アレパンリックス（H1N1）筋注における白色凝集物に関するまとめ

2009 年 12 月 17 日

白色凝集物について

カナダ・ケベック工場製のインフルエンザ抗原製剤（H1N1、H5N1 および季節性）においては、その株の種類により程度の差はあるものの、白色の凝集物を生じることを確認しております。種々の分析結果に基づく検討により、この凝集物は本来抗原製剤中に存在するヘムアグルチニンおよびたん白質から構成されていることが明らかとなっております。このことから、アレパンリックス（H1N1）筋注の抗原製剤においても発生しているこの凝集物は、微生物などの外来性の生物によるものではなく、さらに原料ならびに原薬および抗原製剤の製造工程に由来する外来性の不純物／異物ではなく、スプリットした抗原粒子等が何らかの機構で凝集した内因性の物質であると考えられます。

国内臨床試験用の治験薬について、社内保管品と治験施設への輸送品とを目視検査したところ、凝集物の量および大きさに差が認められないことから、日本国内における輸送の影響はないものと考えております。また、日本に輸送された治験用ロット、その製造元保管品およびカナダ国内向けの市販用ロットを目視検査により比較し、凝集の程度がほぼ同等であることを確認しており、本抗原製剤に認められる凝集物は、カナダから日本および日本国内の輸送により発生するものではないと考えられます。

免疫原性については、日本国内の臨床試験で用いたロットおよびカナダ国内で流通している市販用ロットについて、AS03 アジュバント製剤と混合した後の接種前のワクチンは表示量に相当する HA 含量を示しており、凝集物が及ぼす免疫原性への影響はないことを確認しております。

また、安全性については、凝集が生じている国内臨床試験で用いたロットを用いて異常毒性否定試験および発熱性物質試験を実施することにより確認しています。また、さらに臨床での使用において、これまでに予期しない安全性の問題が発生していないことを確認しております。

以上のことから、本ワクチンにおいて、凝集物の発生が製剤の品質、安全性および有効性に影響を及ぼすことはないと考えております。以下に凝集物に関するこれまでの経緯、種々の検討および今後実施予定の検討を示します。

1. 凝集物発生に関する経緯

2005 年、3 価の季節性インフルエンザワクチン「Fluviral」（当時カナダでのみ承認）の抗原製剤 5 ロット（ロット番号 : 3FV19911、3FV20211、3FV20511、3FV21911 および 3FV23711）において、大きさは様々であるが白色の粒子（凝集物）が認められた。そのため、検体を遠心分離し上清と沈殿物（凝集物）それぞれにつき、光学密度、たん白質含量および HA 含量（SRID 法）の測定ならびに FT-IR による解析を実施し、また、光学密度における保存温度の影響について調査した。現在まで 1mm 以上の凝集物は認められていない。

［平成 21 年 10 月 26 日提出　報告書“Industrial Development Final Report PDI 70.61”］

また、新型 H1N1 インフルエンザワクチン「Arepanrix H1N1（A/California/7/2009 株）」について、カナダ当局による試験時に上記と同様の凝集物が認められ、2009 年 10 月 9 日に当局から報告を受けた。そのため、抗原製剤の外観観察、輸送前後における光学密度、平均粒子径、多分散性指数および HA 含量の測定ならびに SDS-PAGE およびウェスタンブロッドによる解析を実施した。また、検体を遠心分離し上清と沈殿物（凝集物）それぞれにつき、光学密度、たん白質含量および HA 含量の測定ならびに SDS-PAGE により解析を実施し凝集物を調査した。さらに、異常毒性否定試験および発熱性物質試験により安全性を確認した。
［平成 21 年 11 月 13 日提出　報告書“AS03-Adjuvanted Quebec H1N1 Pandemic Vaccine (Arepanrix H1N1) Un-Adjuvanted Monovalent H1N1 Vaccine Briefing Document”］

1.1.　凝集物に関する科学的な調査

- Fluviral について、2005 年以前および以降も凝集物は認められている［10 月 28 日付の照会 3 に対する回答］。
- Arepanrix H1N1 について、すべての臨床用および市販用ロットの抗原製剤について凝集物が認められている［10 月 28 日付の照会 8 に対する回答および EMEA の照会 11 に対する回答］。
- 界面活性剤を含有するドレスデン製の抗原製剤（H1N1、H5N1 および季節性インフルエンザワクチン）には凝集物は認められない［10 月 28 日付の照会 1 に対する回答］。
- 一般的にたん白質を含有する製剤に凝集が発生することがあり、その直接の原因として、温度、pH、塩濃度、輸送および界面活性剤の有無などが挙げられ、たん白質の（静電的性質、疎水性）によるものと考えられている［10 月 28 日付の照会 3 および 5 に対する回答］。
- Arepanrix H1N1 について、SDS-PAGE およびウェスタンブロットによる解析により、遠心分離前と遠心分離後の沈殿物（凝集物）とが同様のたん白質プロファイルであることから、凝集物は抗原製剤中の固形分（ヘムアグルチニンおよびたん白質）と同一の組成からなり、内因性のものであると考えられる。
- Arepanrix H1N1 について、凝集物は微生物などの外来性生物によるものではないことを無菌試験により確認した［EMEA の照会 9 に対する回答］。
- 日本において保管した Arepanrix H1N1 治験薬および DSM サイト（米国）において充てんした Arepanrix H1N1 を、ともにカナダへと輸送した製剤について、アジュバント製剤との混合後にバイアルから 1 回接種分を 10 回抜き取り、それぞれ HA 含量を測定することにより、1 バイアル中の含量均一性を評価した結果、凝集物の存在および輸送の影響がないことを確認した［EMEA の照会 8 に対する回答］、［11 月 12 日付の照会 8 に対する回答（更新版）］および［平成 21 年 12 月 10 日提出　報告書“Factors Impacting the Aggregates in the H1N1 Antigen Lots and in the Adjuvanted H1N1 Vaccine Lots”］。

