naked filled syringes (non-blister packed and not packed into carton) are stored in tubs and placed under 30±2 ^{0}C for up to 4 weeks (accelerated conditions) and at 5±3 ^{0}C for up to 12 months (real time conditions) within constant temperature stability chambers or validated stability rooms. A total of 9 batches of filled syringes are included in the stability testing programs. During stability studies, samples are periodically withdrawn at the designated time points, in sufficient quantities, for the following tests: product appearance, pH, HA content, VES content and sterility in accordance with established stability testing protocols. Details of the stability studies are included in **Tables 19**. The content of Tween® 80 and Triton X-100® in the final bulks and filled syringes are reported as "quantified by input". Filled syringes stored under real time conditions (5±3 ^{0}C) are required to meet the End-of Life Specifications for filled syringes (**Table 20**).

Stability Testing Programs QIV Filled Syringes

Details of the 3 stability programs initiated to assess the stability of the 9 batches of QIV filled syringes in various formulae (A, B, C, D, E, B2, M, N and D2) are shown in **Table 19**. All stability testing programs are performed within temperature controlled enclosures that have been previously validated for the purpose. The samples at real time conditions of 5±3 ^{0}C are expected to meet the End-of-Life specification in **Table 20**.

Table 19: Stability Testing Programs for QIV Filled Syringes

	Stability Testing Program 1	**Stability Testing Program 2**	**Stability Testing Program 3**
Viral Strains	H1N1-179A, H3N2, B/Massachusetts and B/Brisbane strains.	H1N1-74xp, H3N2, B/Massachusetts and B/Brisbane strains.	H1N1-74xp, H3N2, B/Massachusetts and B/Brisbane strains.
Batches	RD-Fill-2014-**A**-0106, **B**-0101, **C**-0102, **D**-0103, **E**-0105	RD-Fill-2014-**B2**-0511, **M**-0512, **N**-0513	RD-Fill-2014-**D2**-0514
Temperature Conditions/Time Points/Tests	5 ± 3 ^{0}C at 0, 1, 2, 3, 6, 9 and 12 months. Only HA Content for H1N1-179A strain, pH and VES Content are tested at 1, 2, 3, 6, 9 and 12 months. All tests are performed at 0 and 12 months. 30 ± 2 ^{0}C at 0, 1, 2, 3 and 4 weeks. All tests except sterility are performed for all test points.	5 ± 3 ^{0}C at 0, 1, 2, 3, 6, 9 and 12 months. Only HA Content for 4 strains, pH and VES Content are tested at 1, 2, 3, 6, 9 and 12 months; All tests are performed at 0 and 12 months. 30 ± 2 ^{0}C at 0, 1, 2, 3 and 4 weeks. All tests except sterility are performed for all test points.	5 ± 3 ^{0}C at 0, 1, 2, 3, 6, 9 and 12 months Only HA Content for H1N1-74xp strain, pH and VES Content are tested at 0, 3 and 12 months; All tests are performed at 0 and 12 months.
Date of commencement	27 Jan 2014	07 May 2014	28 May 2014
Comment	Provides full stability data for QIV in different formulae.	Provides full stability data for QIV with reduced VES in Formulae M and N vs B.	Provides full stability data HA Content for H1N1-74xp strain for QIV in Formula D.

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Table 20: End-of-Life Specifications for Quadrivalent Filled Syringes

Formula	Appearance	pH	H1N1-179A [µg/ml]	H1N1-74XP [µg/ml]	H3N2 [µg/ml]	B-YMG [µg/ml]	B-VIC [µg/ml]	VES [µg/ml]	Sterility
A	Colourless, light opalescent liquid	6.5-8.0	26-40	N/A	26-40	27-41	27-41	0	No growth
B,B2	Colourless, light opalescent liquid	6.5-8.0	26-40 [B1]	26-40 [B2]	26-40	27-41	27-41	426-640	No growth
C	Colourless, light opalescent liquid	6.5-8.0	26-40	N/A	26-40	27-41	27-41	144-216	No growth
D,D2	Colourless, light opalescent liquid	6.5-8.0	26-40 [D1]	26-40 [D2]	26-40	27-41	27-41	160-240	No growth
E	Colourless, light opalescent liquid	6.5-8.0	26-40	N/A	26-40	27-41	27-41	180-270	No growth
M,M2	Colourless, light opalescent liquid	6.5-8.0	26-40 [M1]	26-40 [M2]	26-40	27-41	27-41	104-156	No growth
N	Colourless, light opalescent liquid	6.0-8.0	26-40	26-40	26-40	27-41	27-41	104-156	No growth

Results and Discussion

Stability Testing Program for QIV Filled Syringes in Various Formulae

Stability Data for Fill Syringes at 5±3 ^{0}C and 30±2 ^{0}C

Stability data for Appearance of finished products at 5±3 ^{0}C and 30±2 ^{0}C are included in **Tables 65** and **66** respectively.

Data for pH values of finished products at 5±3 ^{0}C and 30±2 ^{0}C are included in **Tables 67** and **68** respectively (**Figures 31** to **34**). Stability data for HA Content of finished products at 5±3 ^{0}C and 30±2 ^{0}C are included in **Tables 69** to **77 (Figures 35** to **50)**. Stability data for VES

Content of finished products at 5±3 ^{0}C and 30±2 ^{0}C are included in **Tables 78** and **79 (Figure 51** to **54)** respectively. Data for Sterility test on finished products at 5±3 ^{0}C are included in **Tables 80**. Sterility test is not performed at 30±2 ^{0}C.

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Table 65 : Appearance of QIV Filled Syringes for 5±3 ºC

Batch No. (RD-Fill-2014-)	0M	1M	2M	3M	4.5M	5.5M/6M	9M	12M
A-0106	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment
B – 0101	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
C – 0102	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
D – 0103	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
E – 0105	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
B2 - 0511	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
M - 0512	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
N – 0513	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
D2 - 0514	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies

Table 66 : Appearance for QIV Filled Syringes at 30±2 ºC

Appearance for QIV Finished Product at 30±2 ^{0}C Specification: Colourless Light Opalescent Liquid					
Batch No. (RD-Fill-2014-)	**0W**	**1W**	**2W**	**3W**	**4W**
A - 0106	Complies	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment	Small amount of flocculated sediment
B - 0101	Complies	Complies	Complies	Complies	Complies
C - 0102	Complies	Complies	Complies	Complies	Complies
D - 0103	Complies	Complies	Complies	Complies	Complies
E - 0105	Complies	Complies	Complies	Complies	Complies
B2 - 0511	Complies	Complies	Complies	Complies	Complies
M - 0512	Complies	Complies	Complies	Complies	Complies
N - 0513	Complies	Complies	Complies	Complies	Complies
D2 - 0514	Complies	Complies	Complies	Complies	Complies

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Table 67 : pH Results for QIV Filled Syringes at 5±3 ºC

	pH Value for QIV Filled Syringes at 5±3 ^{0}C						**Spec: pH 6.5 – 8.0**	
Batch No. (RD-Fill-2014-)	**0M**	**1M**	**2M**	**3M**	**5.5M**	**6M**	**9M**	**12M**
A – 0106	7.2	7.2	7.2	7.2	7.2	N/A	7.1	7.1
B – 0101	7.0	7.0	7.0	7.0	7.0	N/A	7.0	7.0
C – 0102	7.1	7.1	7.1	7.1	7.1	N/A	7.1	7.1
D – 0103	7.1	7.1	7.1	7.1	7.1	N/A	7.1	7.1
E – 0105	7.1	7.1	7.1	7.1	7.1	N/A	7.1	7.1

B2 – 0511	7.0	7.0	7.0	7.0	N/A	7.0	7.0	7.0
M – 0512	7.2	7.1	7.1	7.2	N/A	7.1	7.2	7.2
N – 0513	7.2	7.1	7.1	7.2	N/A	7.1	7.1	7.2

Table 68 : pH Results for QIV Filled Syringes at 30±2ºC

pH Value for QIV Filled Syringes at 30±3 ºC				**Spec: pH 6.5 – 8.0**	
Batch No. (RD-Fill-2014-)	**0W**	**1W**	**2W**	**3W**	**4W**
A – 0106	7.2	7.2	7.2	7.2	7.1
B – 0101	7.0	7.0	7.0	7.0	7.0
C – 0102	7.1	7.1	7.1	7.1	7.1
D – 0103	7.1	7.1	7.1	7.1	7.1
E – 0105	7.1	7.1	7.1	7.1	7.0
B2 – 0511	7.0	7.0	7.0	7.0	7.0
M – 0512	7.2	7.2	7.2	7.1	7.1
N – 0513	7.2	7.2	7.2	7.1	7.1
D2 – 0514	N/A	N/A	N/A	N/A	N/A

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Table 69 : HA Content for H1N1-179A for QIV Filled Syringes at 5±3 ºC

Batch No. (RD-Fill-2014-)	**HA Content for H1N1-179A at 5±3 ºC**				**Spec: 24 - 36 µg/ml**	
	Reagent Batch No.09/146					
	0M	**1M**	**2M**	**5.5M**	**9M**	**12M**
A – 0106	31	30	29	31	27	26
B – 0101	34	30	30	34	29	29
C – 0102	33	30	30	31	28	29
D – 0103	33	30	30	30	28	28
E – 0105	34	30	30	31	29	29

Table 70 : HA Content (A/Christchurch H1N1-74xp) for QIV Filled Syringes at 5±3 ºC

HA Content for H1N1 at 5±3 ºC				**Spec: 24 - 36 µg/ml**		
Batch No. (RD-Fill-2014-)	**0M**	**1M**	**2M**	**3M**	**6M**	**9M**
B2 – 0511	34	33	33	32	32	33
M – 0512	34	32	34	33	33	33
N – 0513	34	32	33	32	32	32
D2 – 0514	32	35	33	33	33	33

Table 71 : HA Content (A/California H1N1-179A) for QIV Filled Syringes at 30±2 ºC

HA Content for H1N1 at 30±2 ºC					
Batch No. (RD-Fill- 2014-)	**0W**	**1W**	**2W**	**3W**	**4W**
A – 0106	31	27	25	21	17
B – 0101	34	25	20	18	18
C – 0102	33	25	19	18	17
D – 0103	33	25	20	17	17
E – 0105	34	26	20	18	18
B2 – 0511	34	32	32	31	30
M – 0512	34	33	33	32	31
N – 0513	34	32	32	31	30
D2 – 0514	N/A	N/A	N/A	N/A	N/A

Table 72 : HA Content (A/Texas H3N2) for QIV Filled Syringes (5±3 ºC)

HA Content for H3N2 at 5±3 ºC					**Spec: 24 - 36 µg/ml**			
Batch No. (RD-Fill- 2014-)	**0M**	**1M**	**2M**	**3M**	**5.5M**	**6M**	**9M**	**12M**
A – 0106	33	33	30	29	29	N/A	28	28
B – 0101	34	37	34	32	34	N/A	34	35
C – 0102	32	35	33	30	32	N/A	31	32
D – 0103	33	35	33	31	32	N/A	31	33
E – 0105	32	36	33	31	32	N/A	31	32
B2 - 0511	35	35	36	34	N/A	35	36	36
M - 0512	35	35	36	34	N/A	34	34	34
N – 0513	34	35	35	34	N/A	34	34	33
D2 – 0514	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 73 : HA Content (A/Texas H3N2) for QIV Filled Syringes (30±2 ºC)

HA Content for H3N2 at 30±2 ºC					
Batch No. (RD-Fill- 2014)	**0W**	**1W**	**2W**	**3W**	**4W**
A – 0106	33	28	23	24	23
B – 0101	34	36	35	33	34
C – 0102	32	34	32	30	31
D – 0103	33	34	32	30	31
E – 0105	32	35	34	30	31
B2 – 0511	35	35	35	33	34
M – 0512	35	34	34	32	32
N – 0513	34	34	33	31	31
D2 – 0514	N/A	N/A	N/A	N/A	N/A

Table 74 : HA Content (B/Massachusetts B-YMG) for QIV Filled Syringes at 5±3 ºC

HA Content for B-YMG at 5±3 ^{0}C					**Spec: 24 - 36 µg/ml**			
Batch No. (RD-Fill-2014)	**0M**	**1M**	**2M**	**3M**	**5.5M**	**6M**	**9M**	**12M**
A – 0106	36	33	33	32	28	N/A	25	24
B – 0101	34	30	29	27	25	N/A	22	21
C – 0102	34	31	31	30	28	N/A	27	25
D – 0103	34	31	31	30	28	N/A	27	26
E – 0105	35	31	31	30	27	N/A	28	25
B2 – 0511	30	26	25	22	N/A	19	17	17
M – 0512	33	32	32	31	N/A	29	28	24
N – 0513	33	31	32	30	N/A	28	26	24
D2 – 0514	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 75 : HA Content (B/Massachusetts B-YMG) for QIV Filled Syringes (30±2 ºC)

HA Content B-YMG at 30±2 ^{0}C					
Batch No.	**0W**	**1W**	**2W**	**3W**	**4W**
A – 0106	36	24	23	20	18
B – 0101	34	25	24	23	22
C – 0102	34	30	26	26	26
D – 0103	34	31	27	26	26
E – 0105	35	29	27	26	27
B2 – 0511	30	21	20	17	17
M – 0512	33	28	27	24	24
N – 0513	33	28	26	22	22
D2 – 0514	N/A	N/A	N/A	N/A	N/A

Table 76 : HA Content (B/Brisbane B-VIC) for QIV Filled Syringes at 5±3 ºC

HA Content for B-VIC at 5±3 ºC				**Spec: 24 - 36 µg/ml**				
Batch No. (RD-Fill-2014-)	**0M**	**1M**	**2M**	**3M**	**5.5M**	**6M**	**9M**	**12M**
A - 0106	35	31	31	31	29	N/A	26	26
B - 0101	32	28	28	27	24	N/A	22	20
C - 0102	32	29	29	29	26	N/A	25	25
D - 0103	34	30	30	30	27	N/A	26	26
E - 0105	31	29	29	29	26	N/A	26	25
B2 - 0511	29	28	26	24	N/A	20	20	14
M - 0512	32	33	32	32	N/A	29	26	21
N - 0513	32	32	32	32	N/A	28	26	19
D2 - 0514	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 77 : HA Content (B/Brisbane B-VIC) for QIV Filled Syringes at 30±2 ºC

HA Content for B-VIC at 30±2 ºC					
Batch No. (RD-Fill-2014-)	**0W**	**1W**	**2W**	**3W**	**4W**
A – 0106	35	24	21	20	20
B – 0101	32	25	22	20	19
C – 0102	32	26	26	25	26
D – 0103	34	28	26	25	25
E – 0105	31	26	26	25	24
B2 – 0511	29	21	19	18	18
M – 0512	32	28	27	26	26
N – 0513	32	27	26	24	22
D2 – 0514	N/A	N/A	N/A	N/A	N/A

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Table 78 : VES Content (µg/ml) for QIV Filled Syringes at 5±3 ºC

Batch No. (RD-Fill-2014-)	**VES Content at 5±3 ºC**					**Spec: Target ± 20%**			
	Specification (µg/ml)	**0M**	**1M**	**2M**	**3M**	**5.5M**	**6M**	**9M**	**12M**
A – 0106	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
B – 0101	426-640	521	546	528	520	518	N/A	494	492
C – 0102	144-216	162	178	175	175	171	N/A	164	163
D – 0103	160-240	195	204	201	197	192	N/A	183	183
E – 0105	180-270	217	226	224	218	214	N/A	185	182
B2 – 0511	426-640	516	519	524	516	N/A	505	492	483
M – 0512	106-156	125	125	125	123	N/A	121	119	118
N – 0513	104-156	126	125	125	123	N/A	121	118	116
D2 – 0514	150-240	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 79 : VES Content (µg/ml) for QIV Filled Syringes at 30±2 ºC

Batch No. (RD-Fill-2014-)	**0W**	**1W**	**2W**	**3W**	**4W**
A – 0106	N/A	N/A	N/A	N/A	N/A
B – 0101	521	508	501	475	469
C – 0102	162	163	165	160	160
D – 0103	195	188	187	184	181
E – 0105	217	209	213	204	203
B2 – 0511	516	501	485	476	460
M – 0512	125	123	116	114	110
N – 0513	126	123	117	115	111
D2 - 0514	N/A	N/A	N/A	N/A	N/A

Table 80: Sterility Data for Filled Syringes at 5±3 ºC

Batch No. (RD-Fill-2014-)	**Sterility at 5±3 ^{0}C**						**Spec: No growth**	
	0M	**1M**	**2M**	**3M**	**5.5M**	**9M**	**12M**	**18M**
A – 0106	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
B – 0101	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
C – 0102	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
D – 0103	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
E – 0105	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
B2 – 0511	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
M – 0512	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
N – 0513	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A
D2 – 0514	No growth	N/A	N/A	N/A	N/A	N/A	No growth	N/A

Stability Data for Filled Syringes at 5±3 ºC and 30±2 ºC

Appearance

The appearance of batches RD-Fill-2014-B-0101, C-0102, D-0103, E-0105, B2-0511, M-0512, N-0513 and D2-0514 remain unchanged for 12 months at 5±3 ^{0}C. Batches RD-Fill-2014-A-0106 that contains no VES/Tween® 80/Triton X-100®, develops small amount of flocculated sediments after 1 to 3 months. The appearance of batches RD-Fill-2014-B-0101, C-0102, D-0103, E-0105, B2-0511, M-0512, N-0513 and D2-0514 remains unchanged for 4 weeks at 30±2 ^{0}C. At elevated temperature of 30 ^{0}C, flocculated sediments are seen after 2 weeks with batch RD-Fill-2014-A-0106 (Formula A).

Small amount of flocculated sediments is seen at the bottom of all batches with Formula A after 1 month at 5±3 ^{0}C. At elevated temperature of 30±2 ^{0}C, flocculated sediments appear after just 1 week. These floccules appear like protein precipitates. These data show that stabilisers are needed for QIV formulations.

pH

pH values for all batches RD-Fill-2014-A-0106, B-0101, C-0102, D-0103, E-0105, B2-0511, M-0512, N-0513 and D2-0514 remain unchanged at between 7.0 and 7.2 for 12 months 5 ± 3^{0}C. The pH values for all batches RD-Fill-2014-A-0106, B-0101, C-0102, D-0103, E-0105,

B2-0511, M-0512, N-0513 and D2-0514 remain unchanged at between 7.0 and 7.2 for 4 weeks 30±2 ^{0}C.

The pH of all the QIV formulations remain unchanged at between 7.0 and 7.2 throughout the 12 months at 5±3 ^{0}C and 4 weeks at 30±2 ^{0}C. Therefore, different amounts of stabilizer, storage temperature and duration of storage have no impact on the product pH.

HA Content (A/California H1N1-179A & A/Christchurch H1N1-74xp)

HA Contents for A/California H1N1-179A strain for all batches RD-Fill-2014-A-0106, B-0101, C-0102, D-0103, E-0105, B2-0511, M-0512, N-0513 and D2-0514 decline about 10 % during the first month at 5±3 ^{0}C. From the second month onwards the decrease is more gradual until 12 months. The HA Contents (for A/California H1N1-179A) for all batches of filled syringes RD-Fill-2014-A-0106, B-0101, C-0102, D-0103 and E-0105 decline markedly in the 4 weeks at 30±2 ^{0}C. HA Contents are below 24 µg/ml for all batches after 2 weeks at 30±2 ^{0}C except for batch A-0106. These data demonstrate that A/California H1N1-179A is very temperature-sensitive. In contrast, the HA contents for A/Christchurch H1N1-74xp batches RD-Fill-2014-B2-0511, M-0512 and N-0513 and D2-0514 are relatively stable (loss of 10 %) after 4 weeks. The greater thermal stability for the A/Christchurch H1N1-74xp strain is noted

At 30 ^{0}C the HA degrades at a fast rate such that after 2 weeks at 30^{0}C±2 ^{0}C, 5 of the batches have HA Content below 24 µg/ml. There is a large difference between the stability profiles for HA Content of the two H1N1 strains (A/California-179A and A/Christchurch-74xp). The HA from the A/Christchurch H1N1-74xp strains is much more stable at elevated temperature of 30 ^{0}C. However, this difference is not as obvious at the 5±3 ^{0}C where the HA Content for A/California H1N1-179A is stable after initial loss following 1 month of storage.

No real difference in HA Content stability is seen between the different formulae. This result shows that the stability of HA Content is more dependent on the storage temperature than the amounts or types of stabilizer present.

HA Content (A/Texas H3N2)

The HA Contents for A/Texas H3N2 strain for all batches RD-Fill-2014-A-0106, B-0101, C-0102, D-0103 and E-0105 remain within the proposed specifications over 6 months. Due to test method variability HA content for batch B-0101 (Formula B) is above proposed specification at 1 month test point at 5 ± 3^{0}C. HA Contents for batches RD-Fill-2014-B2-0511, M-0512 and N-0513 remain unchanged after 3 months. Batch RD-Fill-2014-D2-0514 is not tested for HA Content for A/Texas H3N2.

HA Contents for A/Texas H3N2 strain for batches RD-Fill-2014-B-0101, C-0102, D-0103, E-0105, B2-0511, M-0512 and N-0513 are relatively stable at 30 ± 2^{0}C for 4 weeks. This is within the method variability (about ±10 %). Batch RD-Fill-2014-A-0106 is much less stable such that after 2 weeks their HA Contents are at or below 24µg/ml. The instability with these two batches is due to absence of stabiliser.

HA Contents for the various batches A/Texas H3N2 appear to be very stable at 5±3^{0}C. All batches are within specifications of between 24 and 36 µg/ml. At the elevated temperature of 30 ^{0}C, one batch RD-Fill-2014-A-0106 is found to be less stable. The results show that A/Texas H3N2 requires inclusion of stabilisers for its stabilisation.

HA Content (B/Massachusetts -YMG)

The HA Contents for B/ Massachusetts B-YMG for all batches RD-Fill-2014-A-0106, B-0101, C-0102, D-0103 and E-0105 remain within the proposed specifications over 12 months at 5 ± 3^{0}C. Batch RD-Fill-2014-B-0101 seems to be declining faster than expected because it has a low starting point. HA Contents for batch RD-Fill-2014-B2-0511 decline by 16% after 2 months while batches RD-Fill-2014-M-0512 and N-0513 remain unchanged. Batch RD-Fill-2014-D2-0514 is not tested for HA Content for B/Massachusetts B-YMG.

HA Contents for B/ Massachusetts B-YMG for batches RD-Fill-2014-A-0106, B-0101, C-0102, D-0103, E-0105, B2-0511, M-0512 and N-0513 are declining relatively steadily at 30 ± 2^{0}C with batches A-0106 and B2-0101 being the most rapid.

Batches RD-Fill-2014-B-0101 and B2-0101 seem to be declining faster than expected because they have lower starting points. The instability with A-0110 is due to absence of stabiliser.

The HA Content results for the B/Massachusetts - YMG are all within specifications. All other stabiliser combinations containing VES, Tween® 80 and Triton X-100® are useful to stabilise the HA. At 5±3^{0}C, batch RD-Fill-2014-A-0106, which does not contain any stabiliser is relatively stable while for the FluLaval® Quadrivalent (RD-Fill-2014-B-0101 and B2-0511) with high contents of VES/Tween® 80 are unexpectedly showing least stability. Higher decrease in HA Contents are also seen at the elevated temperature for batches RD-Fill-2014-A-0106 and B2-0511. These anomalies can be due to analytical variability with the adapted SRID test method and low starting content for this batch. The results show that in QIV formulation HA content for B/Massachusetts-YMG require an addition of a combination of stabilisers in order to meet the proposed product shelf-life.

HA Content (B/Brisbane - VIC)

The HA Contents for B/Brisbane - VIC for batches RD-Fill-2014-A-0106, B-0101, C-0102, D-0103 and E-0105 remain within the limits of 24 to 36 µg/ml of targets for 12 months at 5 ± 3^{0}C. HA Contents for batches RD-Fill-2014-B2-0511, M-0512, N-0513 and D2-0514 remain within acceptable limits after 2 months. Batch RD-Fill-2014-B-0101 appears to trend lower than the other because of low starting point.

The HA Contents for B/Brisbane - VIC for all batches RD-Fill-2014-C-0102, D-0103, E-0105, B2-0511, M-0512, N-0513 and D2-0514 decline at 30 ± 2^{0}C rapidly after the first week then gradually over the 4 weeks. Batches RD-Fill-2014-A-0106 and B-0101 have the most obvious decline in HA Content. Batch RD-Fill-2014-B2-0511 has a low starting value.

All the HA Contents for B/Brisbane - VIC are within specifications. They all seem to show gentle declining trends following a faster decline over the first month of storage at 5±3^{0}C. At 30±2^{0}C, the HA contents are declining fastest for batches RD-Fill-2014-A-0106 (where no stabiliser has been added to the formula), B-0101 (stabiliser system as per FluLaval® Quadrivalent) and RD-Fill-2014-B2-0511 (stabiliser as per FluLaval® QIV). As with the B/Massachusetts strain, the FluLaval® Quadrivalent formula (batches RD-Fill-2014-B-0101 and B2-0511) are unexpectedly showing the worst stability. This anomaly can be due to analytical variability with the adapted SRID test method and low starting content for this batch. The results show that in QIV formulation HA content for B-VIC requires an addition of a combination of stabilisers in order to meet the proposed product shelf-life.

VES Content

VES Contents for all batches RD-Fill-2014-A-0106, B-0101, C-0102, D-0103 and E-0105 remain within the limits of ± 20% of targets for 12 months at 5±3^{0}C. VES Contents of batches RD-Fill-2014-B2-0511, M-0512, N-0513 and D2-0514 remain within the limits of ± 20% of targets for 12 months at 30±2^{0}C. VES Contents for all batches (RD-Fill-2014-) A-0106, B-0101, C-0102, D-0103, E-0105, B2-0511, M-0512, N-0513 and D2-0514 decline about 10% after 4 weeks at 30±2^{0}C. This decline in VES is linked to the increased rates of hydrolysis at high temperatures.

All the VES results are within acceptable limits of ± 20% around the targets. No real declining trend is visible over the 12 months. For the elevated condition of 30±2^{0}C, VES trend is more obvious for batch 0101-B where the VES concentration is the highest. This is to be expected as degradation of VES followed first order kinetics as seen in previous studies. Overall, the limits of ±20% will not be exceeded over the proposed shelf-life of the product of 12 months at 5±3^{0}C.

Sterility

Sterility is performed at the beginning of the stability testing program for all batches RD-Fill-2014-A-0106, B-0101, C-0102, D-0103, E-0105, B2-0511, M-0512, N-0513 and D2-0514. Sterility test is repeated for all batches at the end of the stability programs. All batches pass sterility tests at the start and end of the 12 months stability testing program.

Conclusions

Stability Studies on QIV Filled Syringes

Available 12 months real time (5 ± 3^{0}C) and 4 weeks accelerated (30 ± 2^{0}C) stability data show that QIV formulated in the PBS solution without any stabiliser result in formation of flocculated sediment and unstable HA. There is some decrease in the VES concentration in the formulations but all are within acceptable limits over 12 months.

This study shows that stabilisers are needed in order to prevent aggregations of some strains of HA proteins. There is evidence that some strains (particularly A/Texas H3N2) need stabiliser in order to maintain optimum HA content stability. Overall, batches containing a combination of stabilisers including VES, Tween® 80 and Triton X-100®, on the other hand, provide stabilisation effects to the HA antigens in the formulation.

Those formulae with reduced stabiliser concentrations (Formulae C, D and E) achieve the same or better stabilisation effects of HA when compared with the FluLaval® (Formula B) that has a very high concentration of VES and Tween® 80. These results confirm that reduced stabiliser concentration in the QIV final bulk formulation for splitting agents of Tween® 80 (NMT 80 µg/ml) and Triton X-100® (NMT 300 µg/ml) result in no loss of stability for HA content. Formulae with even lower concentrations in stabiliser (Formulae M and N) although appear promising to ensure product stability.

Available 12 month real time stability data support the proposed shelf-life of 12 months (at 5 ± 3^{0}C) for the QIV filled syringes in Formulae C, D and E. Based on the satisfactory abnormal toxicity and stability data and considering the needs to have minimum additives in a vaccine and a robust stabiliser system to cater for anticipated changes in viral strains from year to year, it is recommended that Formula C be chosen for further stability evaluations.

Claims

1. A vaccine composition comprising a split influenza virus preparation or subunit influenza virus preparation and a pharmaceutically acceptable excipient said excipient comprising polyoxyethylene sorbitan monooleate (Tween® 80 or Polysorbate 80), alpha-tocopherol and t-octylphenoxypolyethoxyethanol (Triton X-100®), wherein the amount of polyoxyethylene sorbitan monooleate per human dose is ≤200 µg and the amount of t-octylphenoxypolyethoxyethanol per human dose is ≥100 µg.

2. The vaccine composition of claim 1 wherein the derivative of alpha tocopherol is alpha tocopherol succinate.

3. The vaccine composition of claims 1 or 2 wherein the amount of polyoxyethylene sorbitan monooleate per human dose is from 20 to 200 µg and the amount of t-octylphenoxypolyethoxyethanol per human dose is from 100 to 600 µg.

4. The vaccine composition of any preceding claim wherein the amount of polyoxyethylene sorbitan monooleate per human dose is from 50 to 150 µg and the amount of t-octylphenoxypolyethoxyethanol per human dose is from 100 to 400 µg.

5. The vaccine composition of any preceding claim wherein the amount of polyoxyethylene sorbitan monooleate per human dose is (i) from 20 to 50 µg per 0.25 ml paediatric dose or (ii) from 60 to 90µg per 0.5 ml adult dose.

6. The vaccine composition of any preceding claim wherein the amount of Triton X-100™ per human dose is (i) from 120 to 170µg per 0.25 ml paediatric dose or (ii) from 250 to 350µg per 0.5ml adult dose.

7. A multi-dose or single dose vial comprising the vaccine composition of any one of claims 1 to 6.

8. A process for producing a vaccine composition comprising the steps of: (i) preparing a split influenza virus preparation or subunit influenza preparation, (ii) optionally inactivating the split influenza virus preparation before or after the splitting step, (iii)

purifying and filtering said preparation and (iv) adding a pharmaceutically acceptable excipient to the preparation, said excipient comprising the following stabilisers: polyoxyethylene sorbitan monooleate, alpha-tocopherol and t-octylphenoxypolyethoxyethanol, wherein the concentration of polyoxyethylene sorbitan monooleate is ≤200 µg/ml and the concentration of t-octylphenoxypolyethoxyethanol is from 300 to 700 µg/ml.

9. The process of claim 8 wherein the wherein the concentration of polyoxyethylene sorbitan monooleate is from 50 to 200 µg/ml and the concentration of t-octylphenoxypolyethoxyethanol is from 400 to 600 µg/ml.

10. The process of claim 8 or 9 further comprising storage of the vaccine composition in bulk after excipient has been added.

11. The process of any one of claims 8-10 further comprising putting the vaccine composition containing excipient into individual or multi-dose vials ready for storage or administration.

12. A vaccine composition according to any one of claims 1 to 6 for use in medicine.

13. A vaccine composition according to any one of claims 1 to 6 for use in the treatment, prevention and/or vaccination against disease caused by influenza.

14. A method of inducing an immune response in a human subject, said method comprising administering to the subject a vaccine composition of any one of claims 1 to 6.

15. A method of treatment, prevention and/or vaccination against influenza disease, comprising the administration of a vaccine composition according to any one of claims 1 to 6 to a person in need thereof (such as an elderly person age 50 or over, particularly age 65 or over).

Figure 1

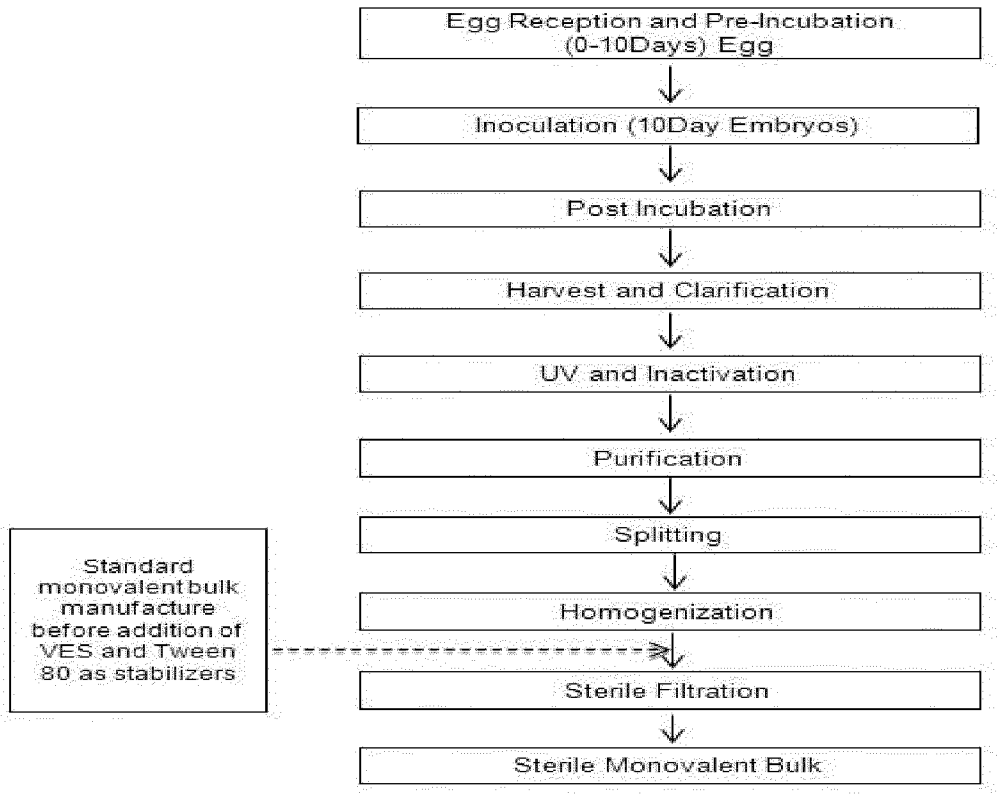

Egg Reception and Pre-Incubation (0-10Days) Egg
Inoculation (10Day Embryos)
Post Incubation
Harvest and Clarification
UV and Inactivation
Purification
Splitting
Homogenization
Sterile Filtration
Sterile Monovalent Bulk
Standard monovalent bulk manufacture before addition of VES and Tween 80 as stabilizers

Figure 2

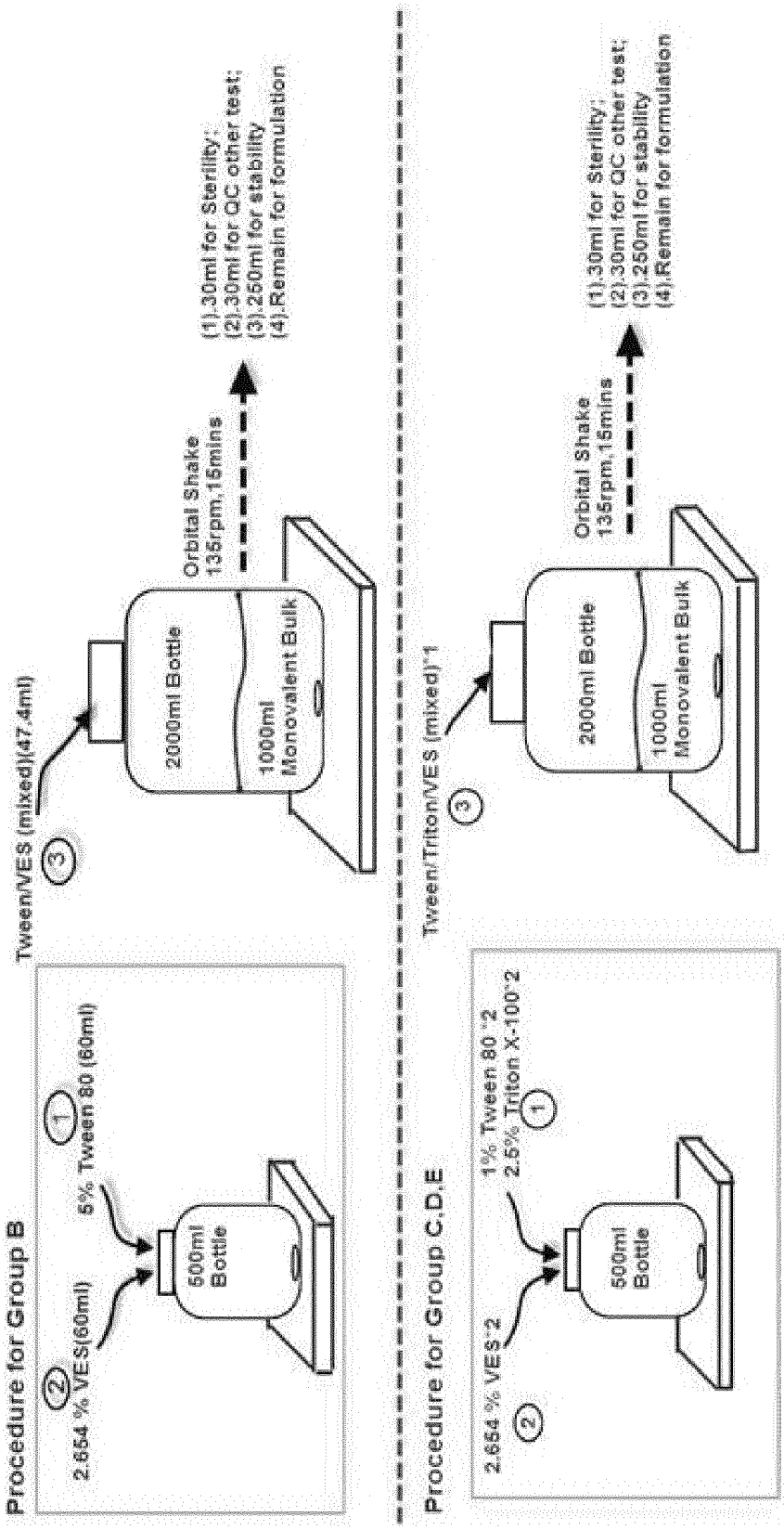

Procedure for Group B
2.654 % VES(60ml)
5% Tween 80 (60ml)
500ml Bottle
Tween/VES (mixed)(47.4ml)
2000ml Bottle
1000ml Monovalent Bulk
Orbital Shake 135rpm,15mins
(1).30ml for Sterility;
(2).30ml for QC other test;
(3).250ml for stability
(4).Remain for formulation
Procedure for Group C,D,E
2.654 % VES*2
1% Tween 80 *2
2.5% Triton X-100*2
500ml Bottle
Tween/Triton/VES (mixed)*1
2000ml Bottle
1000ml Monovalent Bulk
Orbital Shake 135rpm,15mins
(1).30ml for Sterility;
(2).30ml for QC other test;
(3).250ml for stability
(4).Remain for formulation

Figure 3

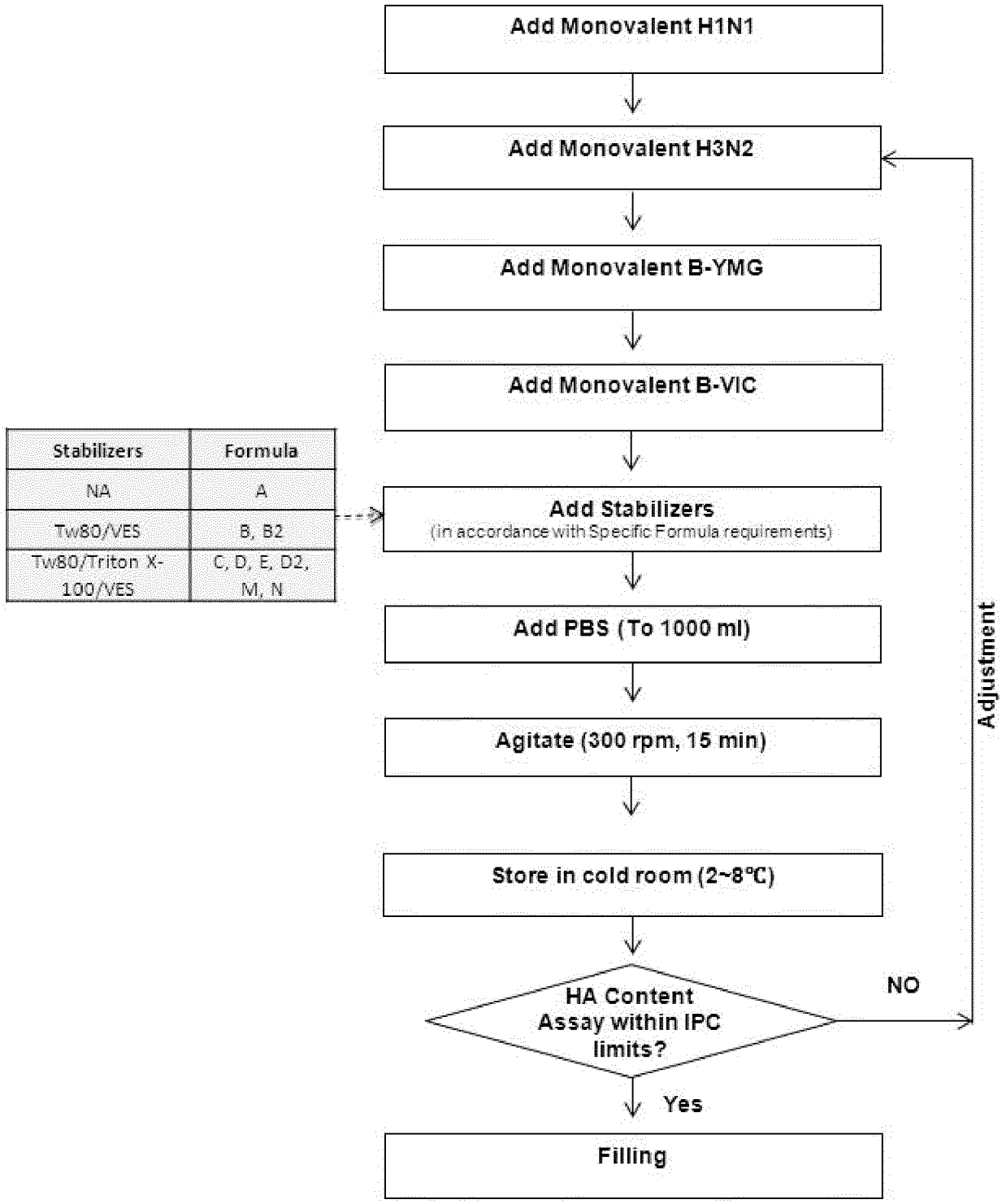

Add Monovalent H1N1
Add Monovalent H3N2
Add Monovalent B-YMG
Add Monovalent B-VIC
Stabilizers
Formula
NA
A
Tw80/VES
B, B2
Tw80/Triton X-100/VES
C, D, E, D2, M, N
Add Stabilizers
(in accordance with Specific Formula requirements)
Add PBS (To 1000 ml)
Agitate (300 rpm, 15 min)
Store in cold room (2~8℃)
HA Content Assay within IPC limits?
NO
Adjustment
Yes
Filling

Figure 4

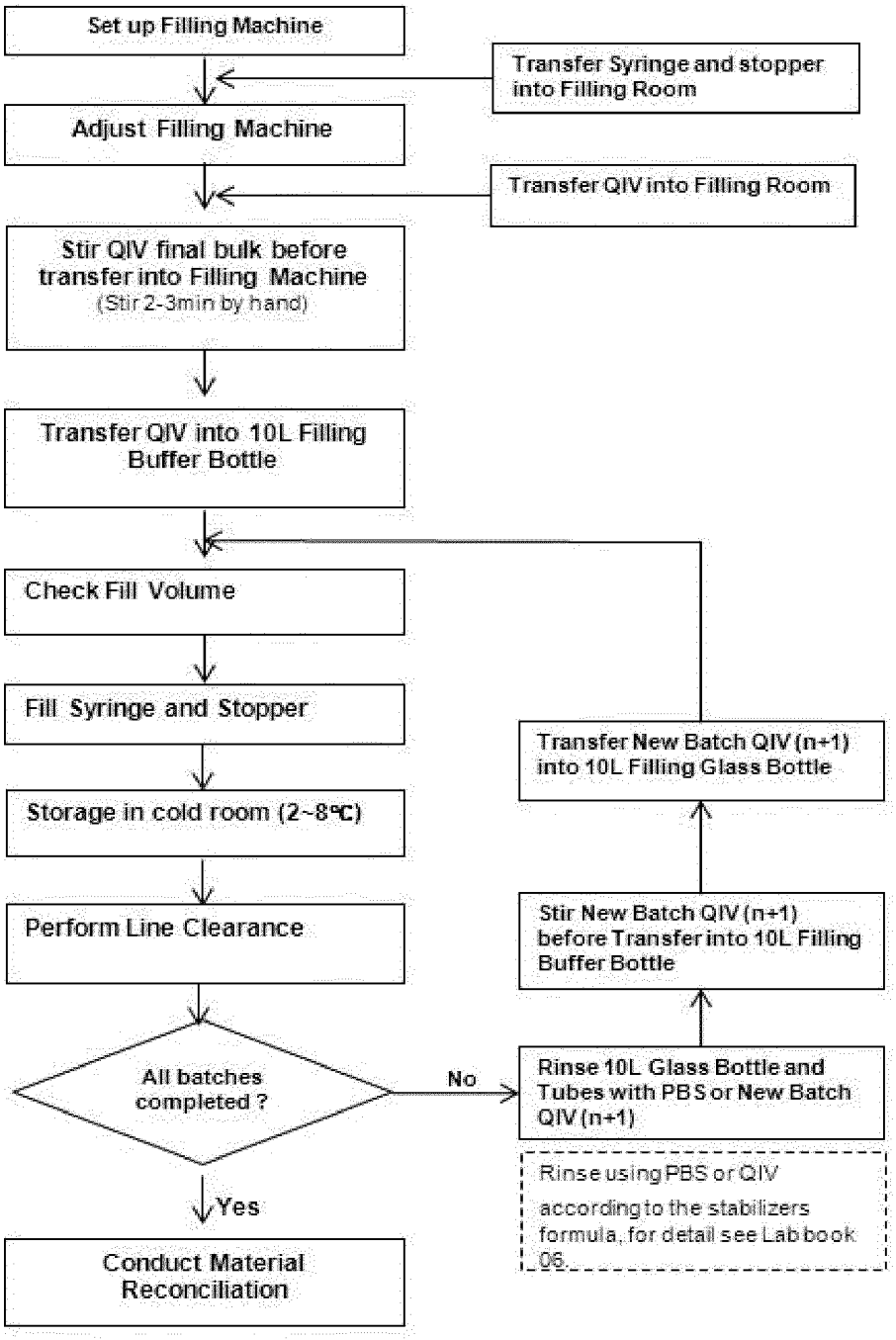

Set up Filling Machine
Transfer Syringe and stopper into Filling Room
Adjust Filling Machine
Transfer QIV into Filling Room
Stir QIV final bulk before transfer into Filling Machine
(Stir 2-3min by hand)
Transfer QIV into 10L Filling Buffer Bottle
Check Fill Volume
Fill Syringe and Stopper
Storage in cold room (2~8℃)
Perform Line Clearance
All batches completed ?
No
Yes
Conduct Material Reconciliation
Rinse 10L Glass Bottle and Tubes with PBS or New Batch QIV (n+1)
Rinse using PBS or QIV according to the stabilizers formula, for detail see Lab book 06
Stir New Batch QIV (n+1) before Transfer into 10L Filling Buffer Bottle
Transfer New Batch QIV (n+1) into 10L Filling Glass Bottle

Figure 5

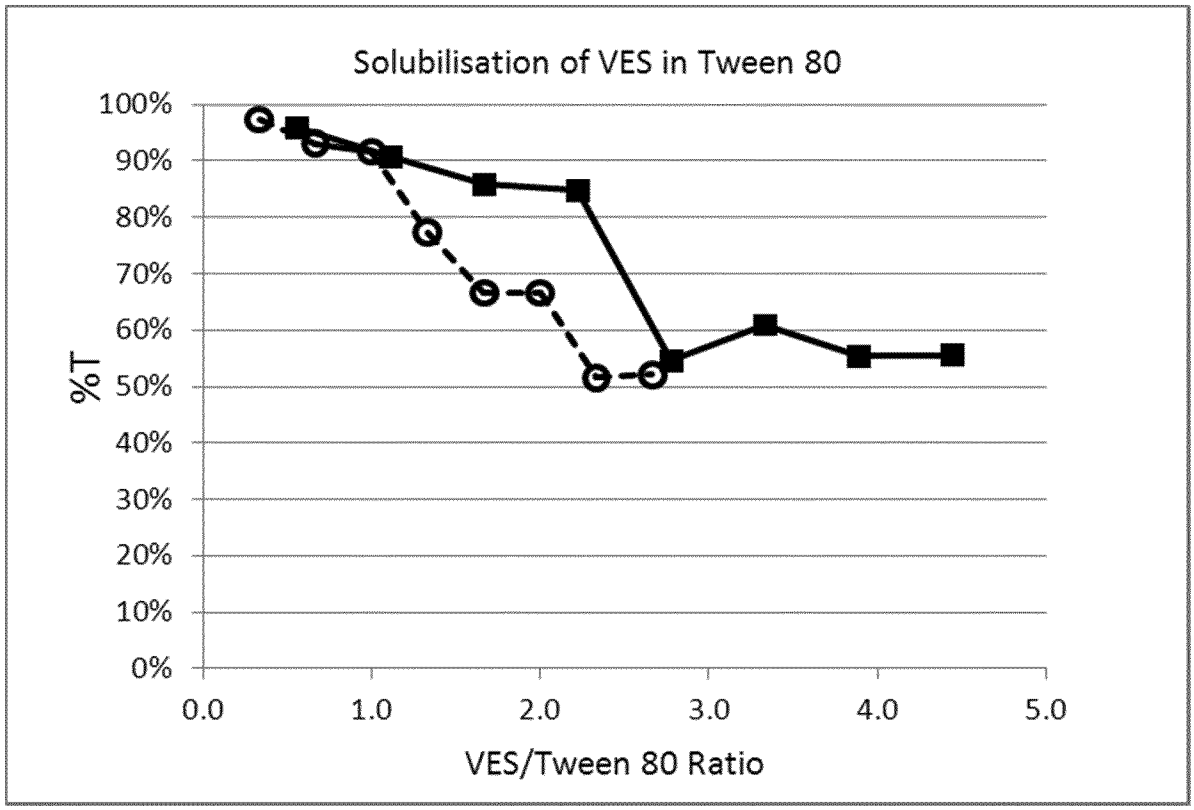


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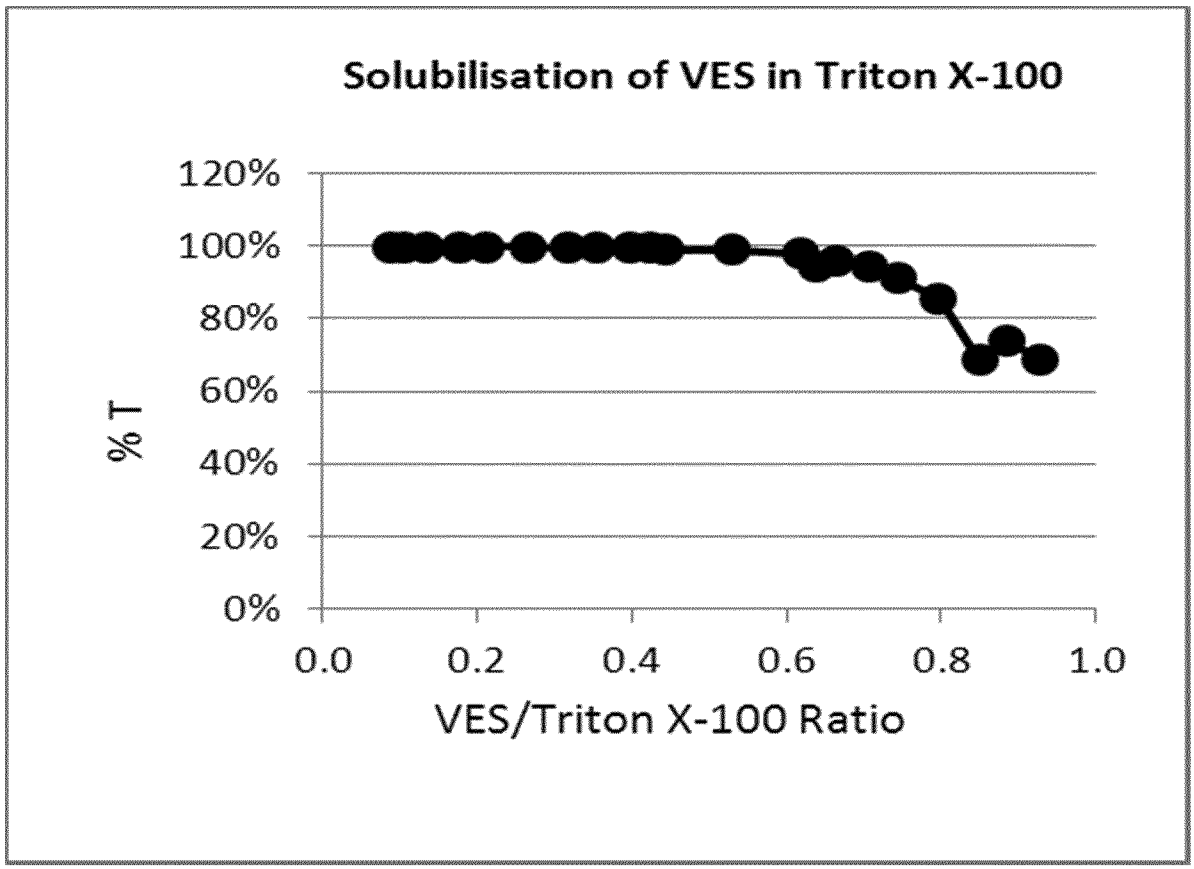