1.1.1.　製造方法の影響

- 2000 年シーズンの Fluviral において、凝集物の発生が認められ、翌年のシーズンにパナマ株のスプリット方法を変更した［EMEA の照会 3 に対する回答］。

- 2005 年以降、製造方法の大きな変更は行われていない［10 月 28 日付の照会 3 に対する回答］。
- Fluviral および Arepanrix H1N1 について、充てん前に 8μm のフィルターによるろ過を実施している［11 月 12 日付の照会 7④に対する回答］。
- 原薬の製造工程で界面活性剤が除去されるため、この段階において凝集が起こる可能性は否定できないが、ろ過滅菌の前後で性状に変化はなく、ろ過滅菌後の原薬はすべて、性状の規格「本品は澄明～乳白色の懸濁液であり，まれにわずかに沈殿を生じる．」に適合している［11 月 12 日付の照会 5 に対する回答］。
- 凝集物が、UV 照射およびホルムアルデヒド処理による不活化に影響を与えることはない。また、万が一、不活化されないウイルス粒子が存在した場合においても、その後のスプリット化工程により十分に不活化される［11 月 12 日付の照会 5 および 11 月 20 日付の照会 2 に対する回答］。

1.1.1.1. 工程管理

- Arepanrix H1N1 抗原製剤は充てんラインにおいて、外来性の異物が混入したバイアルを排除するために教育を受けた検査員による目視による全数検査を実施している。また、AQL を設定し異物が認められた際の管理手順を設定している。その際は、抜き取り検査によりバイアル中の外来性異物の面積を基準円と照らし合わせてその大きさを計測している［11 月 12 日付の照会 1 および 7⑤に対する回答］。

1.1.2. 輸送の影響

- Arepanrix H1N1（AS03-Adjuvanted 製品）の臨床用ロットおよび市販用ロットの抗原製剤ならびに Arepanrix H1N1（Un-Adjuvanted 製品）の市販品ロットについて、トラックによる 20 時間までの輸送の前後においては、平均粒子径、多分散性指数、HA 含量（HPLC 法および SRID 法）ならびに SDS-PAGE およびウェスタンブロットによるたん白質プロファイルに変化はほとんどなく、また、たん白質含量（ローリー法）および HA 含量（HPLC 法および SRID 法）の結果から凝集物の割合もほとんど変化はなかった。さらに、遠心分離前、遠心分離後の上清および沈殿物（凝集物）について SDS-PAGE による解析を実施し、それらは同様のたん白質プロファイルであり、凝集物は抗原製剤に本来含有する成分（ヘムアグルチニンおよびたん白質）であることが示唆された。
- Arepanrix H1N1（AS03-Adjuvanted 製品）の治験用ロットの抗原製剤について、輸送前と比較して輸送後の SRID 法による HA 含量は NIBSC の参照抗原および CBER の参照抗原を用いた際、それぞれ 18%および 10%低下した。しかしながら AS03 アジュバント製剤との混合後の SRID 法における沈降輪は輸送前後において同等であることから、輸送前後の HA 含量に変化は認められなかった［EMEA の照会 5 に対する回答］。一方、HPLC 法による HA 含量も輸送前後において同等であった。
- 凝集の発生は輸送のような外的な要因よりも抗原の性質による要因が強いため、その発生を防止する手段はない［11 月 12 日付の照会 8 に対する回答］。

1.1.3. 温度の影響

- Fluviral について、氷およびアイスパックによる保冷においては光学密度（OD 値）に変化はないものの、-30℃、2 時間の保存により OD 値の上昇が認められた。
- Fluviral について、室温および 37℃において振とうしても凝集物の消失は認められなかった。

1.1.4. 凝集物の経時的な変化

- 製造日より 22～61 日が経過した 4 ロット（市販品 3 ロットおよび臨床試験用 1 ロット）について、抗原製剤において平均粒子径、多分散性指数、遠心分離により採取した凝集物のたん白質含量および HA 含量の測定ならびにアジュバント製剤との混合後の HA 含量の測定を実施した。この結果より、平均粒子径および多分散性指数については経時的な変化は認められなかったものの、経時的に凝集物に占めるたん白質含量は減少し、HA 含量は増加する傾向が認められた。しかしながら、アジュバント製剤混合後の HA 含量については経時的な変化は認められなかった［EMEA の照会（Cycle RR#7.4 Major Objection）1 に対する回答］および［平成 21 年 12 月 10 日提出　報告書“Kinetics of Aggregates Formation”］。

1.1.5. 免疫原性

- Arepanrix H1N1（AS03-Adjuvanted 製品）の治験用ロットの抗原製剤について、SRID 法による HA 含量は輸送により 18%または 10%低下したが、AS03 アジュバント製剤との混合後は輸送前後いずれも規格の範囲内であったことから、Arepanrix H1N1 ワクチンの免疫原性は輸送の有無に影響を受けないことが確認された。