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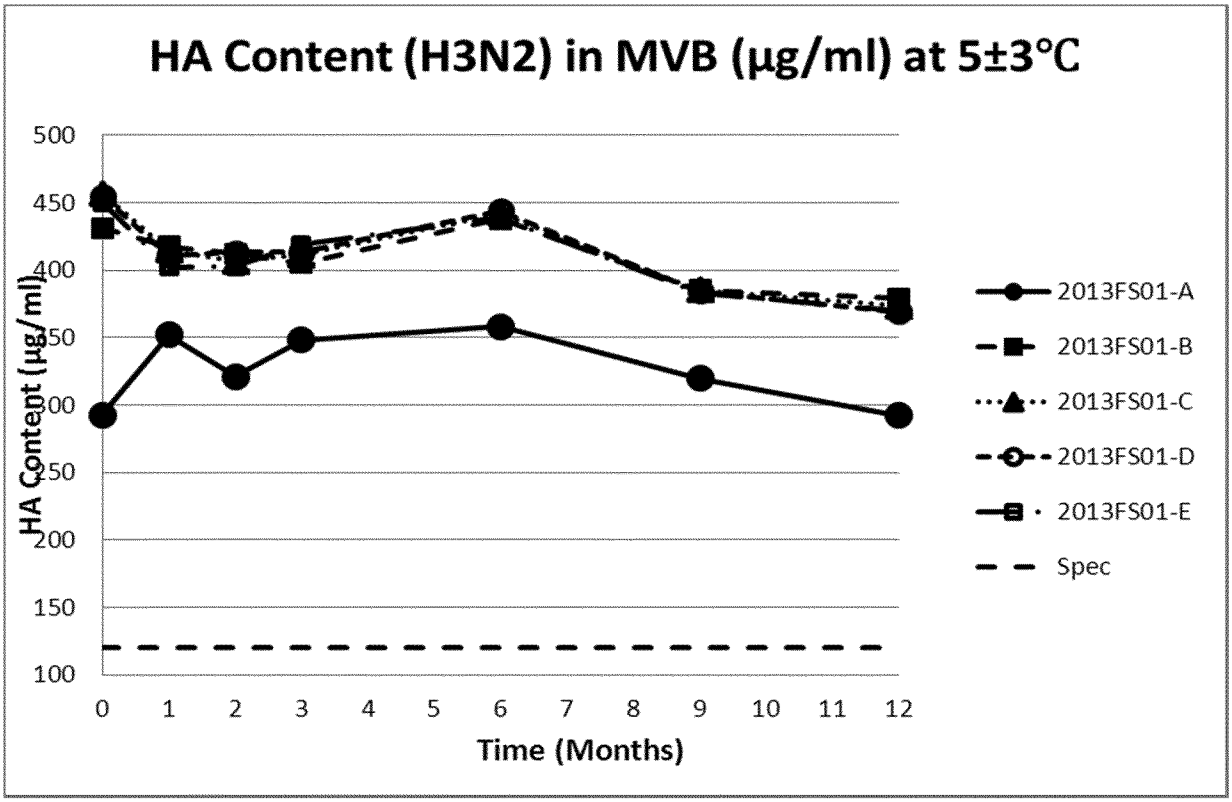


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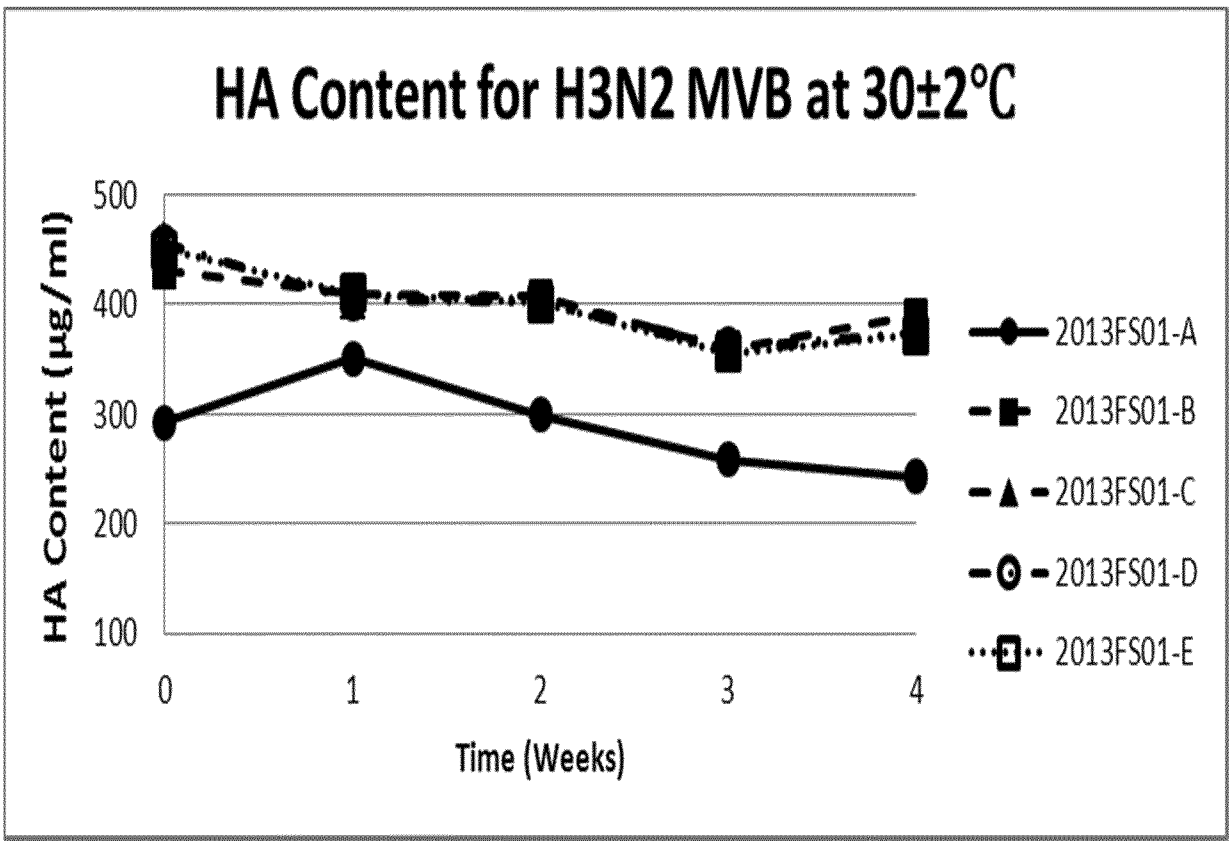


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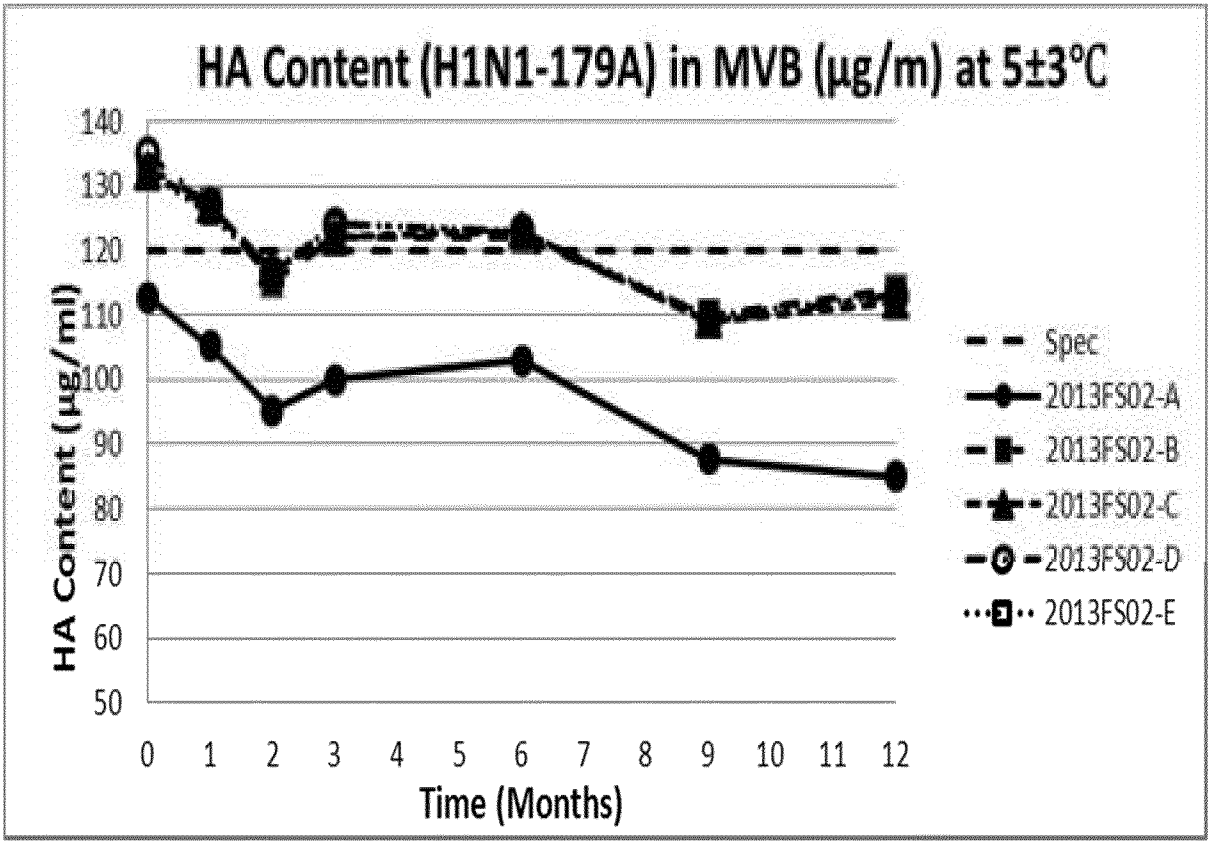


Figure 10

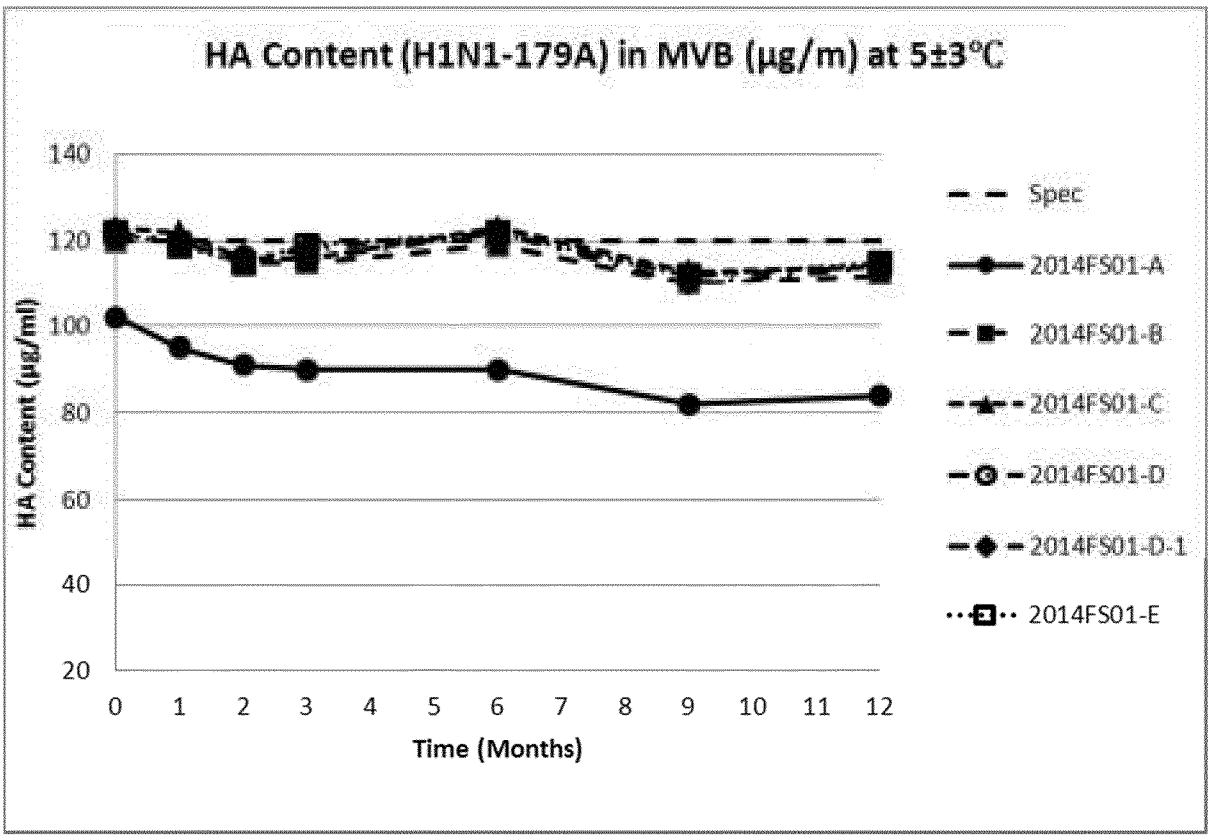


Figure 11

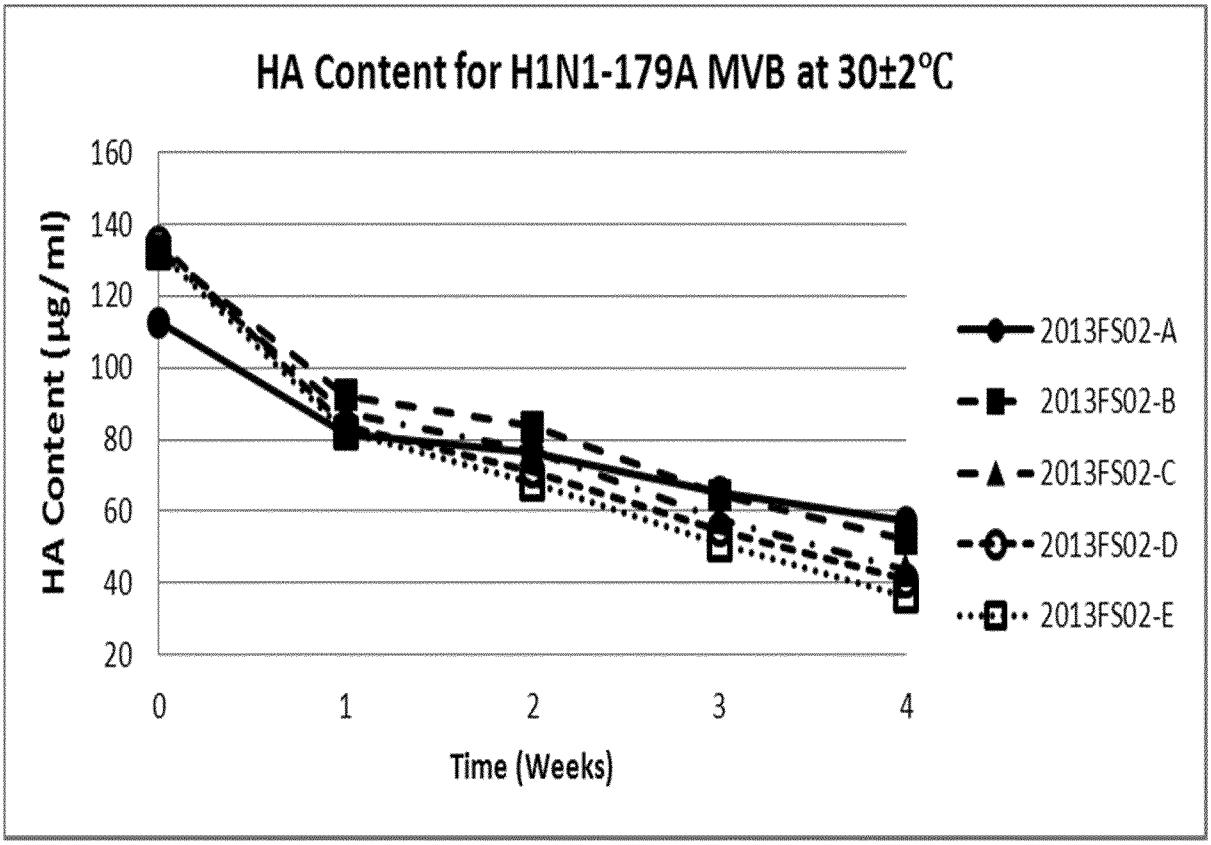


Figure 12

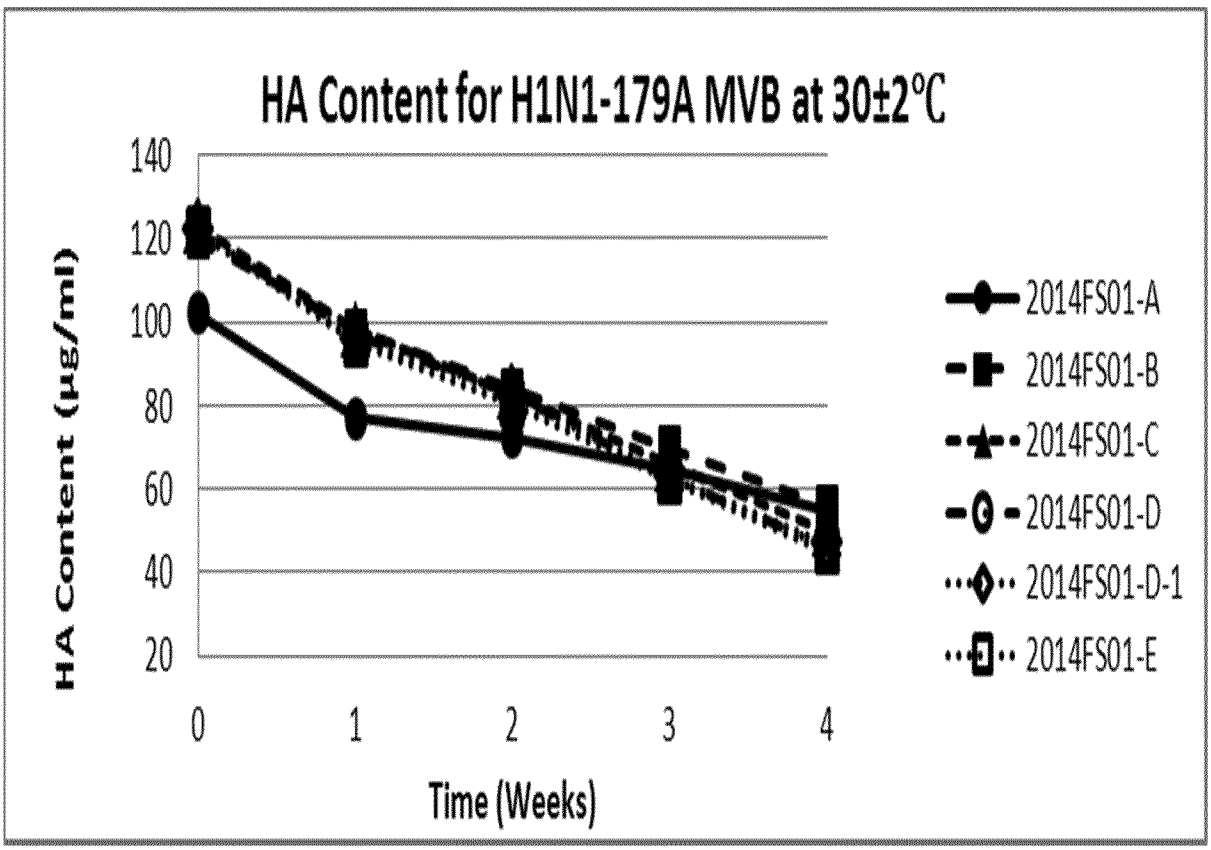


Figure 13

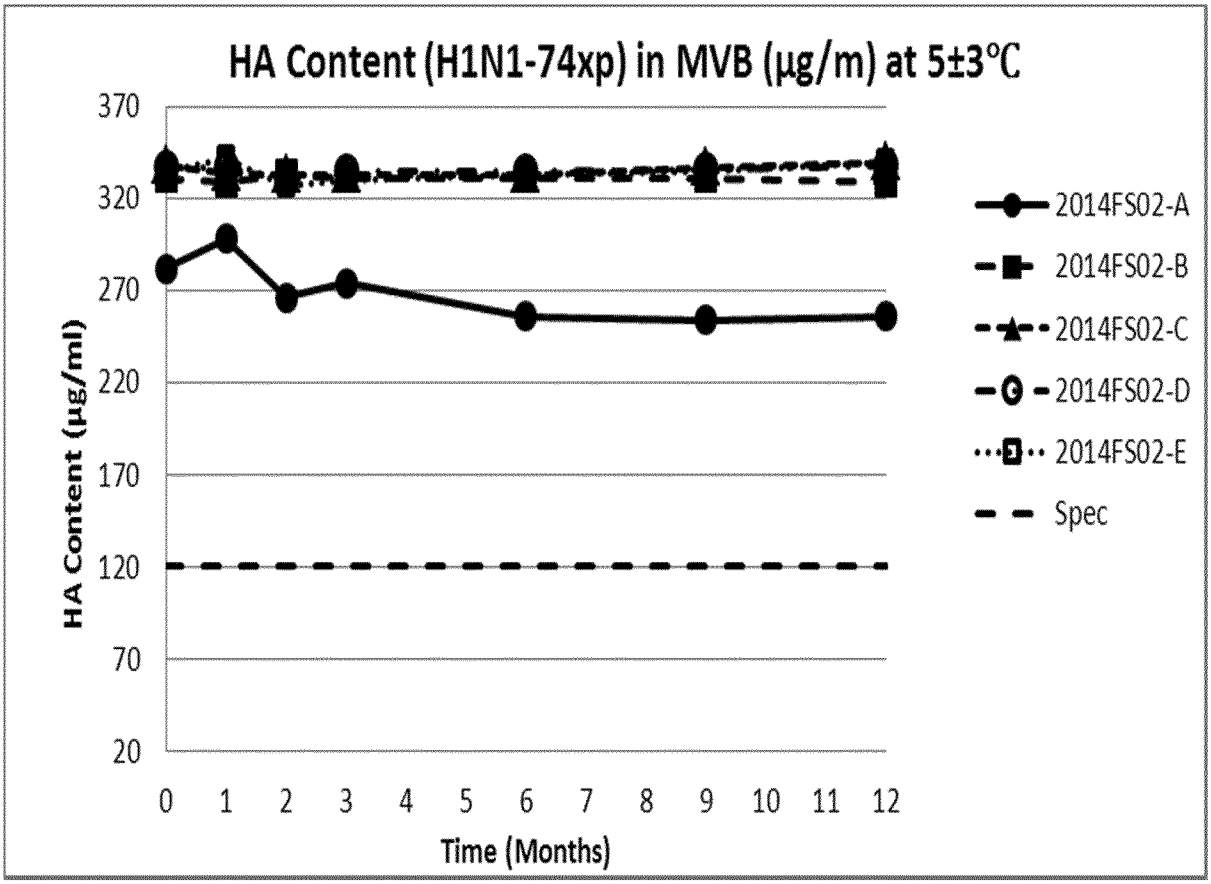


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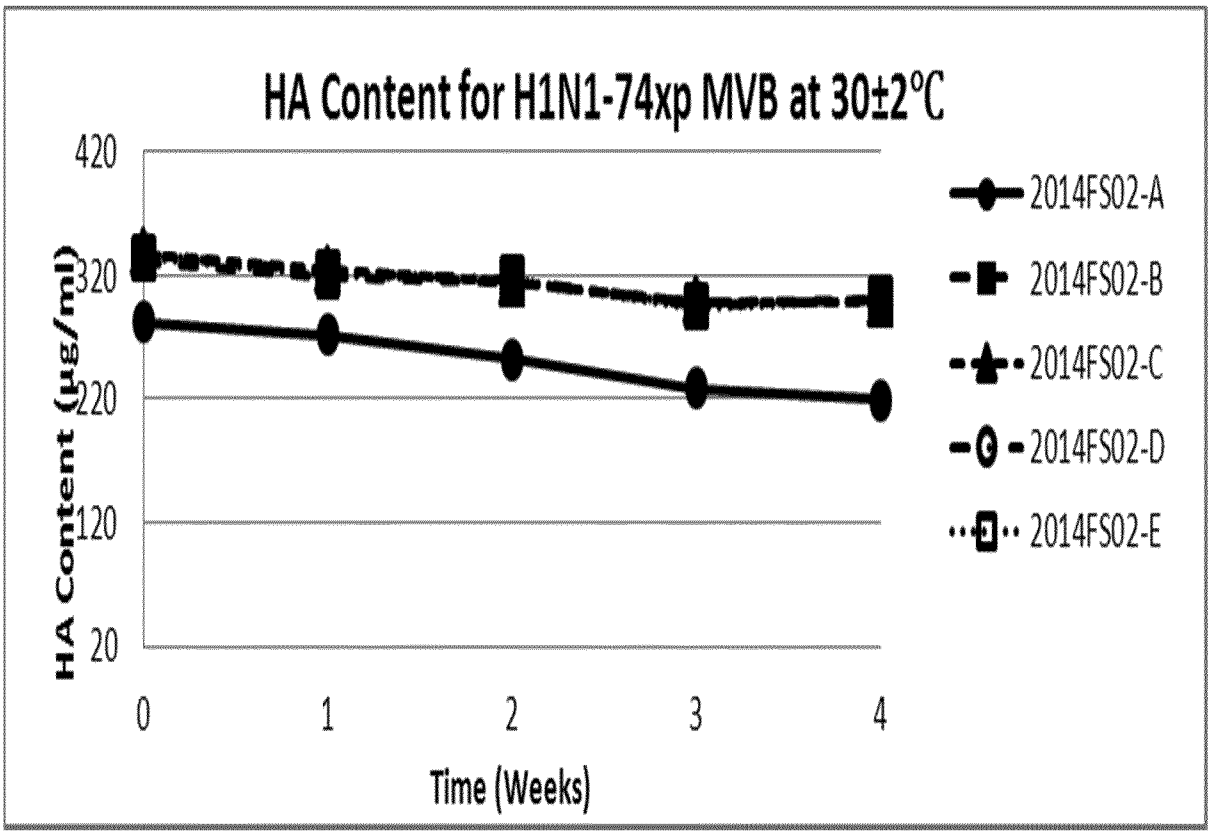


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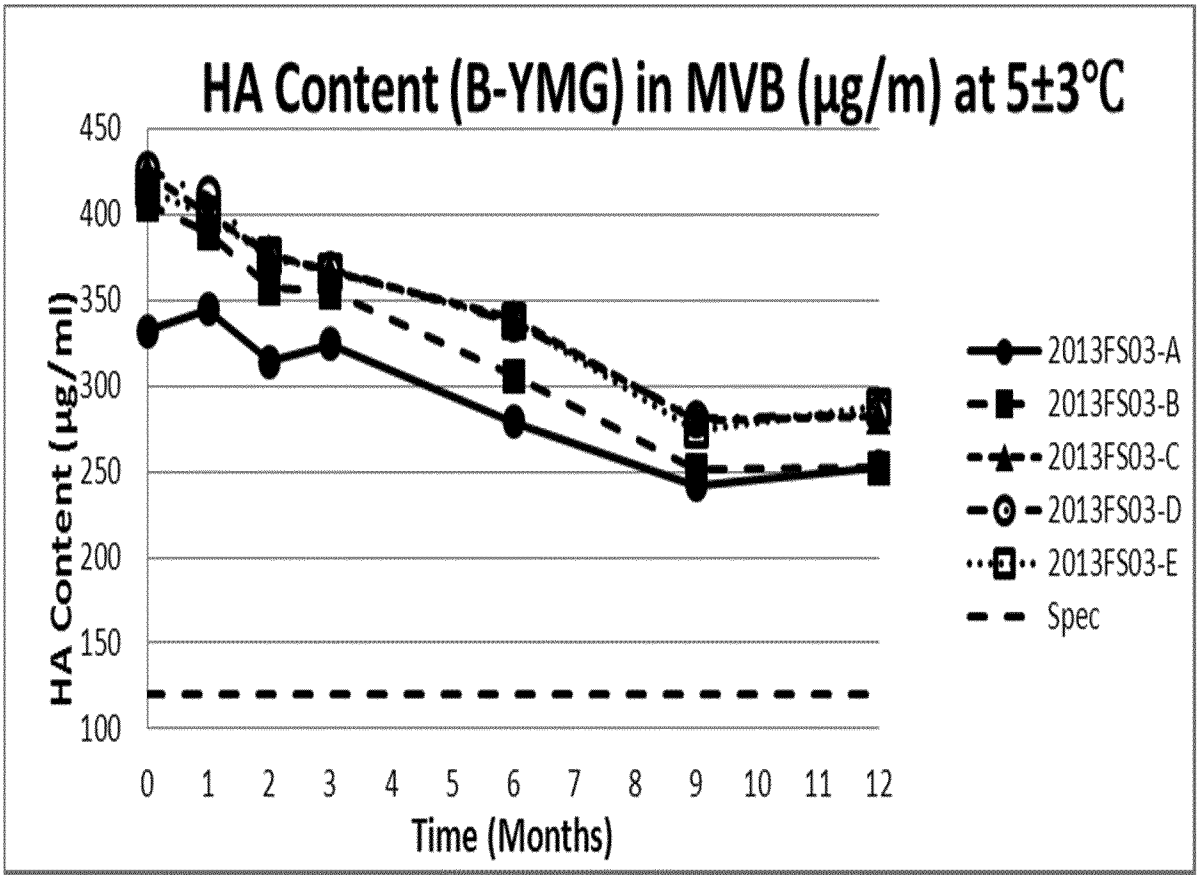


Figure 16

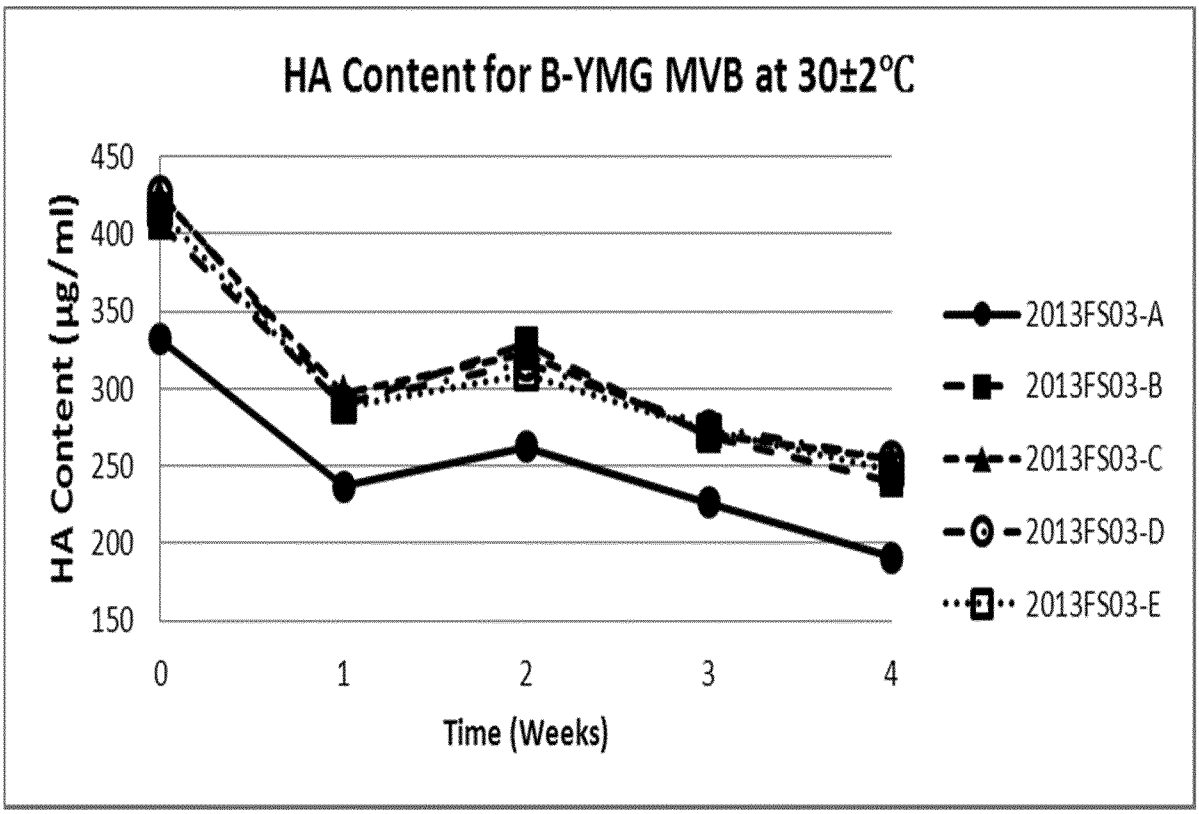


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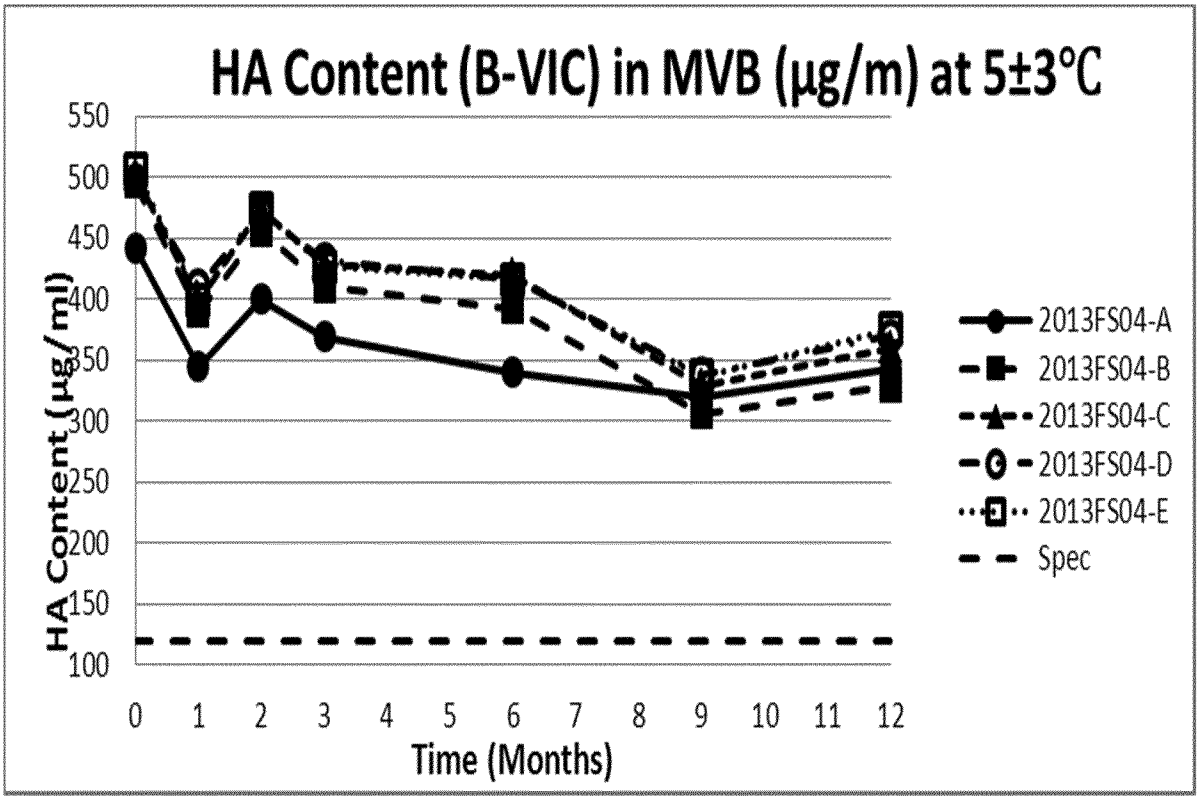


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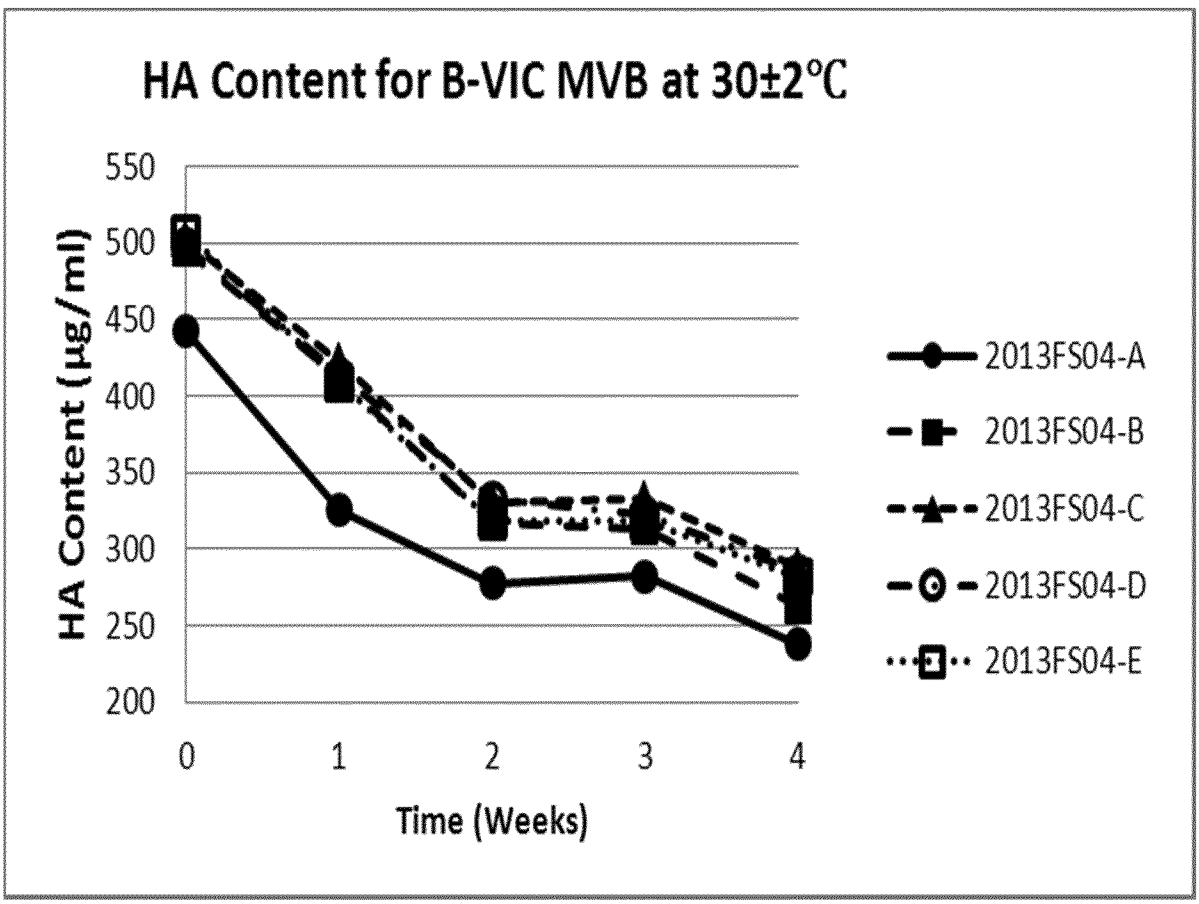


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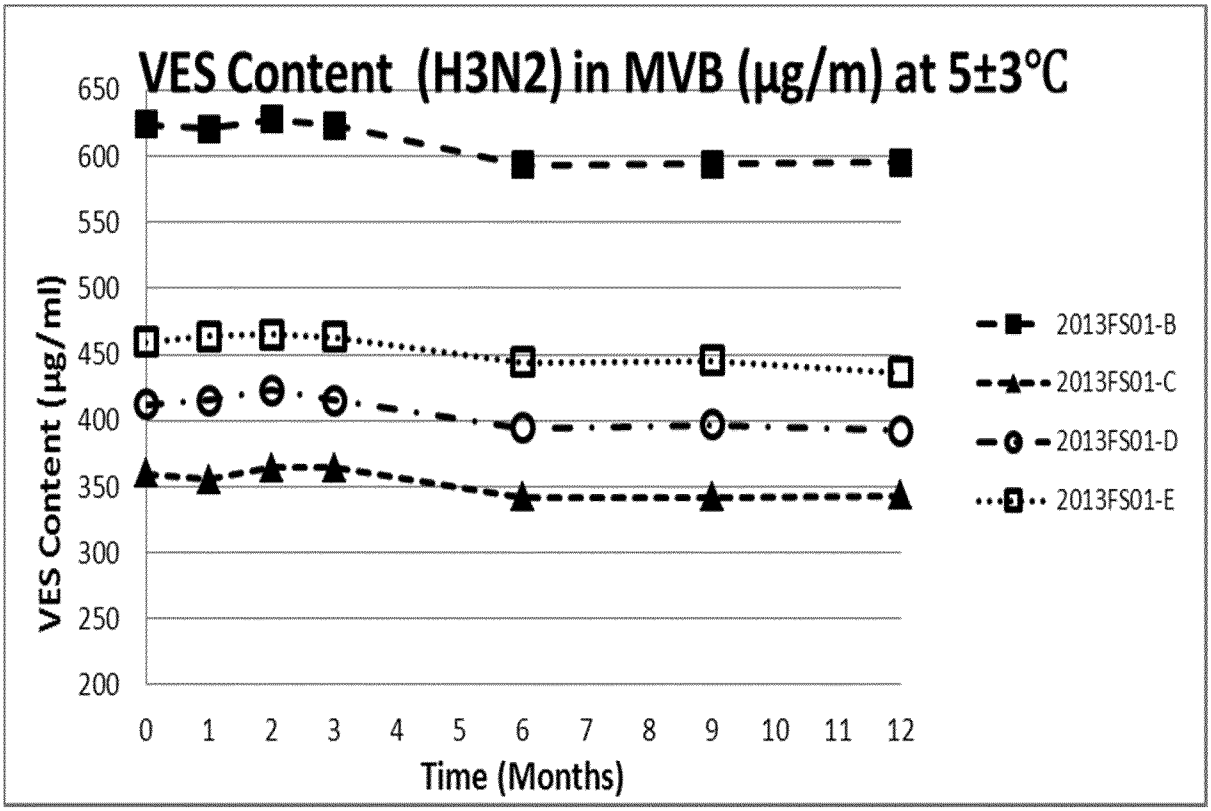


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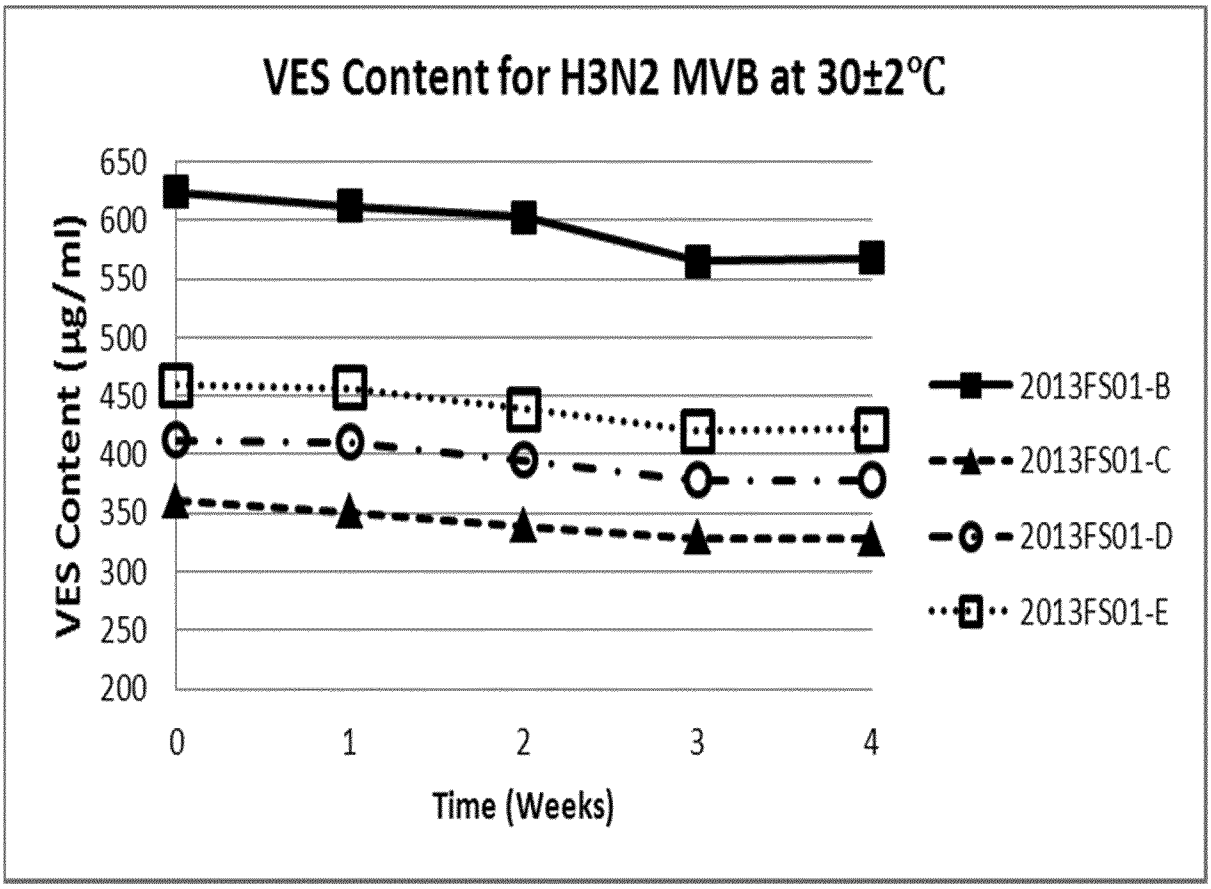


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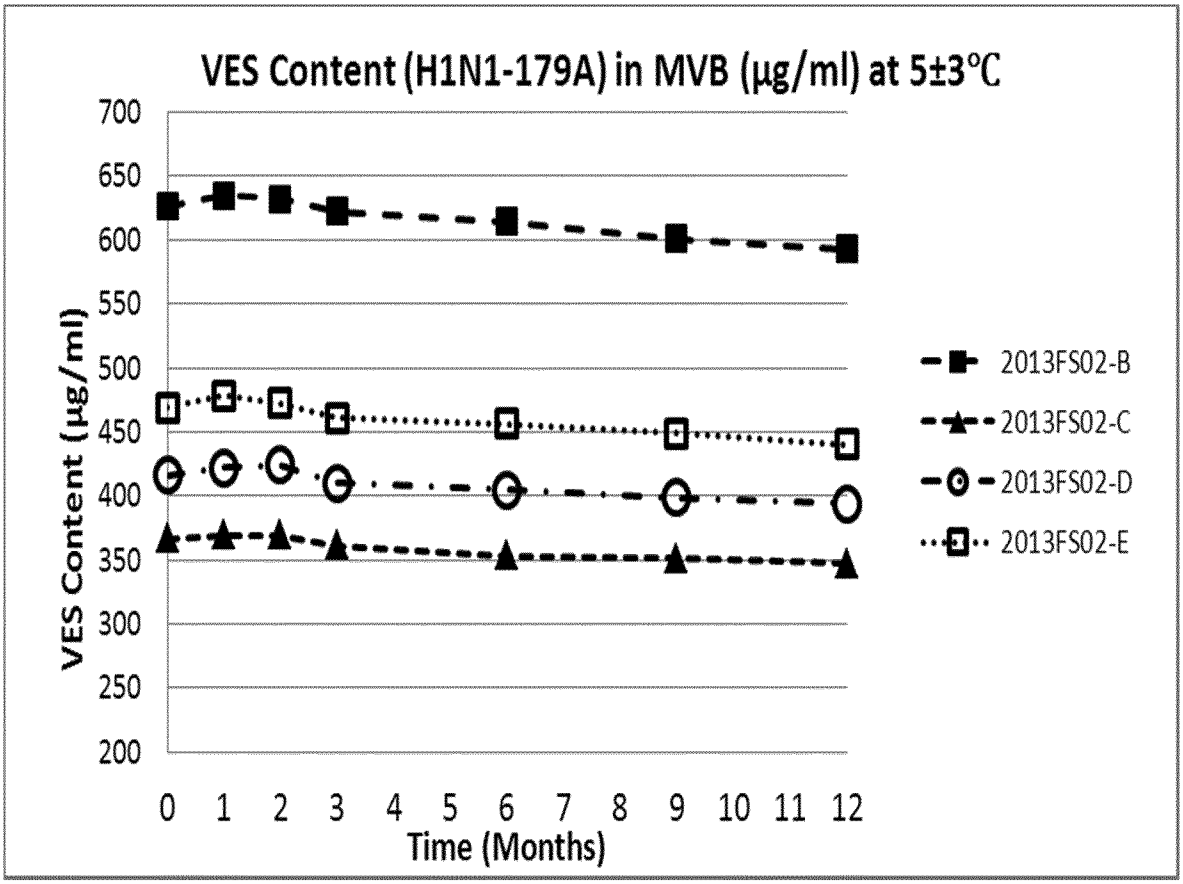