1.1.5.1. アジュバント製剤と混合後の免疫原性

- 凝集が生じている実生産 3 ロットの抗原製剤全量を SRID 法により HA 含量を測定した結果、同様に HA の低下傾向が認められ（12.8μg/mL）、凝集物中の HA 含量は全 HA 含量の 45～50%（遠心分離による方法）であった。さらにそれらのロットを輸送後に SRID 法で HA 含量を測定したところ輸送の影響を受けていなかった（12.8μg/mL）。一方、複数ロットの抗原製剤について同量の AS03 アジュバント製剤との混合後の HA 含量は平均 7.6μg/mL を示した。これは抗原製剤の規格値の半量に相当し、アジュバント混合後に HA 含量が回復していることが示唆された。
- 凝集が生じている治験用 2 ロットおよび市販品 2 ロットについて、0.22μm のフィルターによる凝集物の分取前後に HA 含量を測定した。この結果、抗原製剤のみではフィルターろ過後における HA 含量はろ過前の 73～79%であるものの、アジュバント混合後の製剤においてはろ過前の 91～100%と回復した。このことから、抗原製剤において存在する凝集物はアジュバント混合後にフィルターの目開き以下に小さく分散することが示唆された［11 月 12 日付の照会 8 に対

- カナダにおいてアナフィラキシーの発生原因を調査するために流通を停止したロット（A80CA007A）の製造元保管品について種々の検討を実施したところ、当該ロットは他の市販品ロットと比較し抗原製剤において 50μm 以上の凝集物が多く認められる（ただし平均粒子径については同程度であり、最大粒子径についてはより大きいロットも認められている）ものの、上述の 0.22μm のフィルターろ過による検討において、フィルターろ過後における HA 含量は 90%であった［平成 21 年 12 月 15 日提出　報告書“Arepanrix H1N1 Pandemic vaccine, QC preliminary data on retained samples Dec 10, 2009”］。
- これらの結果から、凝集物が存在していてもアジュバント製剤を加えて抗原成分を分散させた後では、SDS-PAGE 法によるたん白質プロファイルに変化がなく、SRID 法による力価の低下もないことが立証された。したがって、アジュバントで再構成させることにより、凝集物が免疫原性に影響を及ぼさないことが裏付けられた。

1.2. 安全性

- Arepanrix H1N1 の臨床用ロットについて、異常毒性否定試験および発熱性物質試験を実施しており、いずれも試験に適合した。なお、試験を実施するために外部試験機関に輸送している。
- Fluviral において、臨床での使用における予期しない安全性の問題は認められていない。

1.3. まとめ

以上のことから、本凝集物は本来抗原製剤に存在する成分からなる内因性のものであり、温度および輸送による影響はほとんど認められなかった。さらにアジュバント製剤混合後の免疫原性および安全性に対する問題は認められていない。

2. 本ワクチンにおける凝集物の状態

2.1. 目視検査の結果

2.1.1. 国内保管品の状態

- 国内臨床試験用の治験薬について、社内保管品ならびに都内および福岡の治験施設への輸送品における目視検査を実施した。凝集物の量および大きさに差が認められないことから、日本国内における輸送の影響はないものと考えられた。また、社内保管しているケベック工場製の H5N1 の治験薬についても同様に検査を実施したところ、凝集の程度は H1N1 製剤と比較して小さいものの、凝集物を認めた。なお、いずれの検体にも長径 1mm 以上および 0.15mm^2 の凝集物は認められなかった［11 月 12 日付の照会 1 および 2 に対する回答］。

2.1.2. カナダ市販品の状態

- 日本に輸送された治験用ロット、その製造元保管品およびカナダ国内向けの市販用ロットとの凝集状態について目視検査により比較し、凝集物の量および大きさはいずれもほぼ同等であることを確認した。また、カナダにおいてアナフィラキシー反応および ORS の発現が顕著であったロット（A80CA009A）の抗原製剤（ロット番号：AFLPA319BB）についても目視検査を実施したところ、他のロットと比較し凝集の程度は同等であった。なお、いずれの検体にも長径 1mm 以上および 0.15mm^2 の凝集物は認められなかった［11 月 12 日付の照会 1 および 6②に対する回答］。

2.2. まとめ

目視検査の結果から、凝集物の発生において日本国内外の輸送の影響はないものと考えられた。また、日本に輸送された治験用ロット、その製造元保管品およびカナダ国内向けの市販用ロットとの凝集の程度について比較し、凝集の程度がほぼ同等であることを確認した。一方、H5N1 および季節性インフルエンザ株においては凝集の程度が小さいことから、凝集の要因としては、株の内在的な性質による部分が多いと推察された。

3. 今後の実施予定の検討項目

3.1. 日本国内での検討

- 製造元での充てん後の目視検査に加えて、今後輸送される日本向け市販用ロットについて、2.1.1.に準じた方法にて目視検査を実施する。

3.2. 製造元での検討

- 日本国内臨床試験用の社内保管品を製造元に返送し、目視検査を実施し、さらにそれと同一ロットの製造元での保管品との凝集物の割合を比較する。
- 本年12月末までに、抗原製剤の濁度により凝集物の存在を定量的に評価する方法を確立し、出荷時における規格として設定することを検討中である［12 月 9 日付の照会 4 に対する回答］。

3.3. まとめ

凝集物の管理については、上述のように、製造元において出荷時における規格の設定を検討中であり、その規格の設定は 12 月末の予定である。前項までに示すように、凝集物が製剤の品質、安全性および有効性に影響を及ぼすことはないと考えられるが、当社では日本向け市販用ロットについてもいくつかのバイアルにつき目視検査を実施し、各ロットの凝集物の状態をモニターする予定である。

4. 提出資料一覧（凝集物について）

1.1　白色凝集物に関するまとめ（本資料）

1.2　白色凝集物に関するまとめ_別添

- EMEA の照会 3 に対する回答
- EMEA の照会 5 に対する回答
- EMEA の照会 8 に対する回答
- EMEA の照会 9 に対する回答
- EMEA の照会 11 に対する回答
- EMEA の照会（Cycle RR#7.4 Major Objection）1 に対する回答

1.3　Status Aggregate Investigations

1.4　Kinetics of Aggregates Formation

1.5　Factors Impacting the Aggregates in the H1N1 Antigen Lots and in the Adjuvanted H1N1 Vaccine Lots

1.6　Arepanrix H1N1 Pandemic vaccine, QC preliminary data on retained samples Dec 10, 2009

Question 3

Q-Pan Antigen Drug Product - EMEA/H/C/1201/aggregate report

It is understood that changes to the manufacturing processes were made in response to the occurrence of aggregates being linked to the increased ORS cases seen in Canada. This should be detailed and discussed. Discussion should be provided on the suitability of release and stability specifications to control/indicate the likeliness of aggregation.

Upon receipt of a higher number of adverse event reports in the Fall of 2000, the Company immediately initiated a thorough investigation to identify a potential cause. Investigations focused on all aspects of manufacturing, including raw materials, manufacturing process and the final vaccine product.

Extensive testing using Transmission Electronic Microscopy (TEM) revealed that the split monovalent concentrates of the A/Panama/2007/99 strain as well as the Fluviral/FluLaval trivalent vaccine produced in 2000 contained large aggregates with a size of more than 500nm of unsplit virions. These aggregates of unsplit virions were practically absent from the previous year's Fluviral/FluLaval. In the following season, 2001-2002, the manufacturing process was modified to improve the splitting process of the Panama strain. This modification resulted in a decrease of the number of ORS cases. Hence, it was concluded at that time that there might have been a link between presence of unsplit split virion particles and the increased rate of ORS.

Table 1 presents results of fragmentation assessment from the referred production seasons as well as the previous and the following seasons

Table 1 Fragmentation Assessment by TEM – Fluviral Monovalent (1999 to 2002)

Year	Strains	% of Fragmented Virions	% of Non-Fragmented Virions
Year 1999-2000 (Pre-ORS Season)	A/Bejing/262/95	95	5
	A/Sydney/5/97	99	1
	B/Yamanashi/166/98	98	2
Year 2000-2001 (ORS Season)	A/Panama/2007/99	78	21
	A/New Caledonia/20/99	97	3
	B/Yamanashi/166/98	98	2
Year 2001-2002 (with Revised Splitting Process)	A/Panama 2007/99	99.7	0.3
	A/New Caledonia/20/99	98.4	1.6
	B/Victoria/504/2000	99.4	0.6

The revised splitting process consisted of an increased dilution of the monovalent (from 8L to 17L) before splitting, and an increased DOC content, from 0.3% to 0.5% for all strains. In addition, for the A/Panama strain exclusively, a second splitting treatment with Triton X-100 at 0.3% was added to ensure a greater level of assurance of disruption efficiency while the investigation was still ongoing. This additional step of Triton treatment for the A/Panama strain was later removed from the manufacturing process for the Panama strain, as it was no longer necessary, as confirmed by testing of each monovalent by electron microscopy.

Fragmentation assessment by TEM was also introduced as QC release test for all drug substance monobulks. A minimum of 95% of fragmented virions was established as a specification for this test in order to indicate the effectiveness of the splitting process.

The revised splitting process has been in use since then and since then over 1200 monovalent bulk batches of 10 different strains have been produced and used for the manufacture of Fluviral/FluLaval lots. All batches were tested and were found to be over 95% fragmentation, irrespective of the strain, demonstrating the robustness of the revised process.

In the case of Arepanrix H1N1, the effectiveness of the process to fragment the virions was clearly demonstrated during the production of consistency batches. Table 2 shows fragmentation assessment by TEM for three batches representative of the commercial scale.

Table 2 Fragmentation Assessment by TEM – Arepanrix H1N1 (2009)

Batch Number	% of Fragmented Virions	% of Non-Fragmented Virions
1M9091CL	99.2	0.8
1M9092CL	99.6	0.4
1M9093CL	>99.9	<0.1

All three batches met the specification for the fragmentation confirming the efficacy and consistency of the splitting step.

In conclusion, the production process of Arepanrix H1N1 has demonstrated to consistently produce an adequately fragmented drug substance. Therefore, the presence of aggregates observed in final containers of Arepanrix H1N1 has no connection with the presence of unsplit virions.

Hence, given that white aggregates have been observed in Fluviral/FluLaval lots after implementation of the revised splitting method, for which no specific increased rate of ORS was observed and given that H1N1 monovalent bulk batches have been shown to be properly fragmented, the presence of the aggregates in H1N1 antigen vials is likely to have no impact on the ORS incidence rate.