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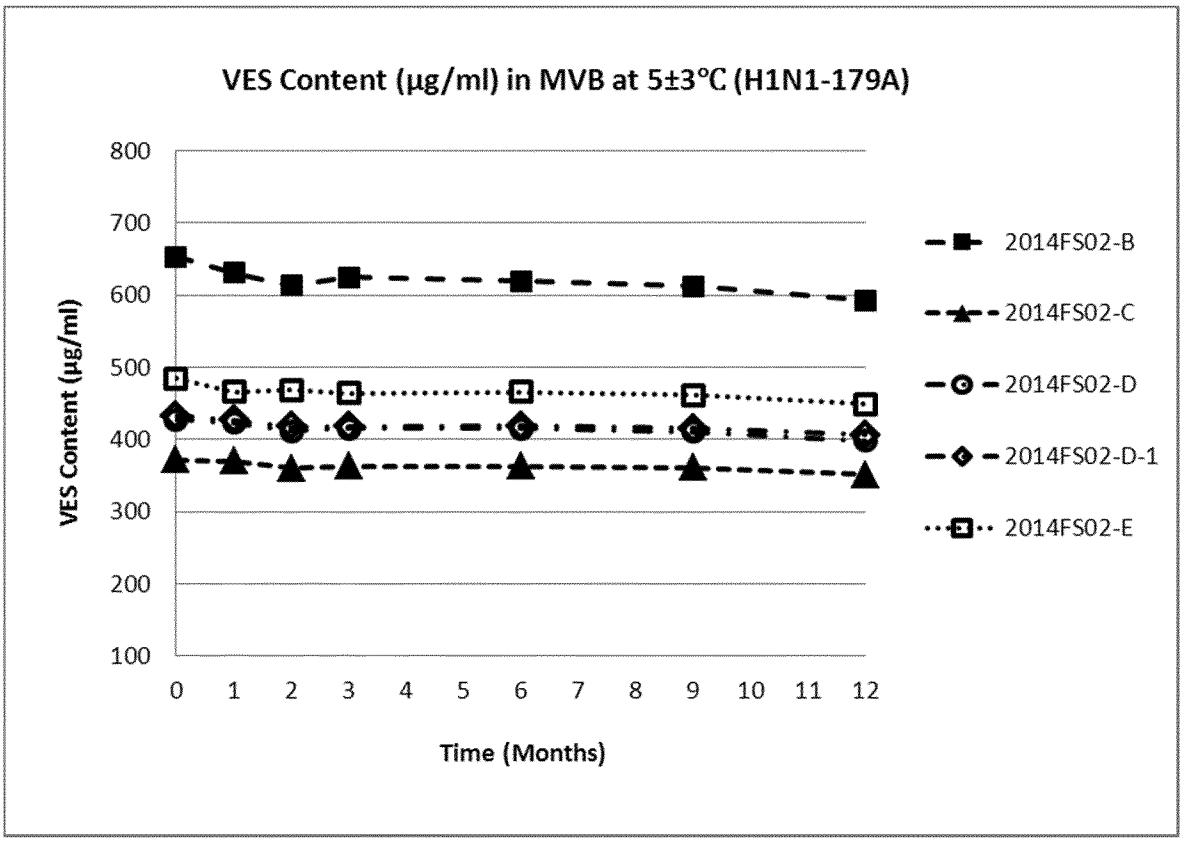


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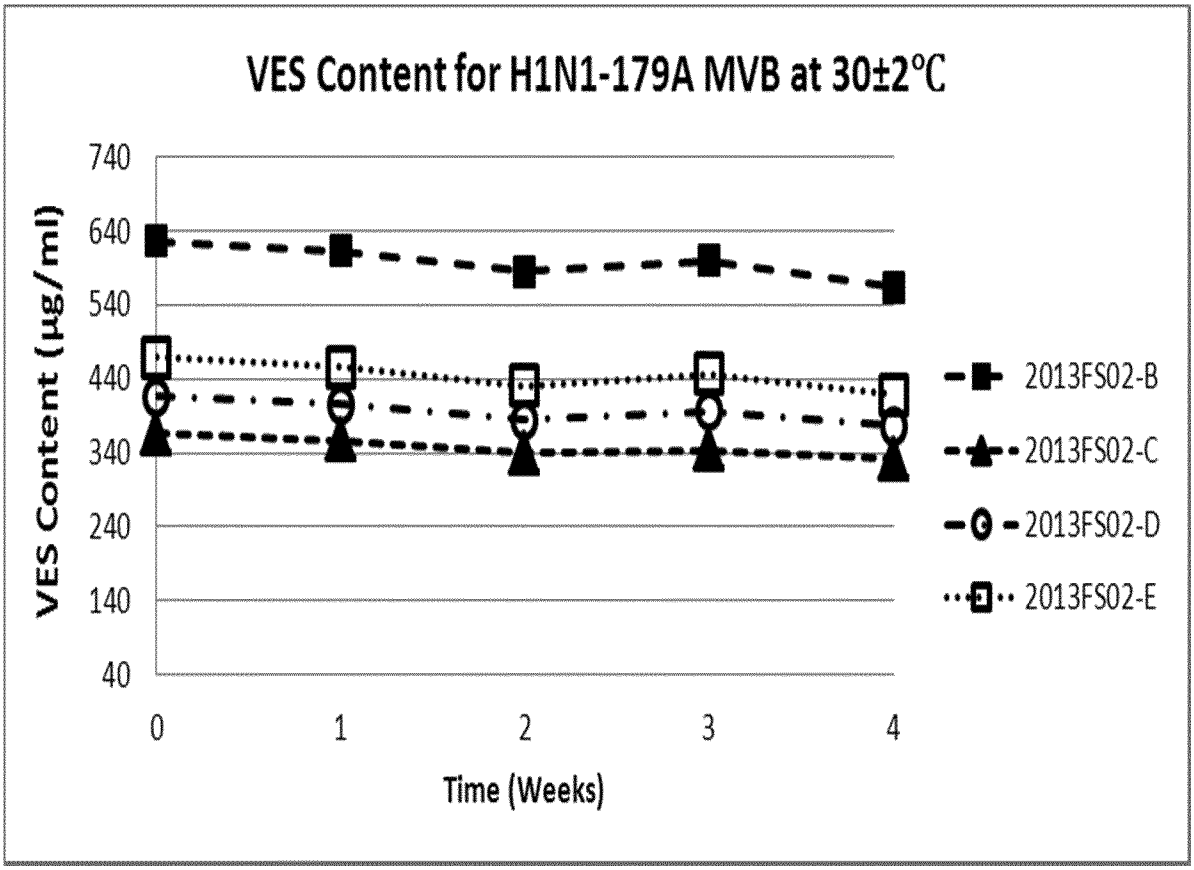


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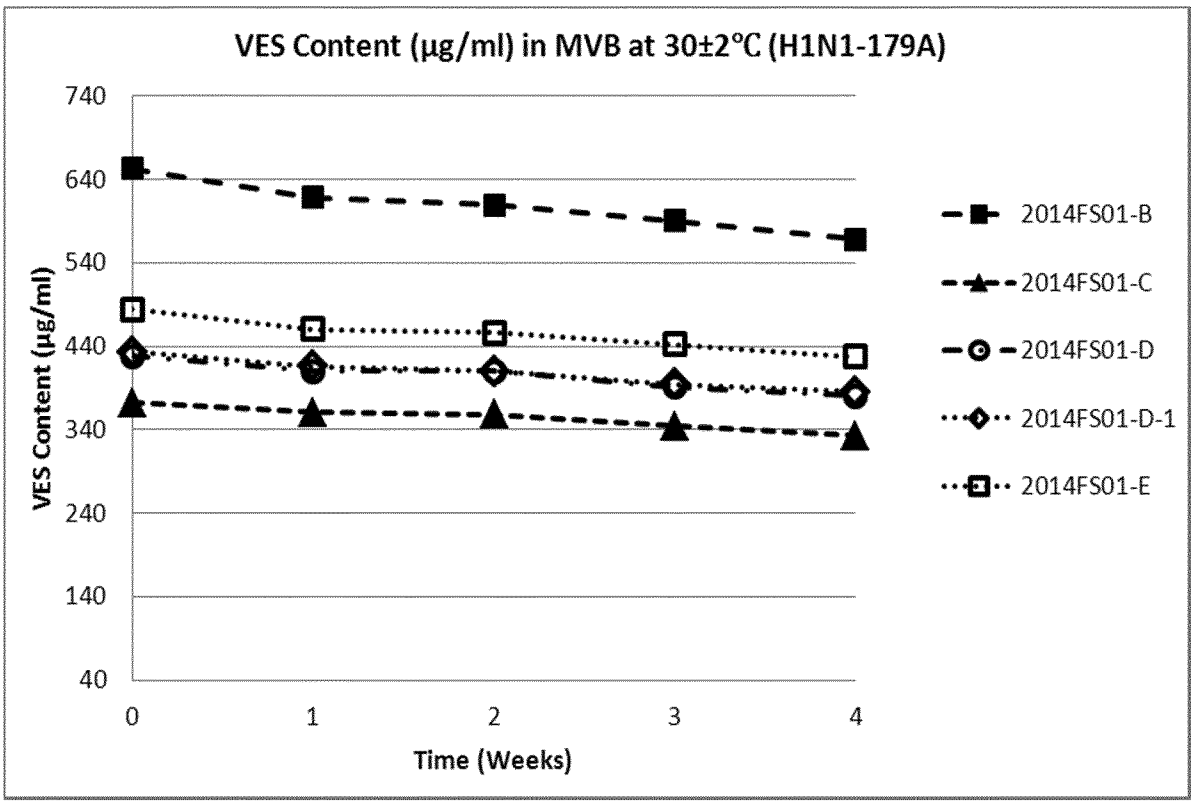


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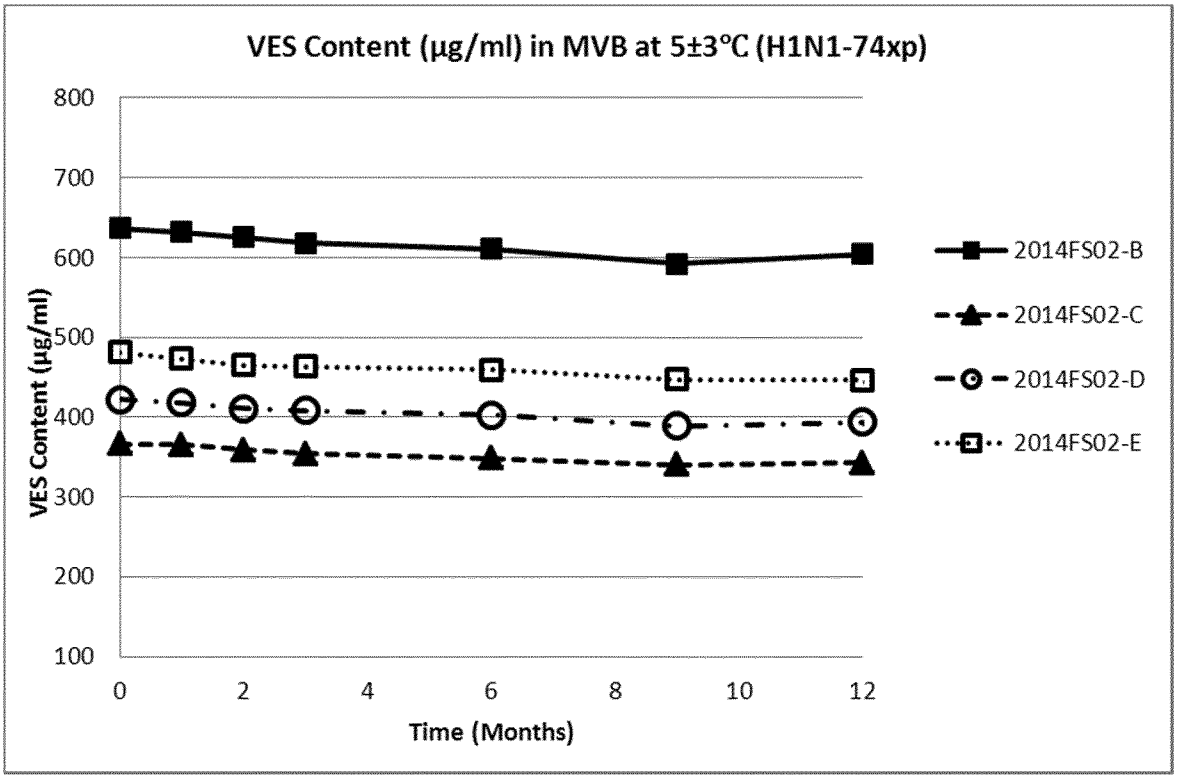


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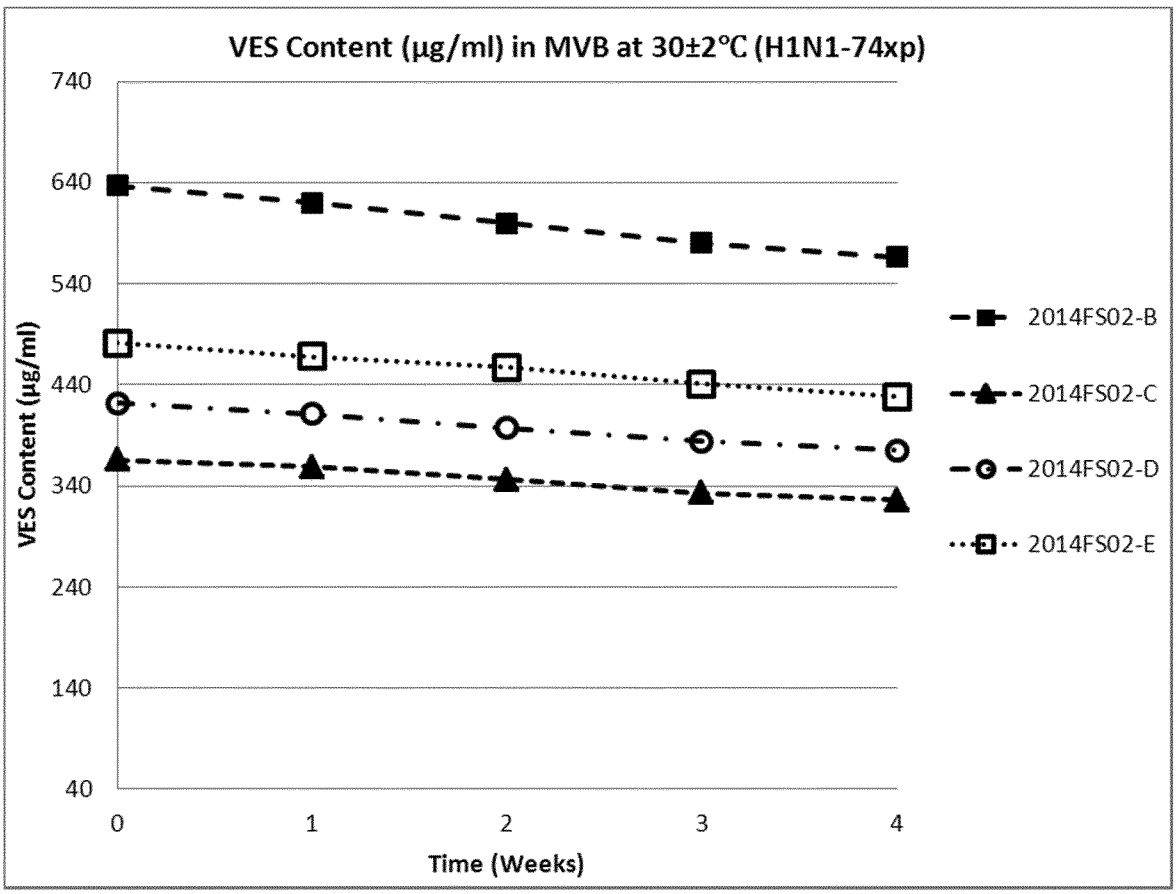


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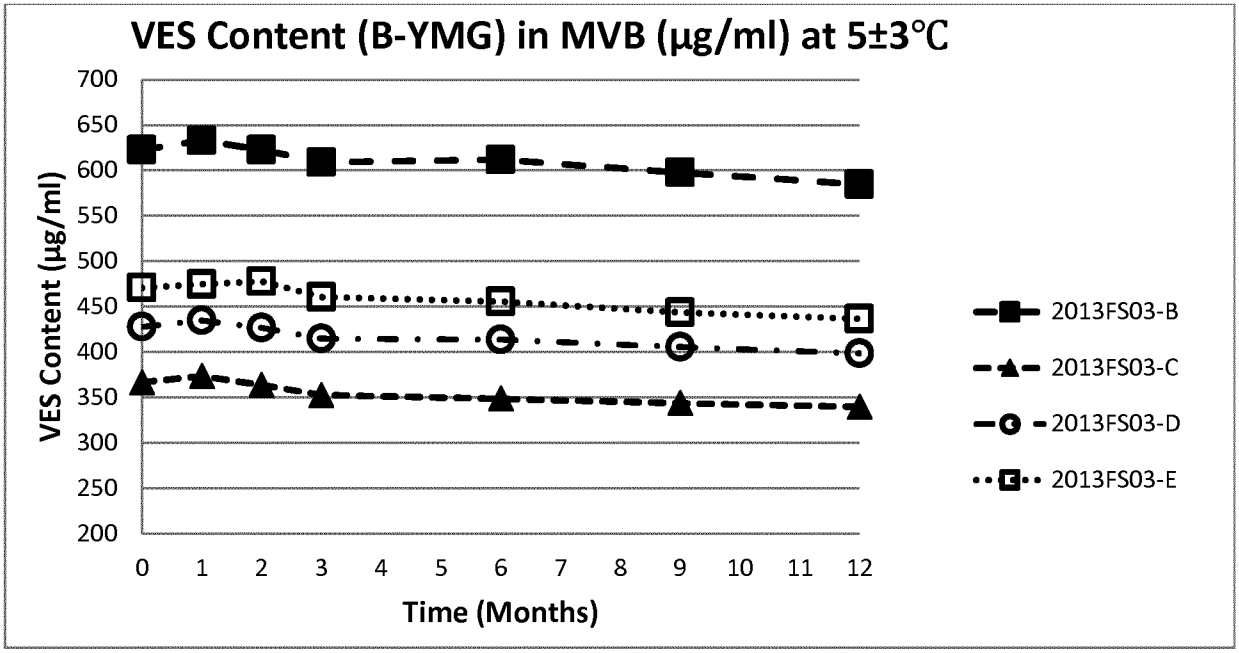


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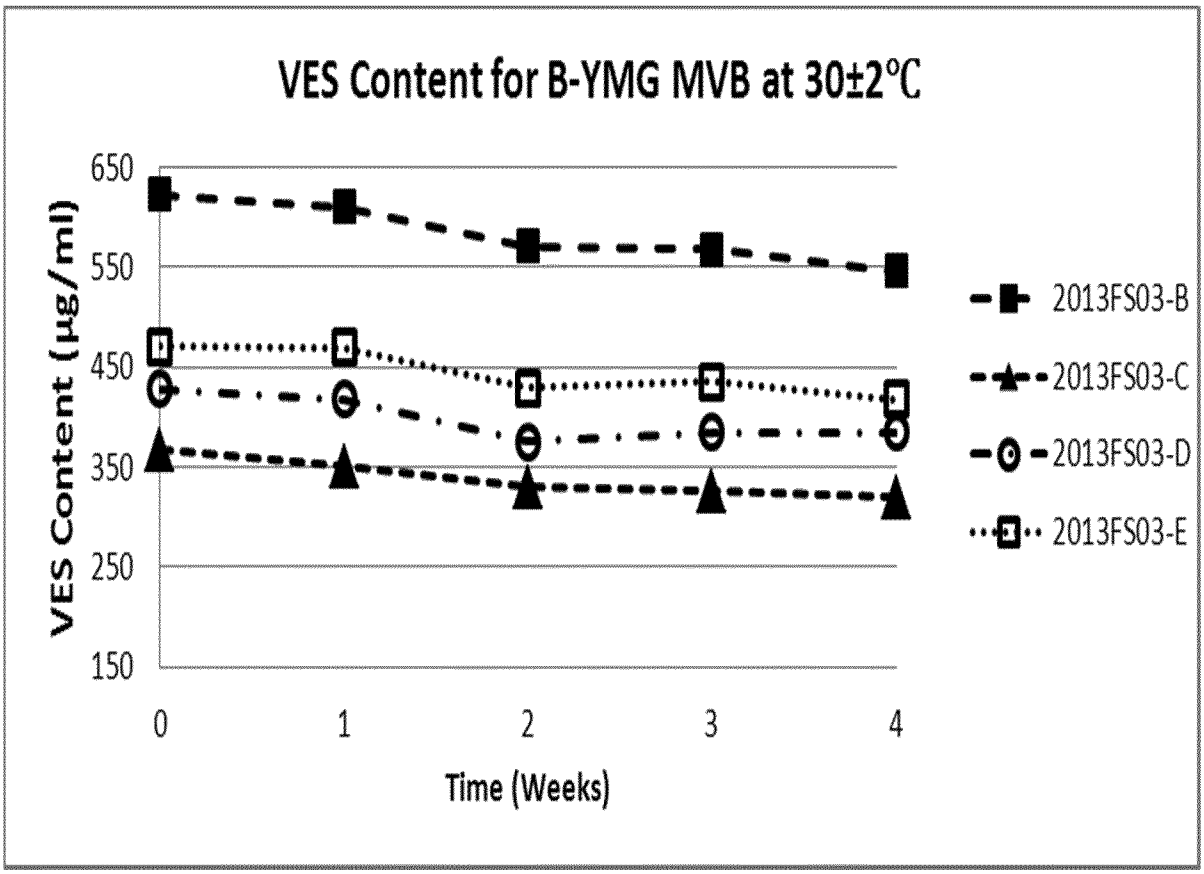


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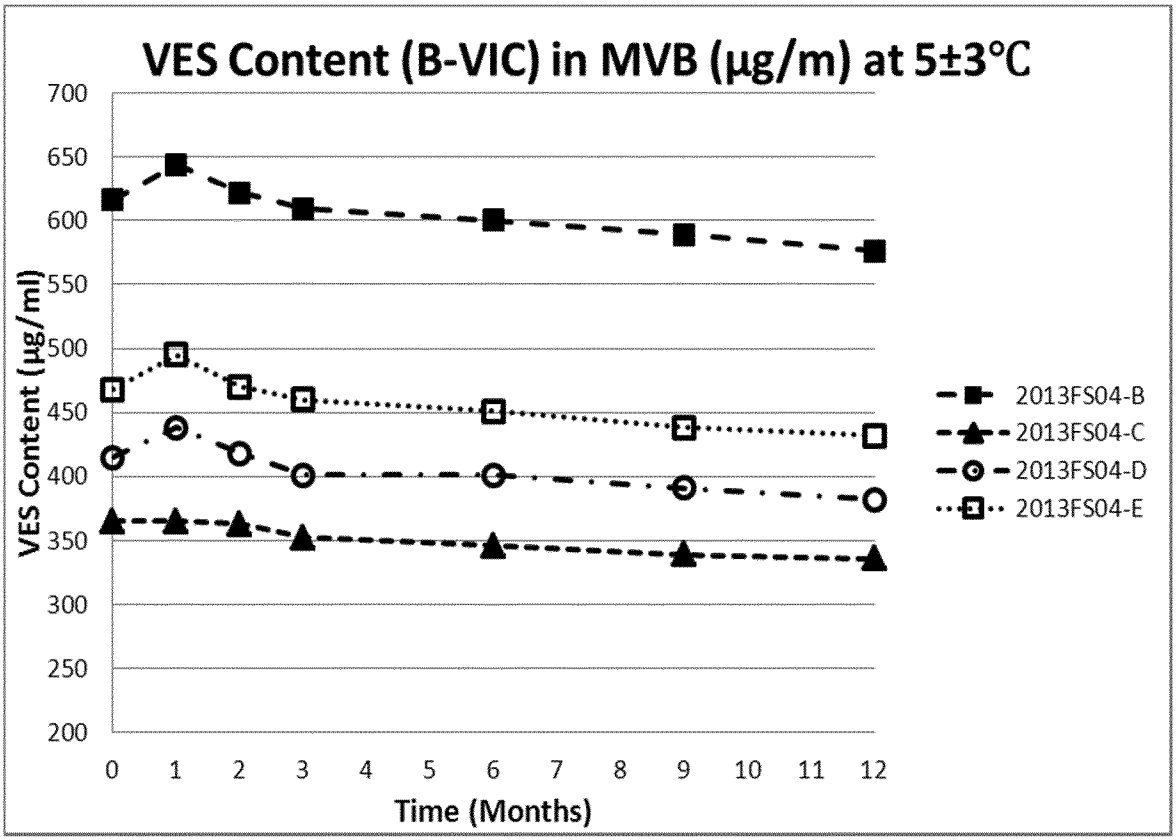


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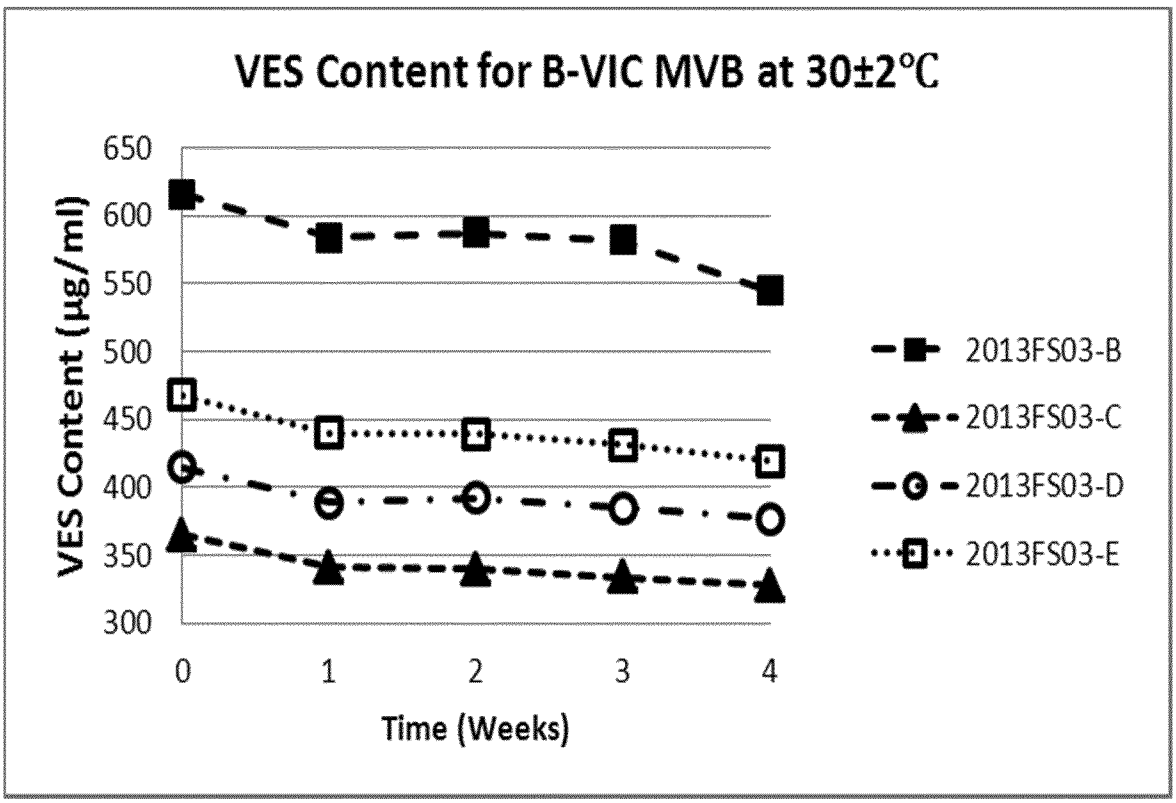


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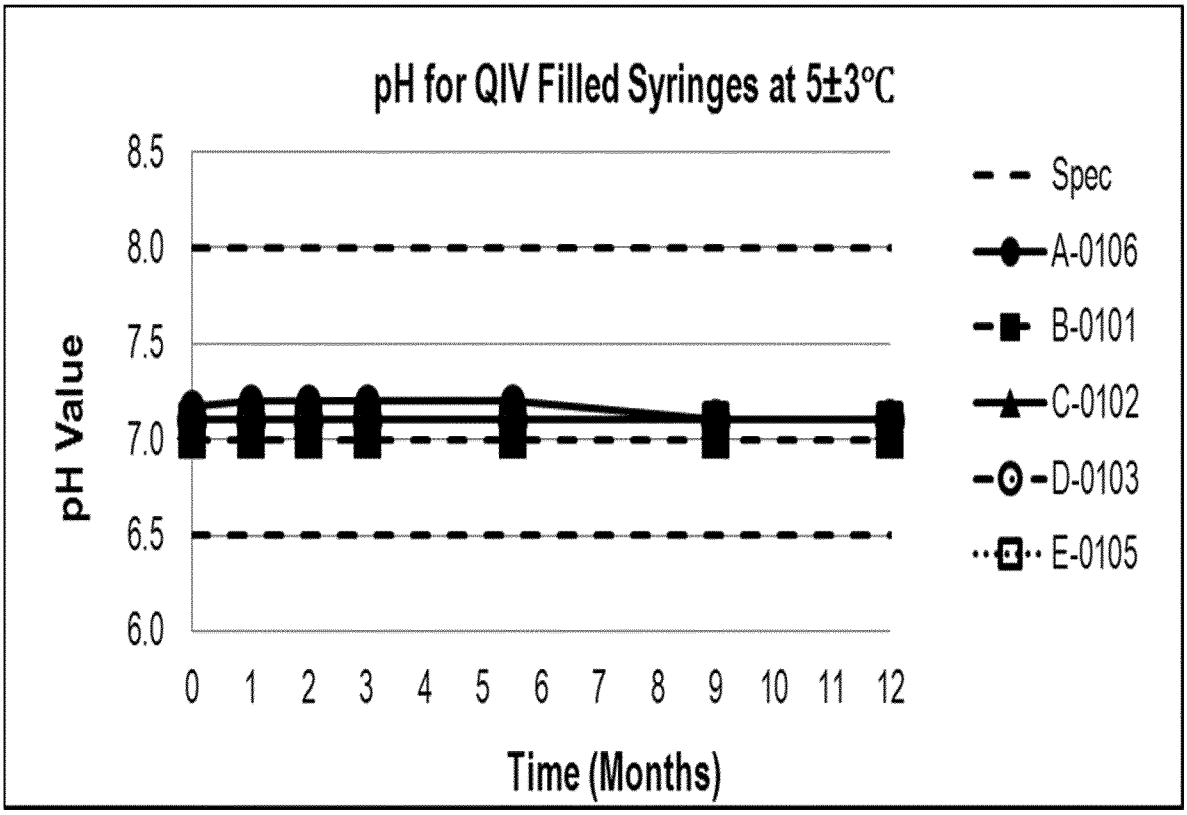


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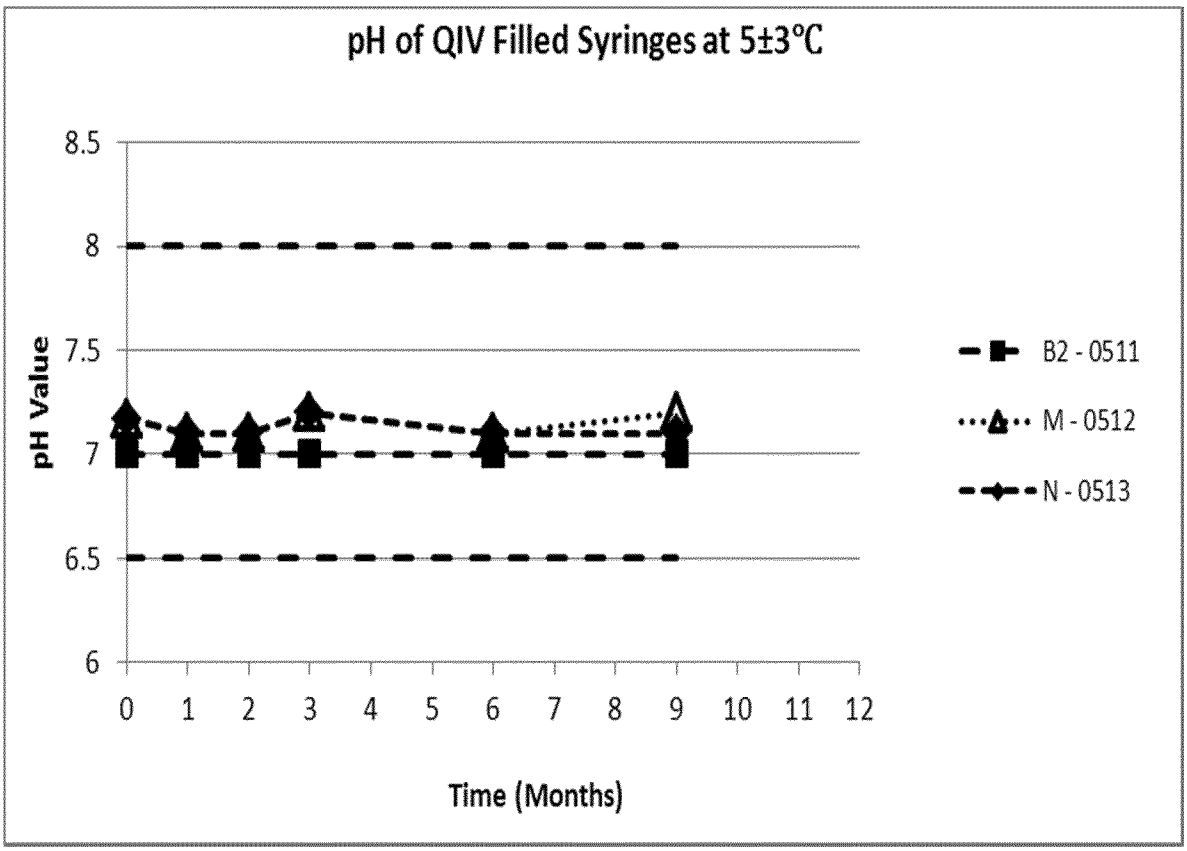


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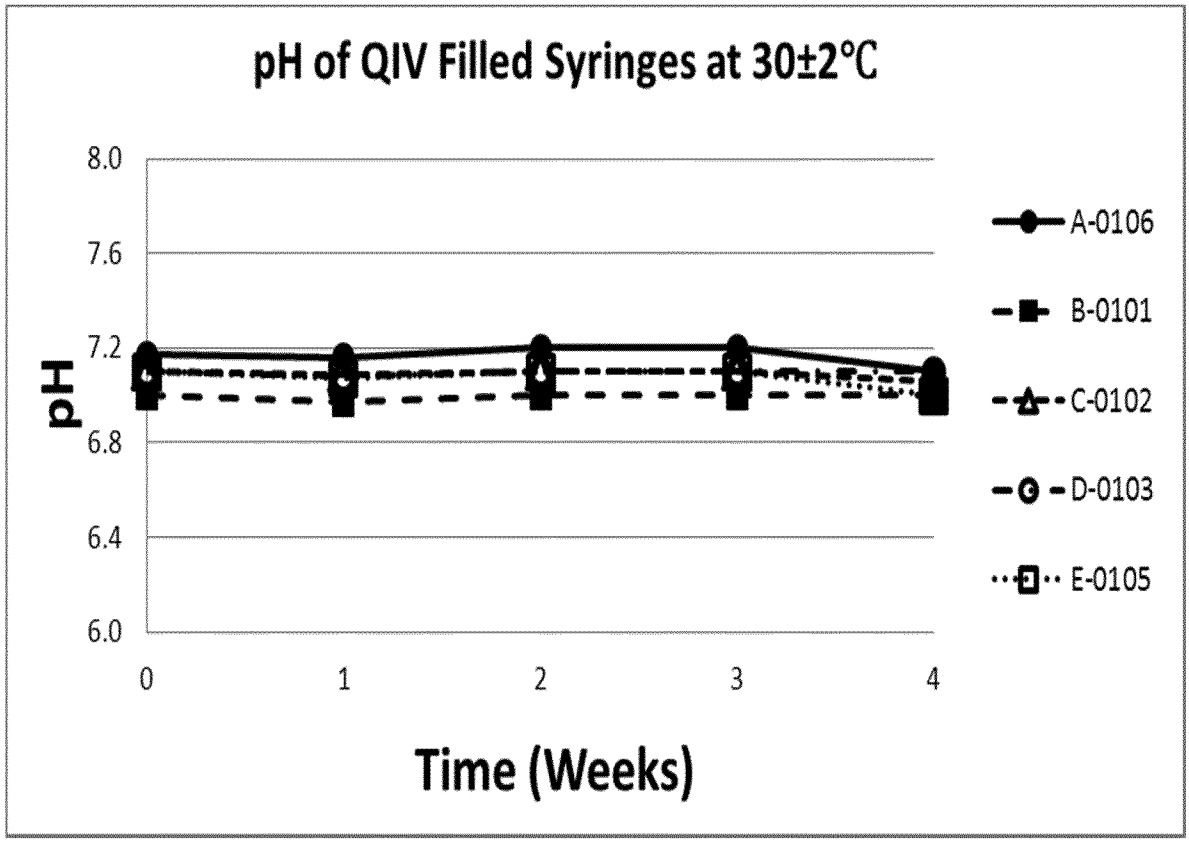


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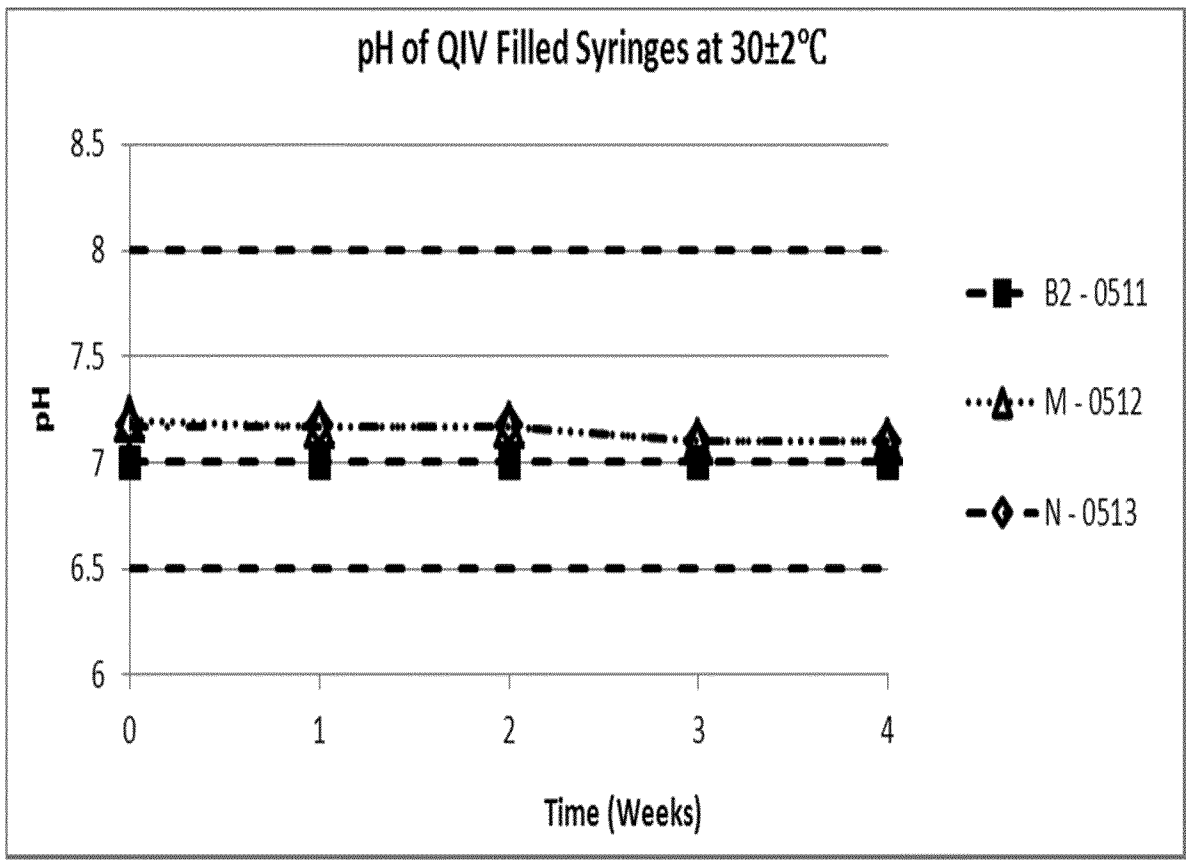


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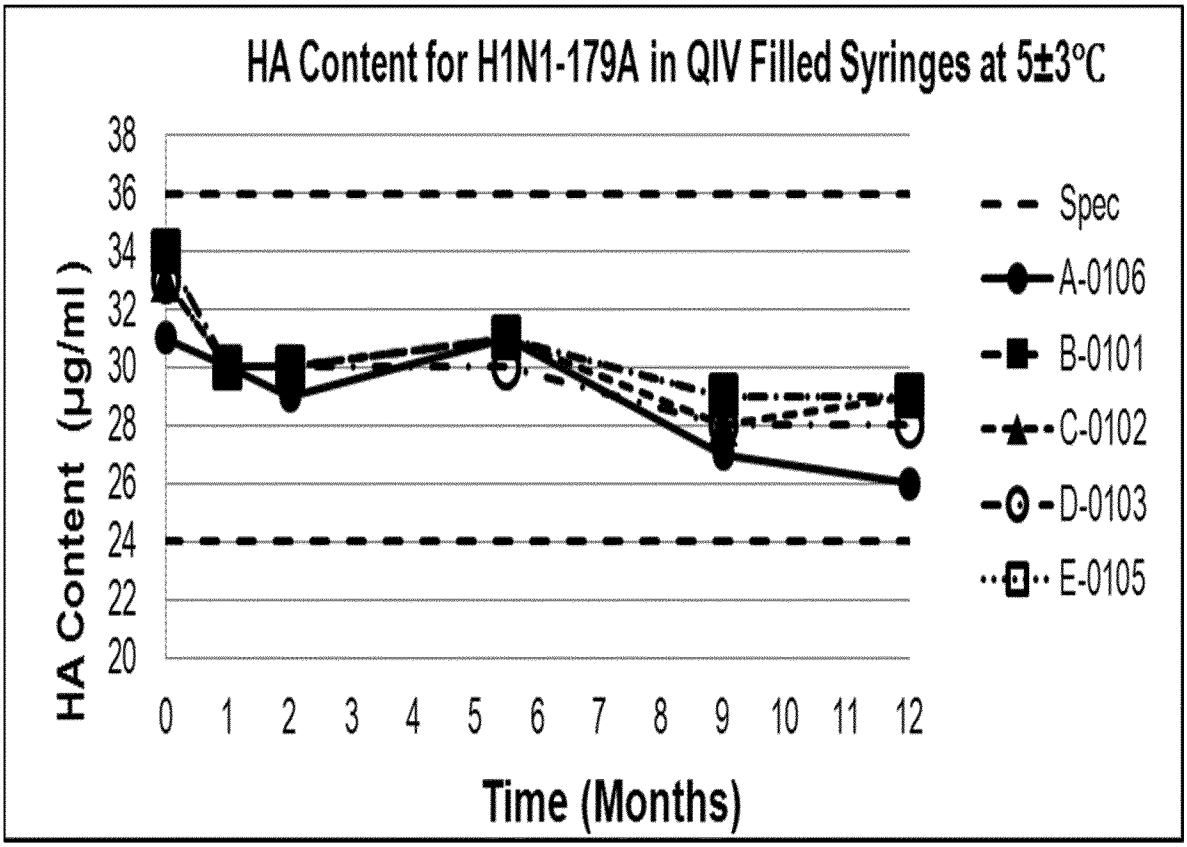


Figure 36

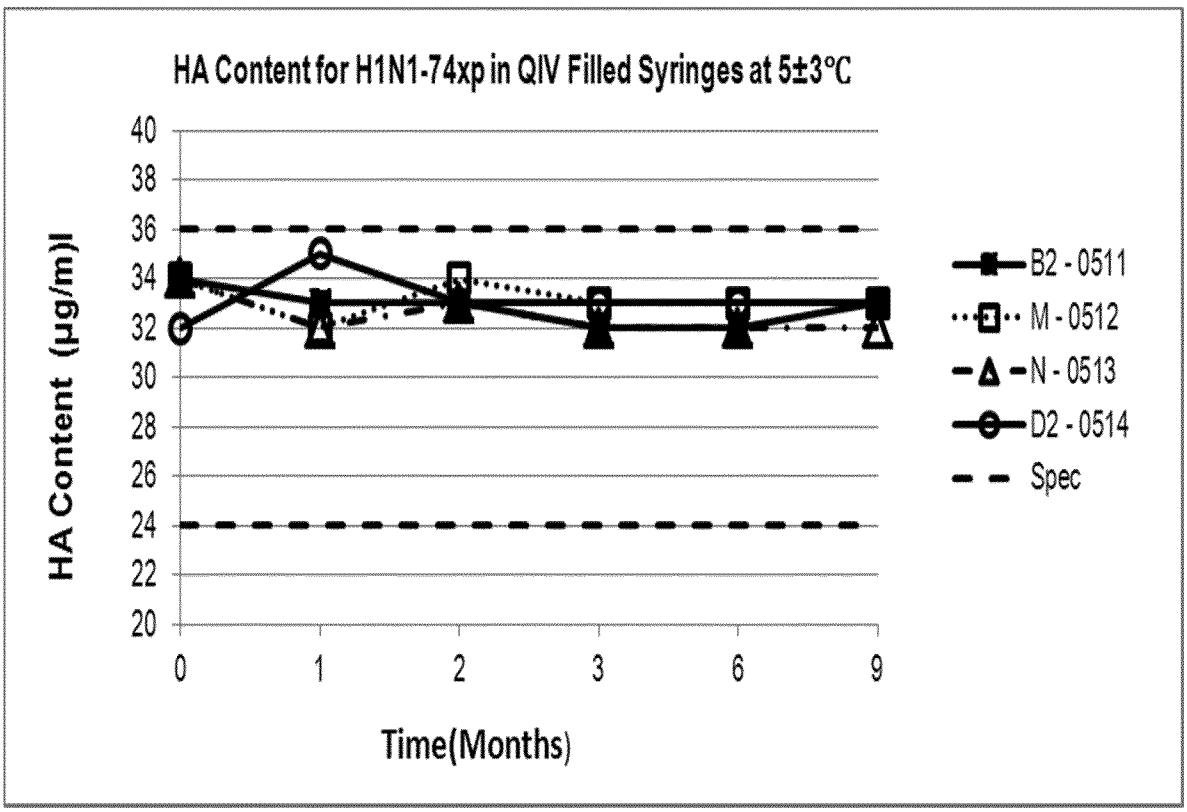


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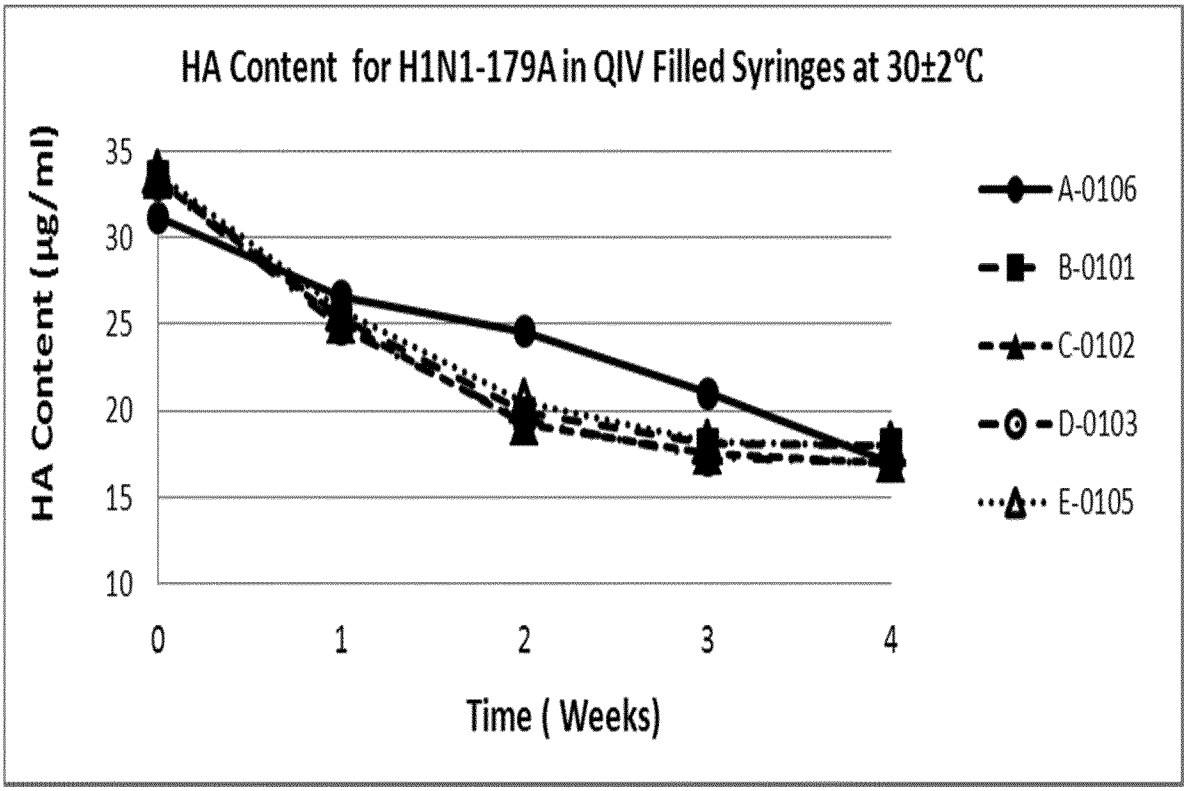


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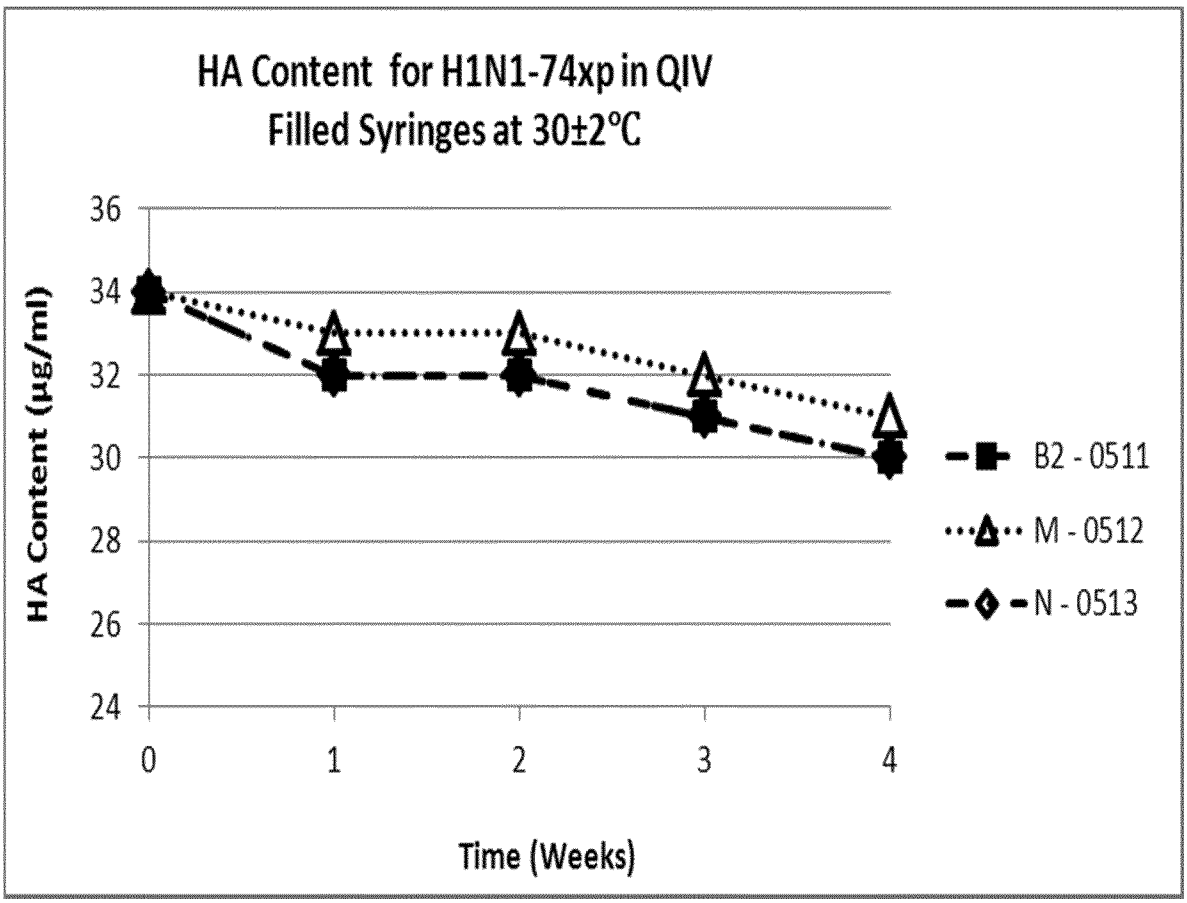