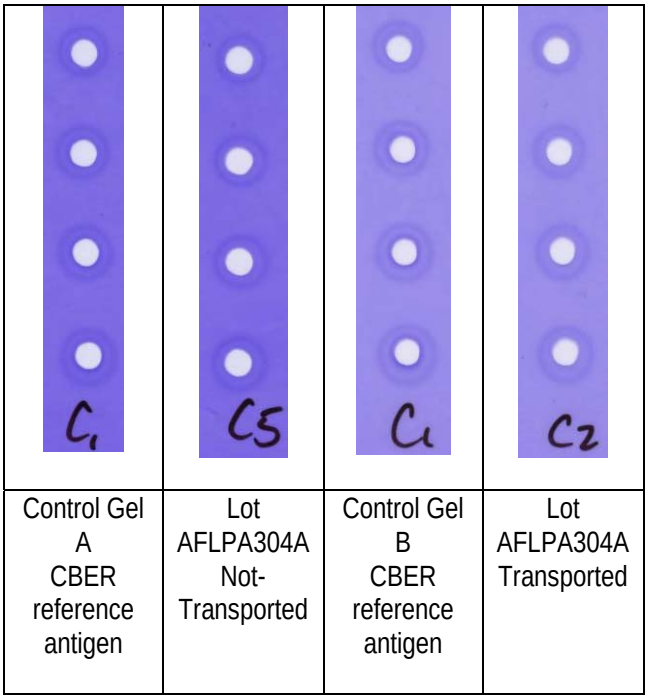
Question 5

Q-Pan Antigen Drug Product - EMEA/H/C/1201/aggregate report

The MAH should provide photographic pictures of the SRD gel for the aggregated and non-aggregated samples. The possibility of aggregates not being able to diffuse through the SRD matrix should be excluded.

Photographic pictures of SRD gels are provided in Figure 1. The figure shows rings from the four dilutions (higher to lower dilution from top to bottom) tested for HA content in each sample. Lot AFLPA304A, where aggregates were observed in both transported and not-transported samples, is presented. The Reference antigen (lot 69) provided by the Centre for Biologics Evaluation and Research (CBER) is presented as a control for non-aggregated material.

Figure 1 Pictures of SRD gels from aggregated and non-aggregated samples



Control Gel A CBER reference antigen	Lot AFLPA304A Not-Transported	Control Gel B CBER reference antigen	Lot AFLPA304A Transported

The analysis of the images of SRD gels shows that there is no evident difference in the appearance of the rings generated with both aggregated and non-aggregated material.

Question 8

Q-Pan Antigen Drug Product - EMEA/H/C/1201/aggregate report

It could be foreseen that product towards the end of it's shelf-life might contain more aggregates than that seen so far with product transported to and from Rixensart. This might have a more significant effect for this multidose product (e.g. failure of uniformity of content for mixed product for each vaccine dose or change in antigen-adjuvant interaction). This should be fully discussed with supporting evidence.

The company is currently evaluating the feasibility of the implementation of a physicochemical method to monitor the presence of aggregation. This assay would allow to assess the quality of the product towards the end of its shelf-life. The definition of a specification at release and during stability studies is also considered in the scope of this project. The target for completion of these activities would be by end of December 2009.

With respect to the effect of the presence of aggregates in this multidose presentation, GSK has conducted a dose uniformity study with a Arepanrix H1N1 vaccine lot that contains aggregates. The experimental design of this study was similar to the dose uniformity study reported in m3.2.P.2. Pharmaceutical Development (AS03-adjuvanted Quebec H1N1 influenza vaccine).

Clinical lot of antigen DFPLA304A and AS03 adjuvant lot number AA03A209C were used in this study. Using a syringe, the entire content of the AS03 adjuvant vial was removed and injected into the antigen vial to produce approximately 6 ml of final vaccine (approximately 7.5 µg HA/ml). The reconstituted vaccine was then hand shaken briefly. Ten 0.5 ml doses were then withdrawn using a syringe and the HA content was determined in each dose. The same operator conducted all the tests.

The following acceptance criterion was applied following guidance from the Ph. Eur monograph 2.9.40 - Uniformity of dosage units. The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to 15.

Calculation of acceptance value (AV) is done using the following formula as described in the Ph. Eur:

$$AV = | M - X | + ks$$

Where :
M = Reference value (case 2 where T>101.5, M=X))
X = Mean of individual contents of the dosage units, expressed as a percentage of the label claim
k = Acceptability constant (for n=10 dosage units, k=2.4)
s = Sample standard deviation

The results presented in Table 1 show that the content in each dose is close to the content claimed on the label (4.3(g/dose vs. 3.75(g/dose). The relative standard deviation across doses was 2.12%.

Uniformity of dosage units acceptance value (AV) was 0.2 and therefore the criterion of the test (less than or equal to 15) has been met, and uniformity of dosage has been demonstrated.

This confirms that the presence of aggregates has no effect on the uniformity of content in the Arepanrix H1N1 multidose presentation.

Table 1 Uniformity of dose of H1N1 adjuvanted vaccine following extemporaneously mixing

Test performed	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	Dose 10	Mean (n=10)	SD	RSD	AV (15
HA content (μg/mL)	8.6	8.4	8.6	8.9	8.4	8.6	8.4	8.6	8.9	8.6	8.6	0.18	2.09	N/Ap
HA content (μg)*	4.3	4.2	4.3	4.45	4.2	4.3	4.2	4.3	4.45	4.3	4.3	0.09	2.12	0.2

* Results expressed as content per delivered dose

Question 9

Q-Pan Antigen Drug Product - EMEA/H/C/1201/aggregate report

The possibility of microbial (or other) contamination should be excluded for the H1N1v samples.

Microbial (or other contamination) was excluded during the investigation on the presence of aggregates in Arepanrix H1N1 antigen final container:

- The final step in the drug substance purification process is a sterile filtration. Filters are integrity tested. All batches of Quebec H1N1 drug substance are tested for sterility per Ph. Eur. 2.6.1. No growth was observed in any of the 199 H1N1 drug substance batches produced from July to October 2009.
- Quebec H1N1 drug product final bulks are formulated and filled under a Class A environment. All lots of Quebec H1N1 drug product final bulk and final container are tested for sterility per Ph. Eur. 2.6.1. No growth was observed in any of the 59 H1N1 drug product lots produced from September to October 2009.