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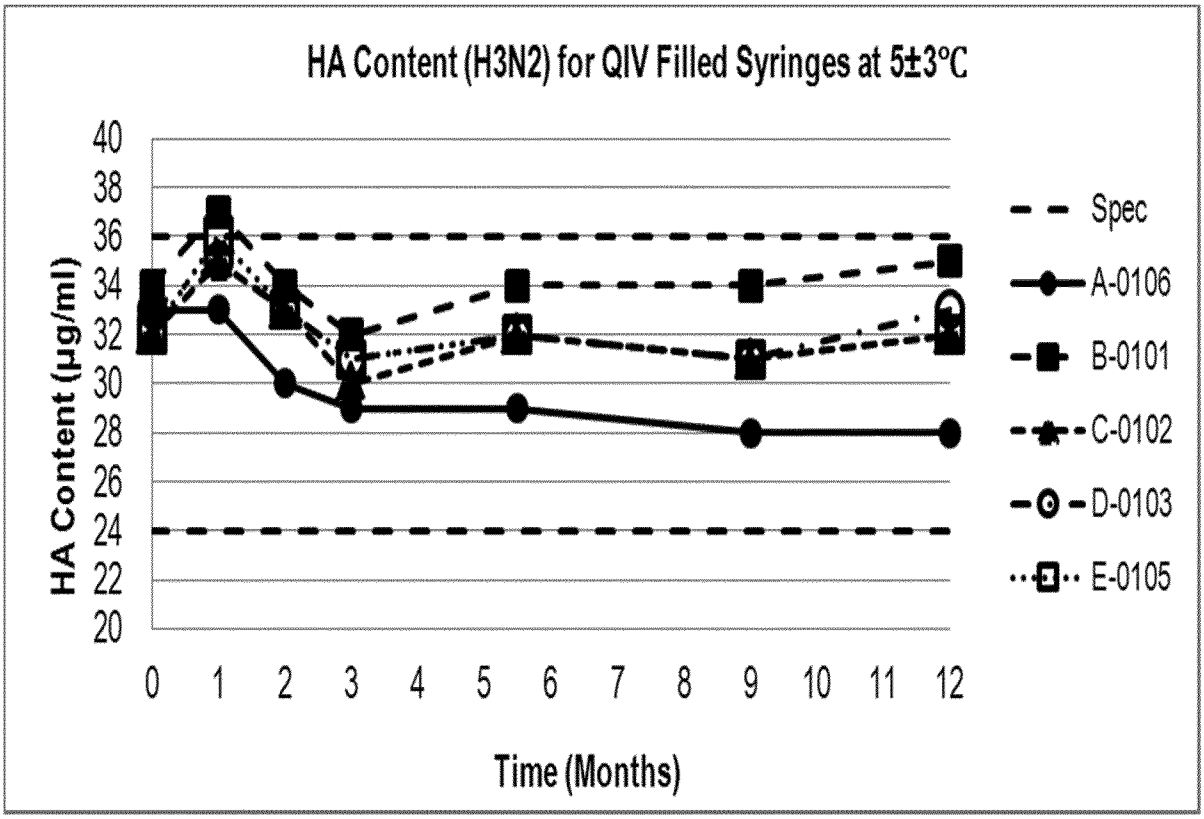


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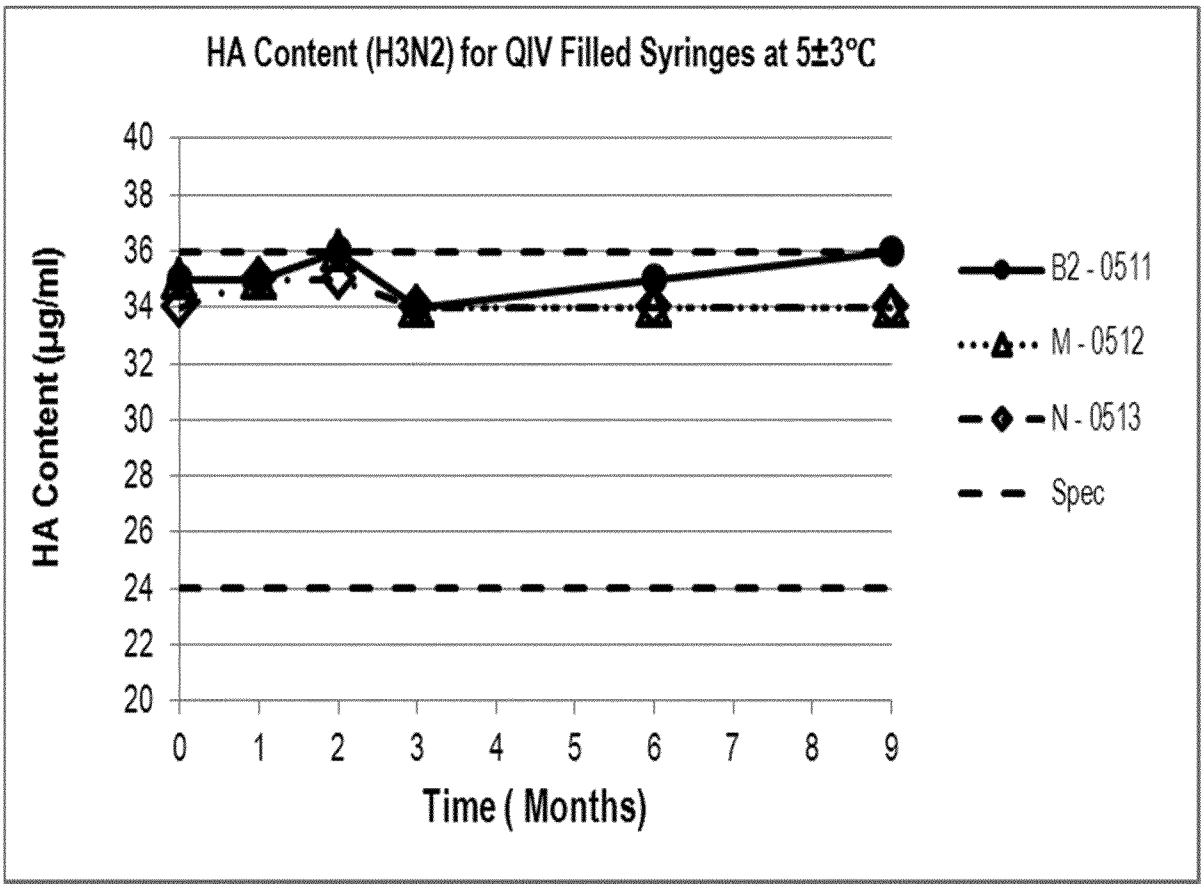


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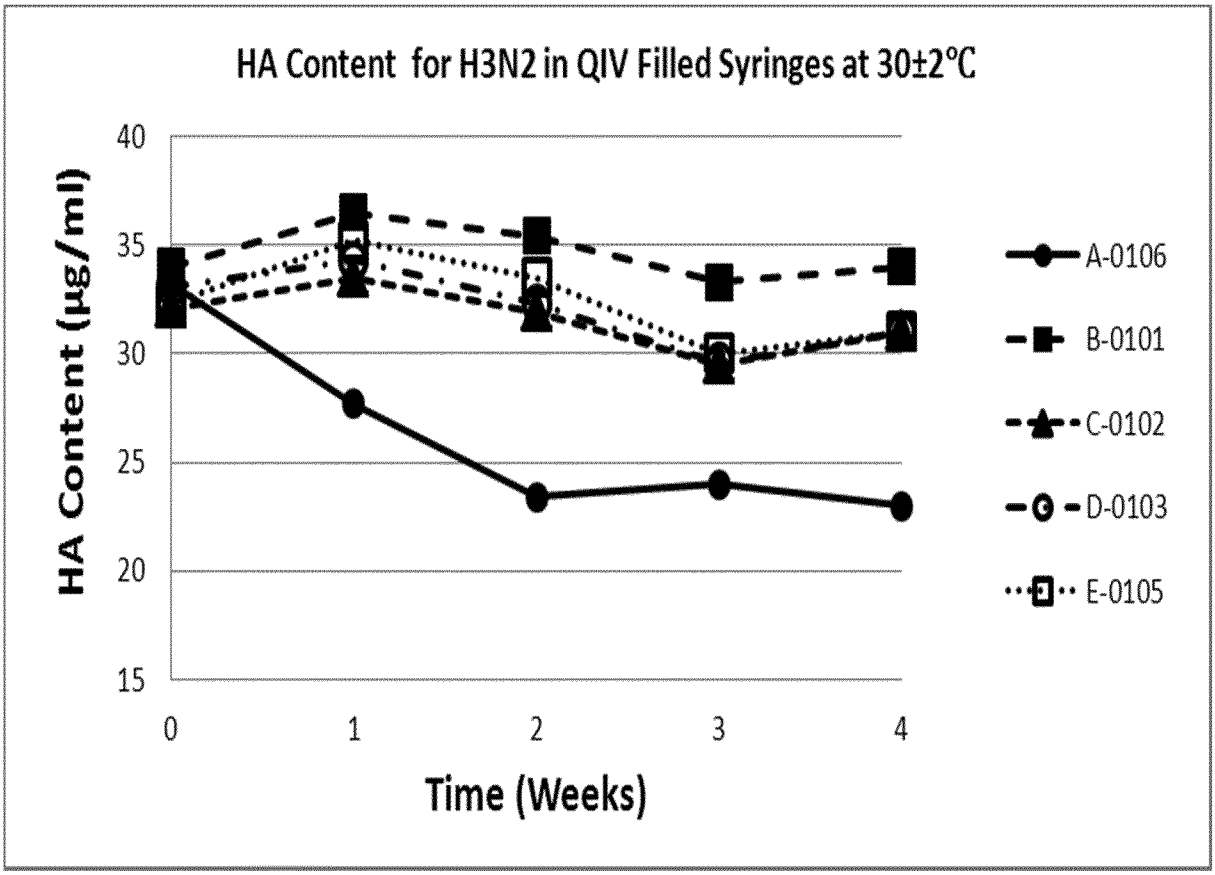


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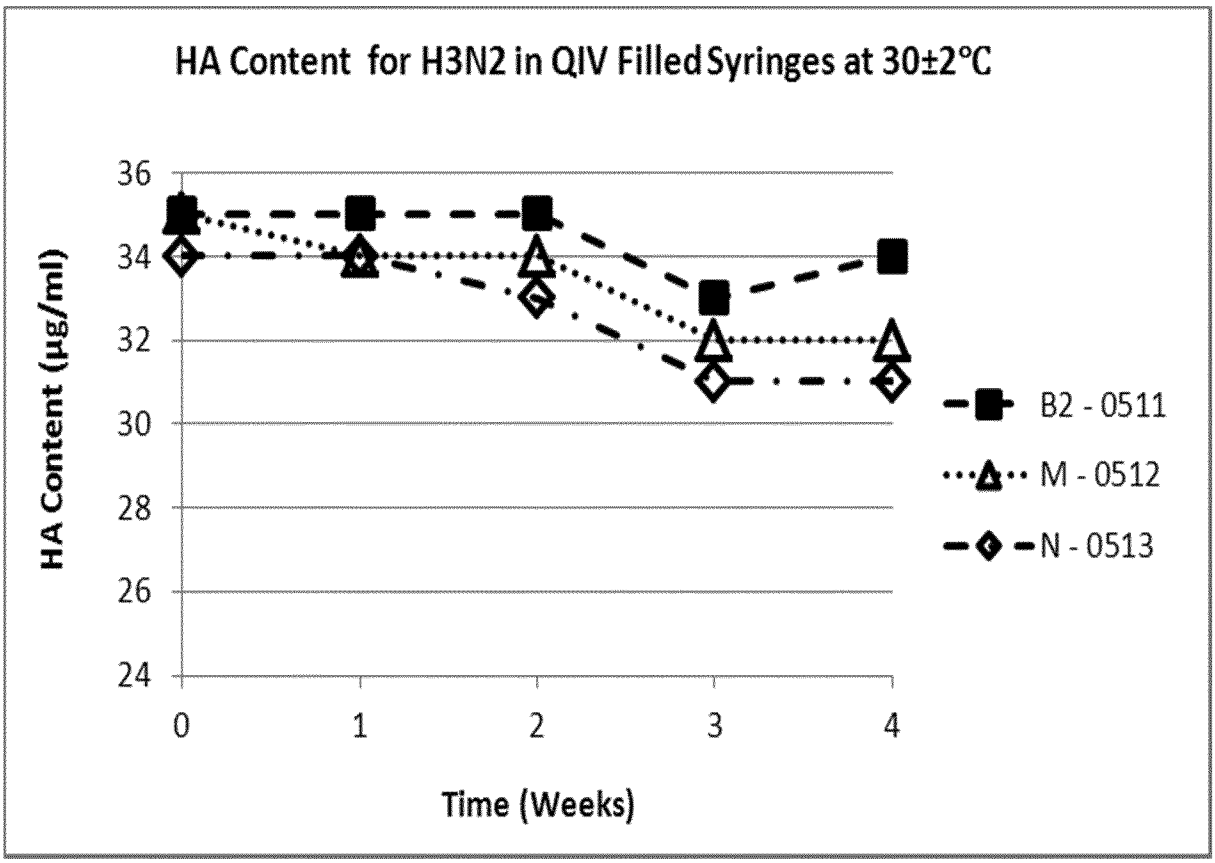


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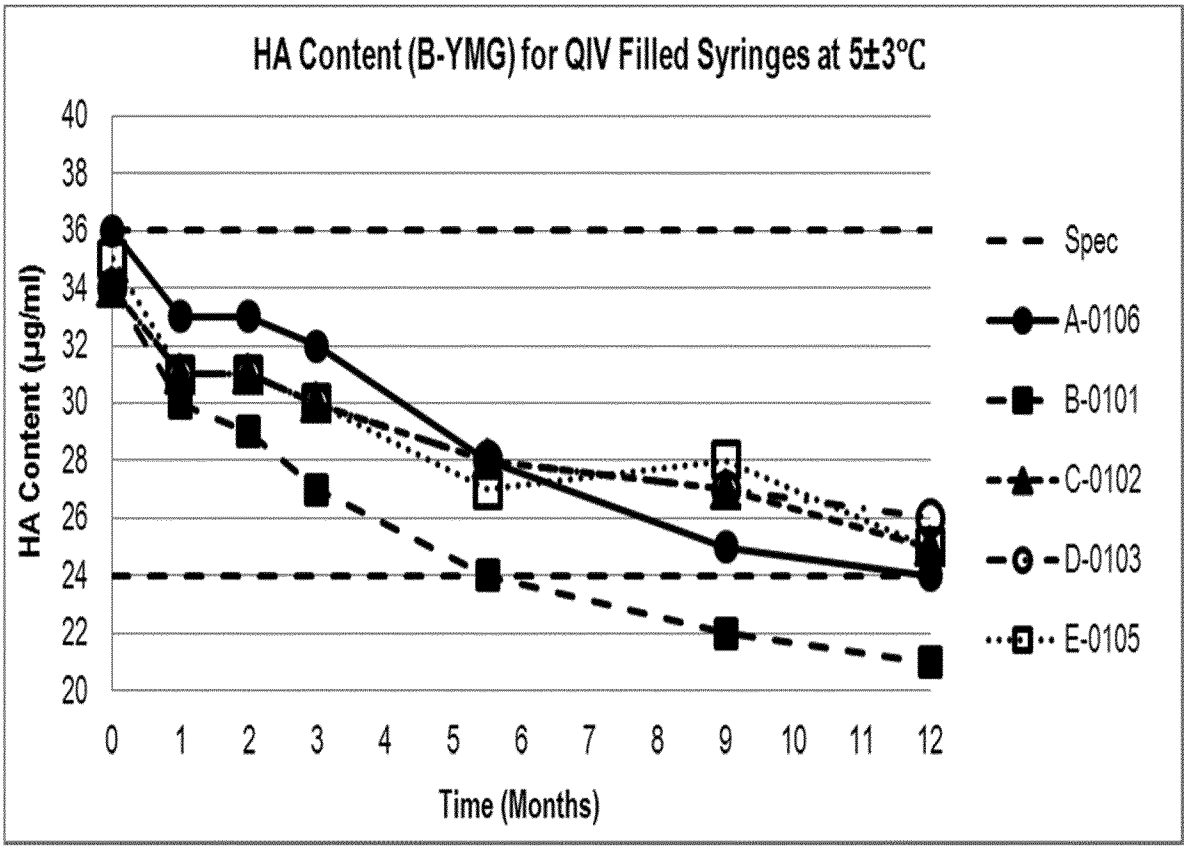


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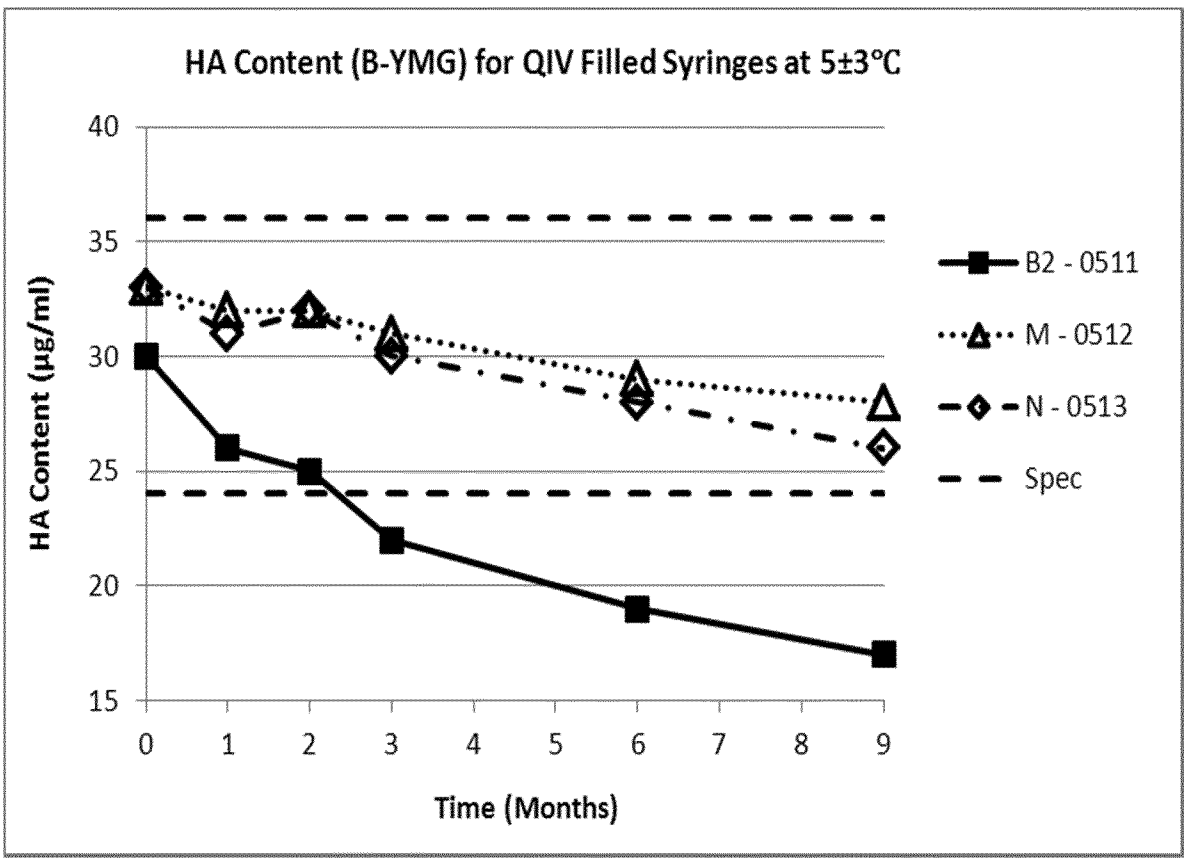


Figure 45

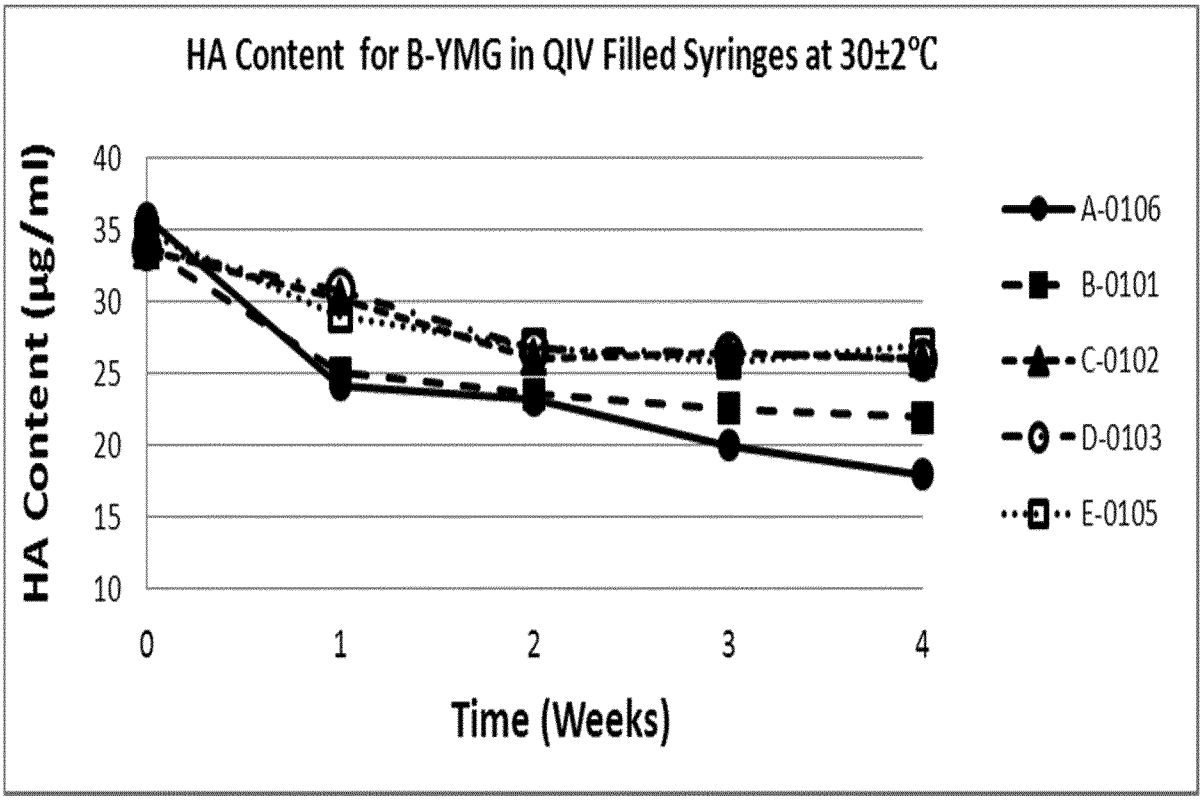


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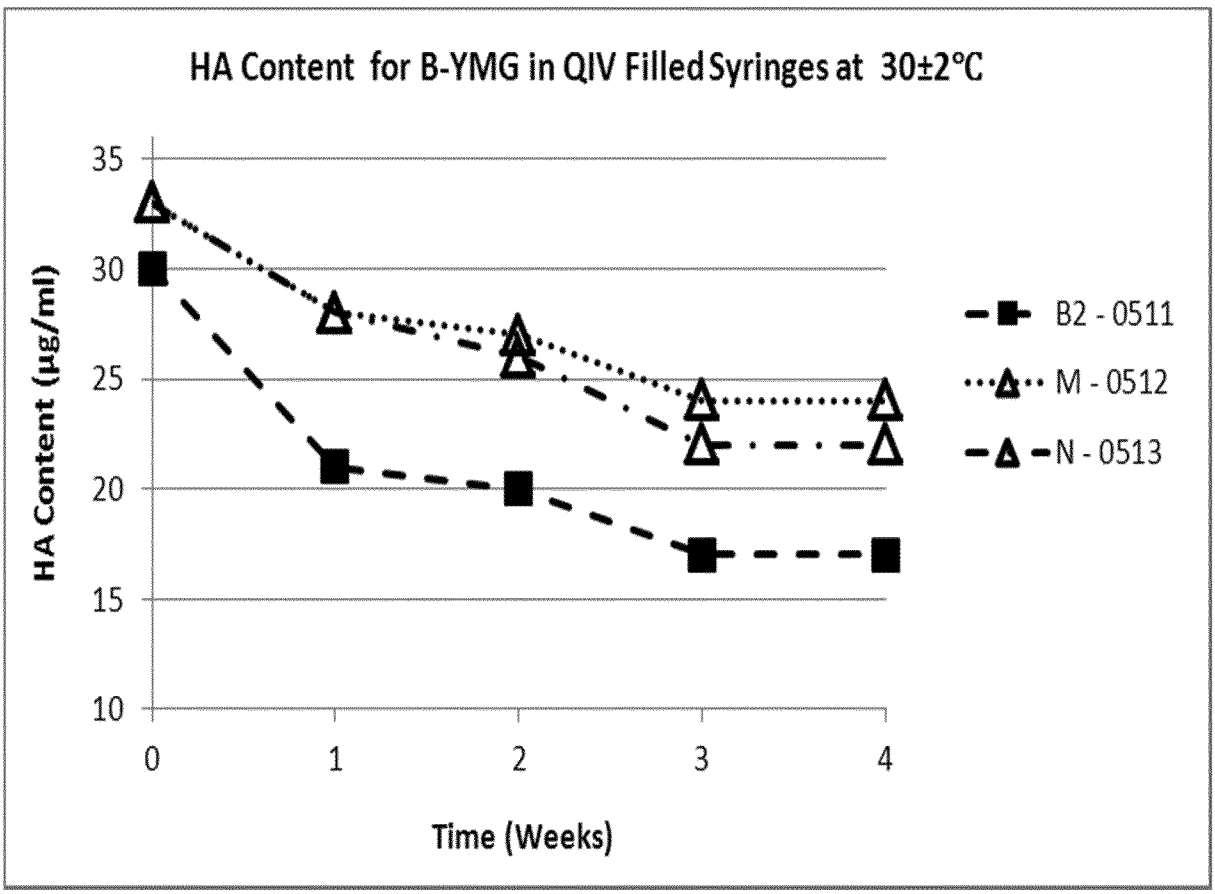