A comprehensively analysis of total protein, HA content and SDS-PAGE gels was presented in Questions 6. This study demonstrates that the white aggregates that have been observed in clinical and commercial lots of A/California/7/2009 adjuvanted H1N1 antigen formulation are constituted of the same components as those found in the antigen suspension (hemagglutinin and proteins).

Question 11

Q-Pan Antigen Drug Product - EMEA/H/C/1201/aggregate report

In what proportion of batches of Arepanrix have aggregates been observed?

In the affected batches what proportion of vials contain aggregates?

Presence of white aggregates has been observed in all clinical and commercial lots of A/California/7/2009 antigen vials of Arepanrix H1N1.

Arepanrix H1N1, likewise *Fluviral* seasonal influenza vaccine, is a suspension that is known to contain aggregates that may sediment. The extent of aggregation or sedimentation may vary, from lot to lot and from strain to strain. This feature is inherent to the nature of the vaccine, which is a suspension of split, inactivated virus particles and proteins.

Cycle RR#7.4 - Major Objection

Question 1

The presence of aggregates containing up to 30% of the protein and 50% of HA is a serious quality observation which may impact the safety and efficacy of the vaccine. The company should address this satisfactorily before a positive opinion can be given. (Arises from Q6 & Q7 RR#7.4)

- **The Applicant should address this satisfactorily before a positive opinion can be given. It should be clear that the clinical batches at the time of the clinical studies contained similar aggregated-HA amounts (in the mixed solution administered to the patient) as the commercial lots. It has been demonstrated that transporting does not contribute to aggregation, but it is likely that aggregation increases over time. Therefore, the level of aggregation at the time of clinical studies would have been lower than currently found in the same clinical batches.**

- **The issue can be resolved by demonstrating that aggregates re-dissolve upon mixing with the adjuvant as previously suggested. Alternatively, it could be demonstrated that the clinical batches at the time of the clinical studies contained similar aggregated-HA amounts (in the mixed solution administered to the patient). In the latter kinetics of aggregates formation should be taken into account: aggregate formation may be rapid making it likely aggregates had formed in clinical batches at time of administration. On the other hand, if aggregate formation is slow the clinical material was not aggregated. Also, variability between vials and batches should be taken into account.**

Clinical batches were used in clinical trials approximately between 30 days to 60 days after their manufacturing. The kinetics of aggregate formation in the H1N1 antigen alone or in combination with the AS03 adjuvant were studied using H1N1 antigen lots of different ages spanning that period. This study indicated that the H1N1 antigen aggregate profile is not significantly affected between 22 and 61 days after manufacturing; although a potential trend for a limited increase in the aggregation profile over time could not be excluded. In contrast, when mixed with the adjuvant, no impact on the antigen content as measured by SRD was observed over time. These data suggests that the clinical batches at the time of the clinical studies contained similar aggregated-HA amounts, both in the antigen alone and in the mixed solution administered to the patient. Details regarding the kinetics experiments are provided hereafter.

1. KINETICS OF AGGREGATES FORMATION

1.1. Principle

Given that kinetics experiments starting from fresh lots would take too much time prior having the results, the kinetics of aggregates formation was studied using H1N1 antigen lots of different ages spanning the 22 to 61 days period, within which the clinical material was administered (30-60 days). The aggregation profiles of these lots at different ages were studied through a number of methods as summarized in the Table 1.

Results were plotted as a function of the age of each lot to evaluate the evolution of the aggregation over time.

Table 1 Analyses and method

Materials	Analyses	Method
H1N1 antigen	Particle size and polydispersity profiles	[redacted] instrument
	Percentage of total protein content in aggregates	H1N1 samples were centrifuged and the supernatant fractions were collected. The supernatant fractions as well as the samples prior to centrifugation were tested for total protein (by Lowry) and HA content (by HPLC). The difference between the total protein or HA contents found in the whole suspension before centrifugation and the values observed in the supernatant fractions correspond to the amount of materials present in the aggregates. The quantity of total protein or HA in the aggregate is expressed as a percentage relative to the quantity of total protein or HA present in the whole suspension before centrifugation
	Percentage of HA content in aggregates	
AS03-adjuvanted H1N1 vaccine	HA content in the mixed vaccine	Standard SRD method

1.2. Materials and Method

1.2.1. Materials

Three commercial lots (AFLPA323B, AFLPA324BA and AFLPA328AA) and the clinical lot DFLPA304A were used. Some vials of these lots were subjected to transportation. Hence some commercial lot vials were transported by truck during 20 hours; vials of the clinical lot were shipped by plane from GSK Ste-Foy (Canada) manufacturing site to GSK Belgium and back to Canada. The manufacturing dates for these lots were as follows: 30 Sep 09 (AFLPA323B), 02 Oct 09 (AFLPA324BA) and 04 Oct 09 (AFLPA328AA) and 13 Sep 09 (DFLPA304A). Lot ages were calculated from the manufacturing date to the testing date.

1.2.2. Methods

The protein content was determined using the standard Lowry method. The HA content determination in AS03-adjuvanted H1N1 vaccine is described in details in m3.2.P.3.5. Pharmaceutical Development (AS03-Adjuvanted Quebec H1N1 Influenza Vaccine) of the initial file. All other methods are briefly described below.

1.2.2.1. HA content by HPLC

This analytical method determines the hemagglutinin content based on the measurement of the HA1 subunit after separation of the proteins in a mixture by hydrophobic interaction chromatography. The quantification is obtained by comparing the area of the HA1 subunit peak at 214nm in the samples against the area generated with a reference antigen preparation.

Zorbax Poroshell 300SB-C3 Narrow-Bore column, 75 mm x 2.1 mm ID, 5µm, 300Å; and Zorbax 300SB-C3 Narrow-Bore Guard Column, 12.5 mm x 2.1 mm ID, 5µm, 300Å are used as stationary phase. Samples are pre-treated with Dithiotreitol (DTT) and boiled for 15 minutes at 95ºC prior to being analysed. A linear gradient from 23% to 95% acetonitrile, 0.1% trifluoroacetic acid w/v is the mobile phase used for the elution.

1.2.2.2. Particle Size with [redacted] Instrument

[redacted] Instruments' Zetasizer uses light scattering techniques to measure hydrodynamic size of proteins and nanoparticles. This technique is based on the principle that the intensity of light scattered is proportional to the sixth power of the diameter ($I \mu d^6$) for small molecules and particles.

The instrument offers an analytical range from approximately 0.6 to 6000 nm. It does not take into account particles larger than 6000 nm.

1.2.2.3. Pellet and Supernatant Preparation

For each lot, the volume of 4 vials was pooled in a 50 ml Falcon and mixed to ensure homogeneity. Samples were collected for analysis of the whole suspension by Lowry or by HPLC. From the pooled material, 9 ml were centrifuged (6 x 1.5 ml in eppendorf tubes) at 13 000 rpm (16,060 g) for 30 min. From each eppendorf tube, 1.4 ml of supernatant was collected and transferred to a fresh tube. Samples of the supernatant fraction were collected for analysis by Lowry or HPLC.

The speed of the centrifuge was selected with the aim to separate all potential aggregates in suspension, whether visible or sub-visible, from the soluble fraction of the H1N1 antigen drug product. This speed is not believed to be fast enough to allow pelleting soluble proteins (which require ultracentrifugation forces).

1.3. Results

1.3.1. Evolution of the Aggregation Profiles of the H1N1 Antigen over Time

Results of the particle size and polydispersity analyses as well as the determination of percentage of the total protein and total HA content in aggregates are presented in Table 2. These parameters were plotted as a function of the time and are presented in Figure 1 (Particle size), Figure 2 (Polydispersity Index), Figure 3 (Total proteins in aggregates) and Figure 4 (Total HA content in aggregates).

Table 2 Particle size, Polydispersity Index, Total Proteins and Total HA in Aggregates as a Function of Time

Lot Number	Lot Age (days)	Particle Size	Polydispersity Index	Total Proteins in Aggregates (%)[1]	Total HA in Aggregates (%)[1]
AFLPA328AA	22	133	0.1483	31.4	48.5
AFLPA328AA (T)	22	142	0.2265	25.9	47.9
AFLPA324AB	24	136	0.2094	27.7	43.1
AFLPA324AB (T)	24	139	0.1948	28.9	46.8
AFLPA323B	27	135	0.1736	26.3	51.1
AFLPA323B (T)	27	136	0.1583	29.3	49.6
DFLPA304A	31	203[2]	0.2660	ND	ND
DFLPA304A (T)	31	155	0.2900	ND	ND
AFLPA328AA	39	137	0.2289	17.6	48.4
AFLPA324AB	41	135	0.2197	32.6	48.6
AFLPA323B	44	163	0.2657	26.4	49.4
DFLPA304A	61	150	0.2401	18.9	58.2

Note:
(T) identifies vials that were transported
1. The total proteins or HA in aggregates is expressed as a percentage relative to the total amount of proteins or HA found in the whole suspension prior to centrifugation
2. Outlier value. No explanation to date for this value