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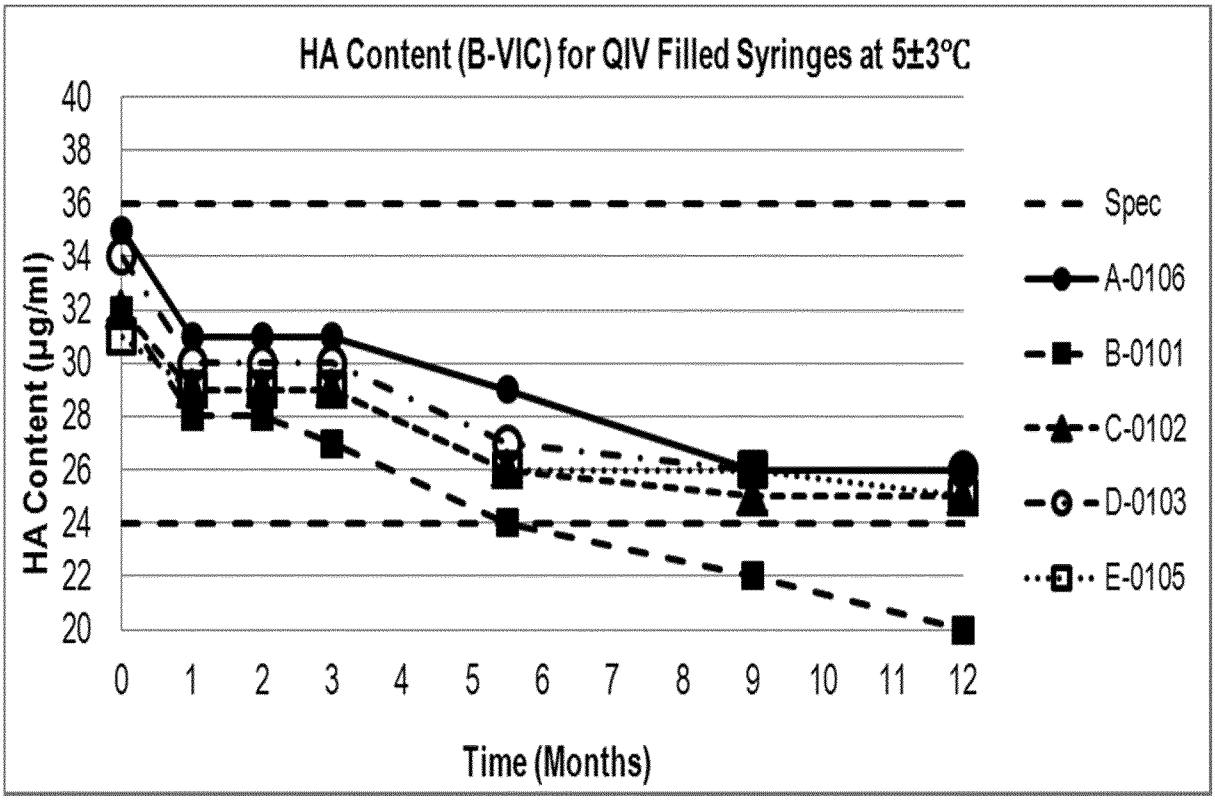


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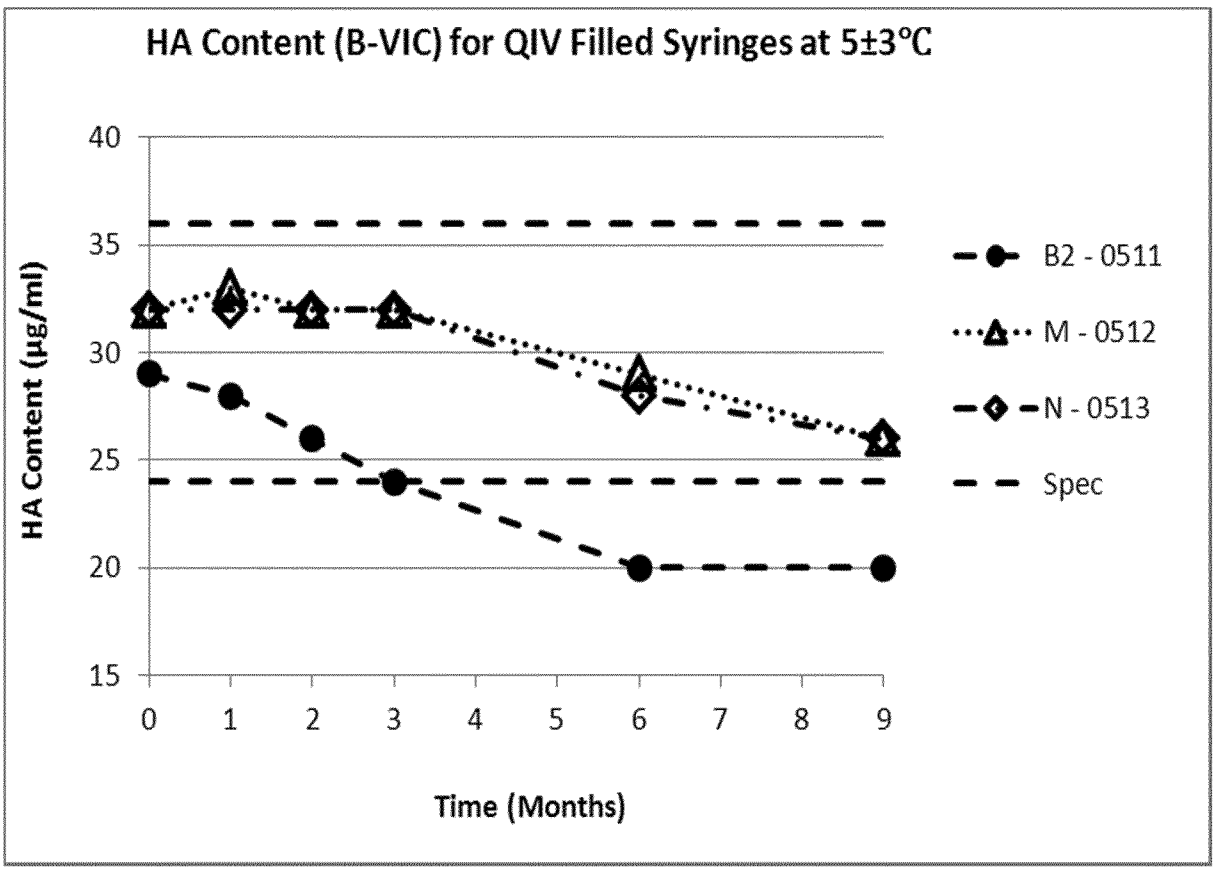


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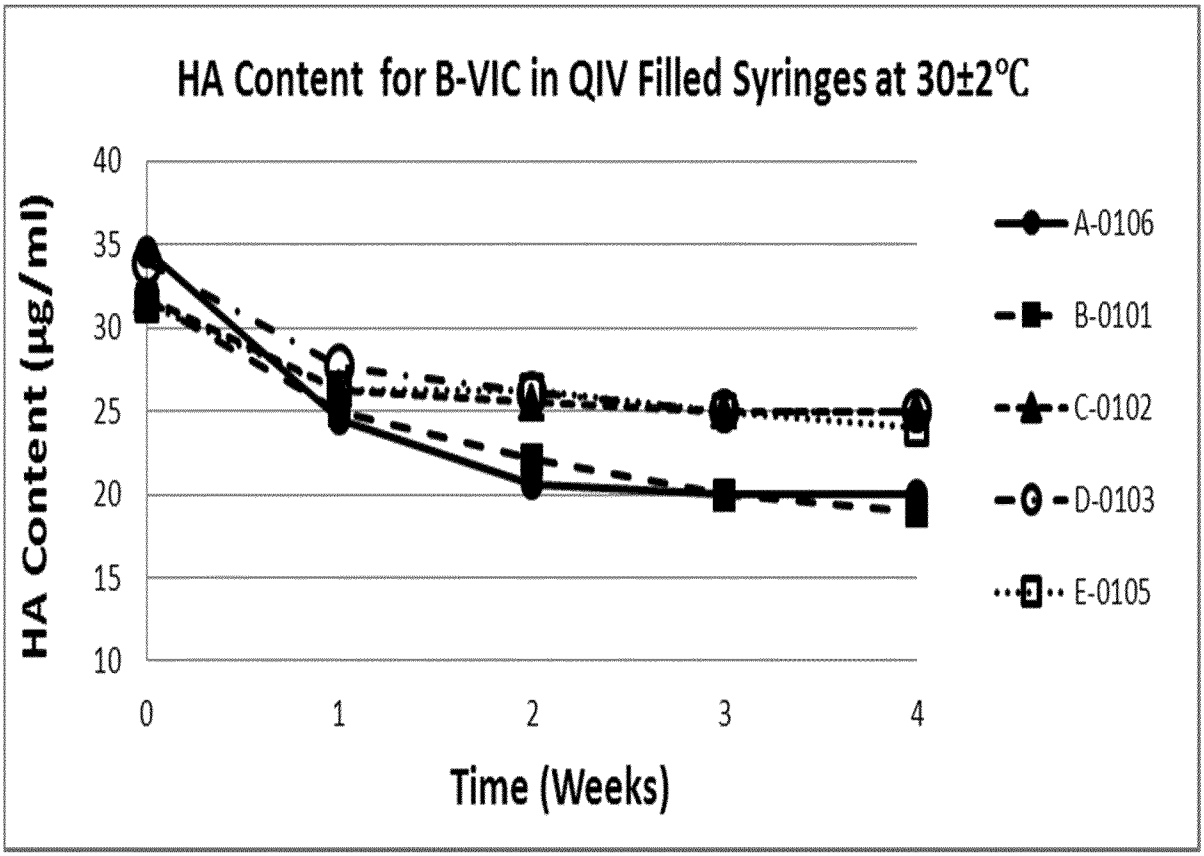


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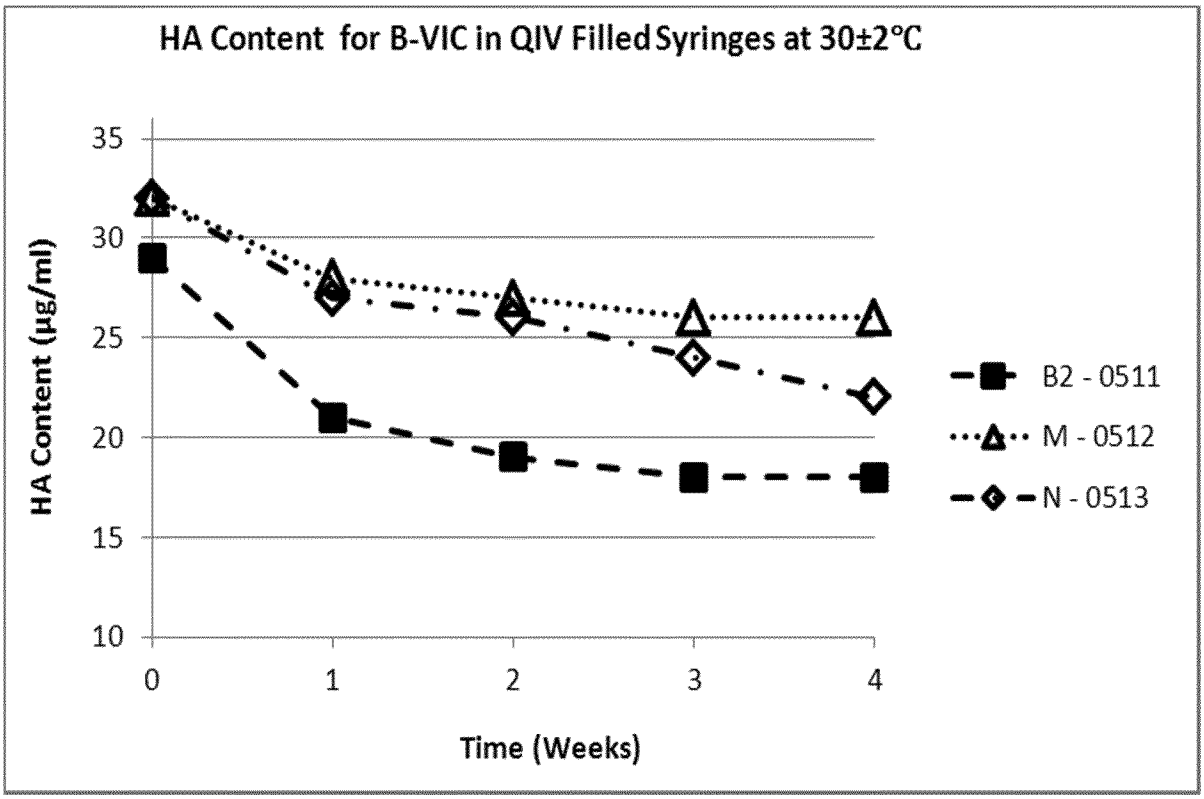


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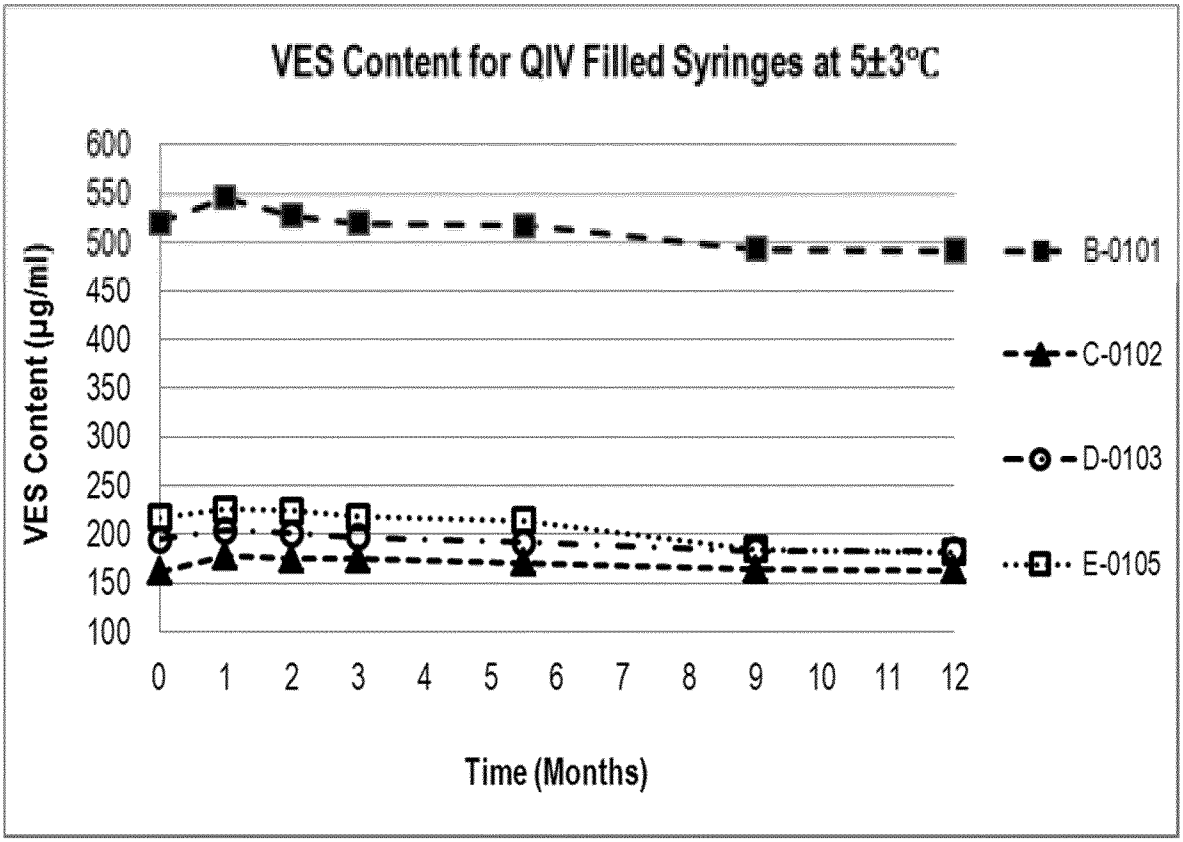


Figure 52

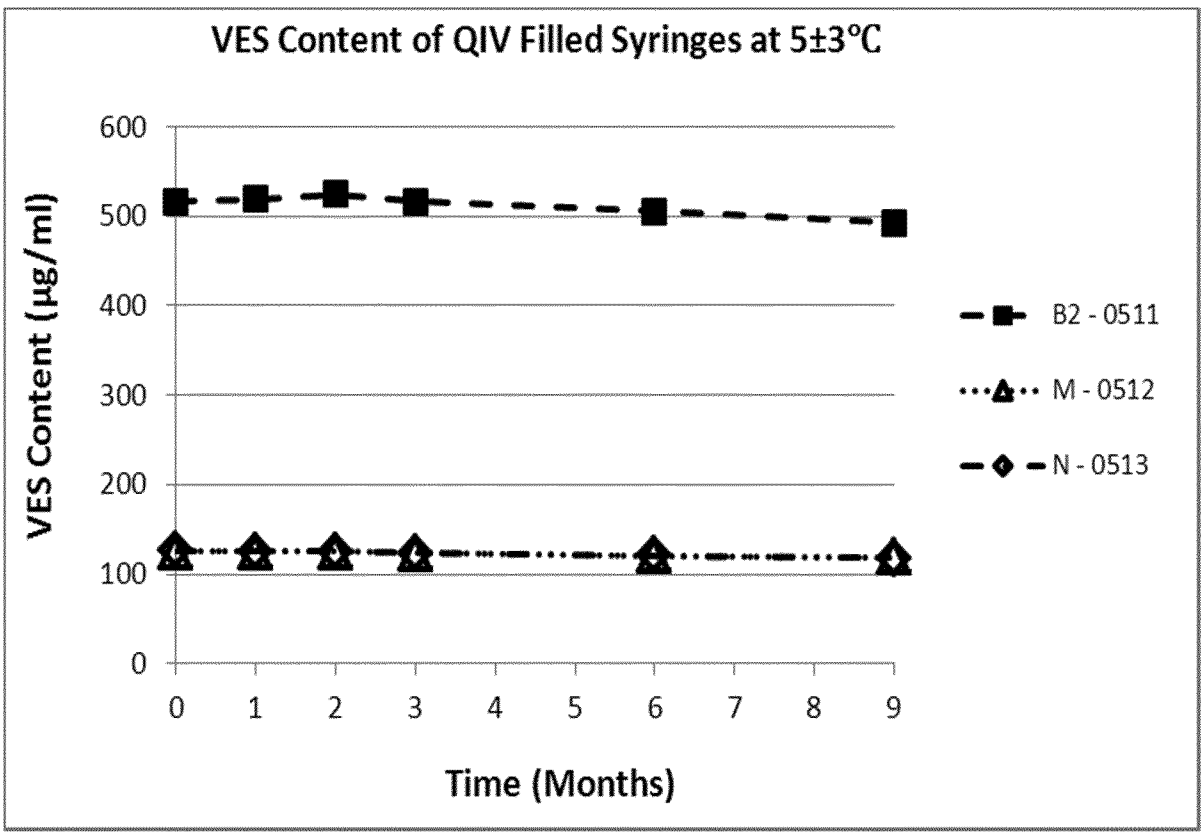


Figure 53

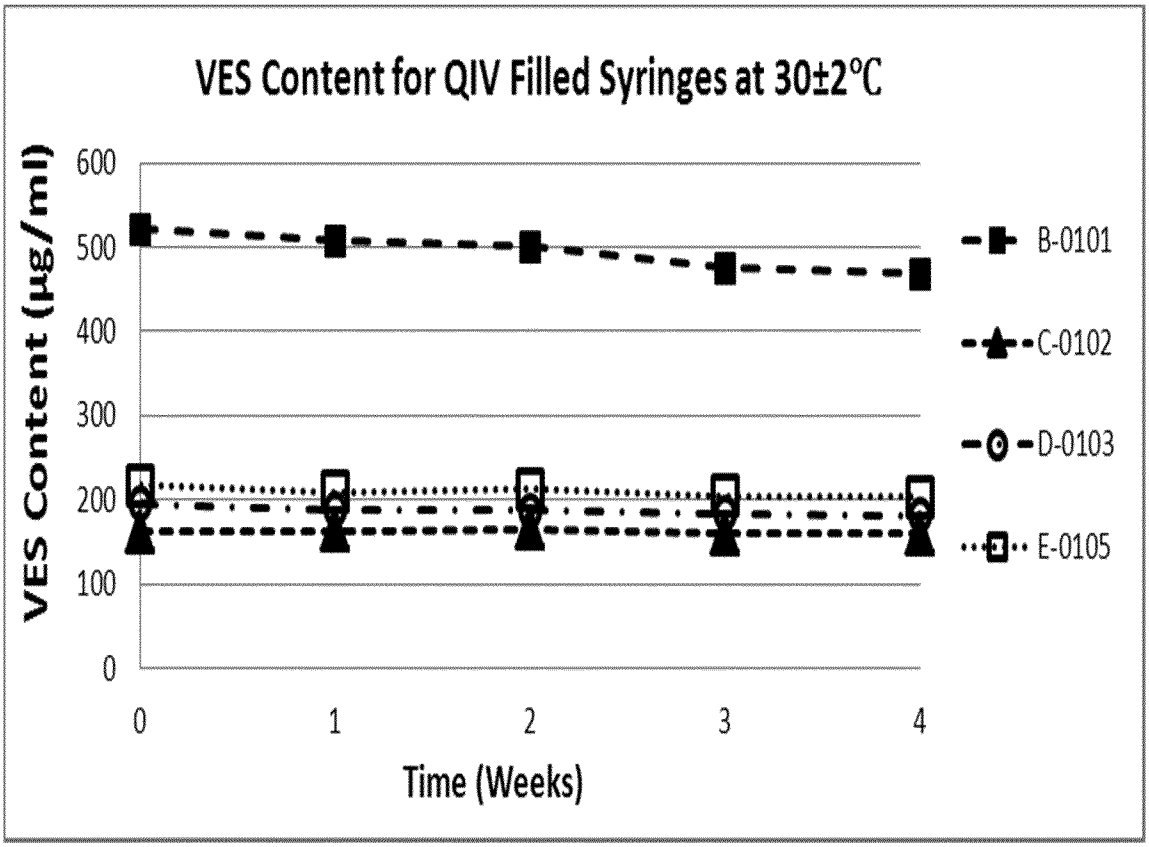
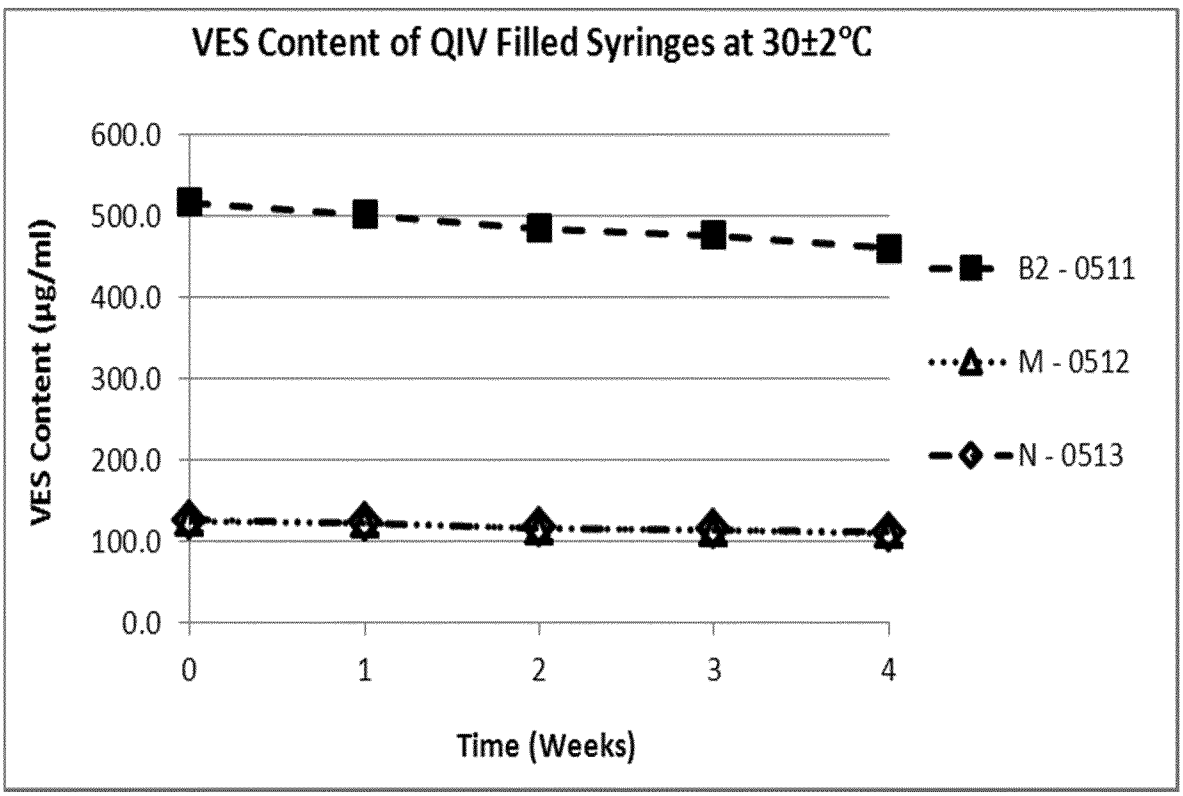

VES Content for QIV Filled Syringes at 30±2°C
VES Content (µg/ml)
600
500
400
300
200
100
0
0
1
2
3
4
Time (Weeks)
B-0101
C-0102
D-0103
E-0105

Figure 54


VES Content of QIV Filled Syringes at 30±2°C
VES Content (µg/ml)
600.0
500.0
400.0
300.0
200.0
100.0
0.0
0
1
2
3
4
Time (Weeks)
B2 - 0511
M - 0512
N - 0513

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/057344

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12 A61K39/145 A61K47/22 A61K47/26
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2010/221284 A1 (EICHHORN UWE [DE] ET AL) 2 September 2010 (2010-09-02) para. 34, 39, 49, 76, 79, 84, 85, 88-107, 153, 189	1-6, 12-15 7-11

X Y	WO 02/097072 A2 (SAECHSISCHES SERUMWERK [DE]; EICHHORN UWE [DE]) 5 December 2002 (2002-12-05) cited in the application pages 8, 14, 16, 37	1-6, 12-15 7-11

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2017	06/06/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Heder, Andreas

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/057344

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