Figure 1 Average Particle Size over Time

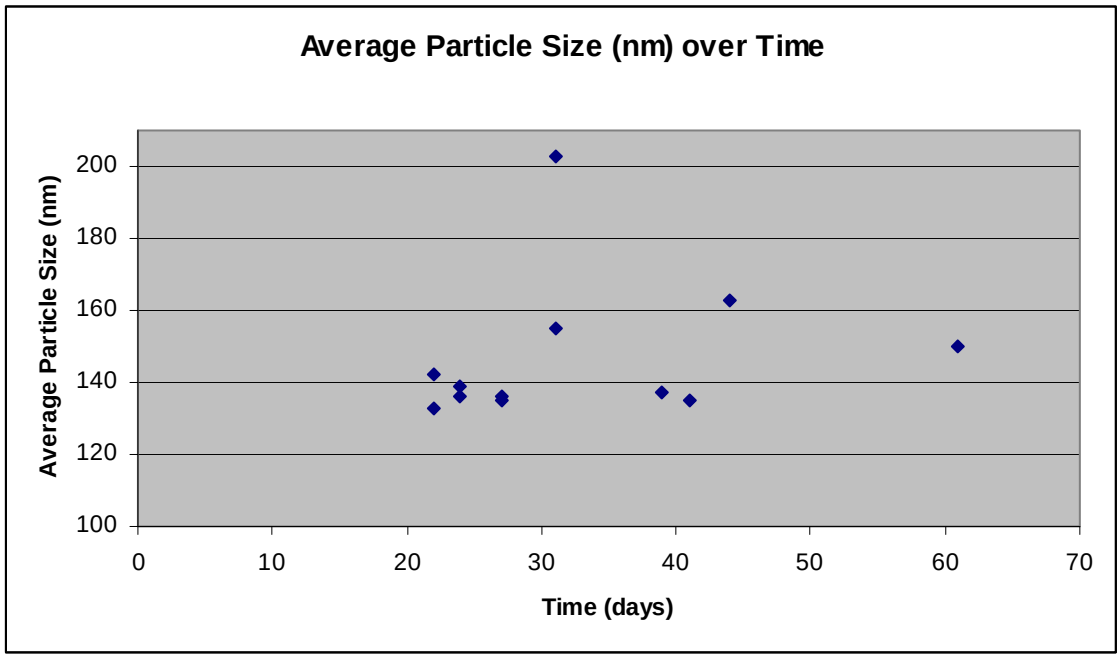


Figure 2 Polydispersity Index over Time

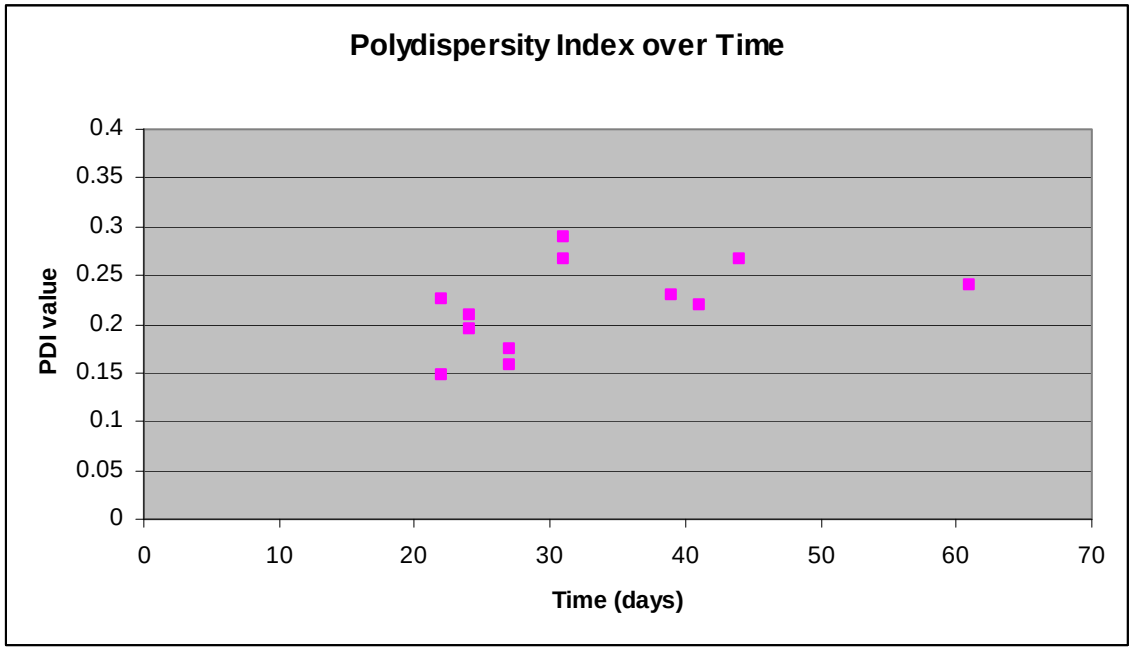


Figure 3 Percentage of Total Protein Content in Aggregates over Time

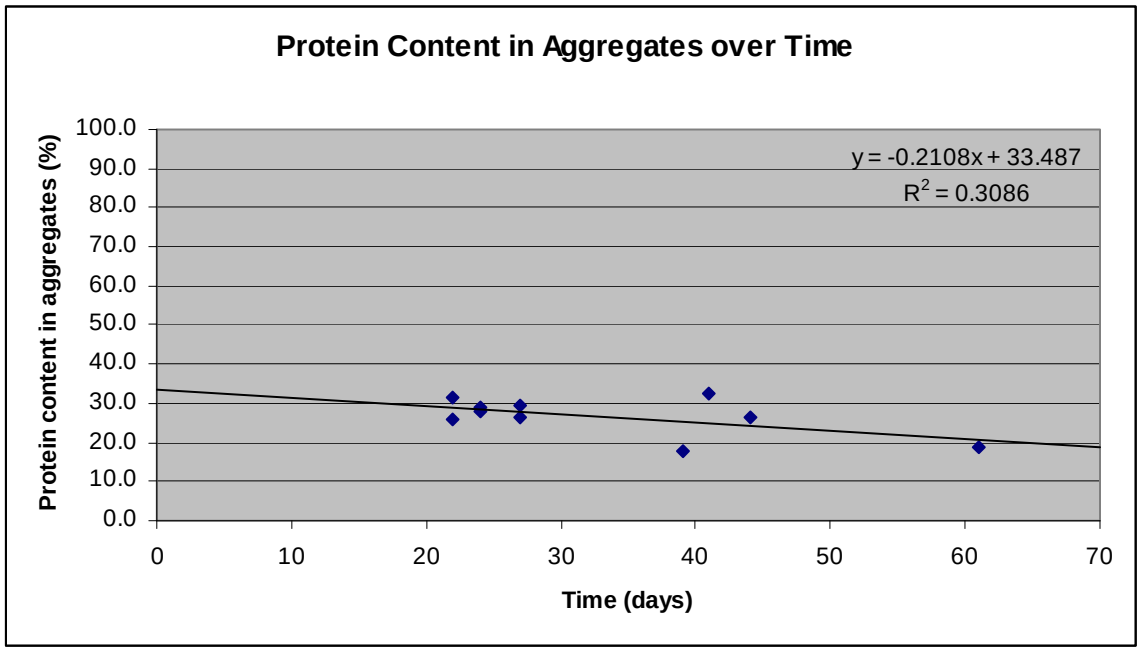
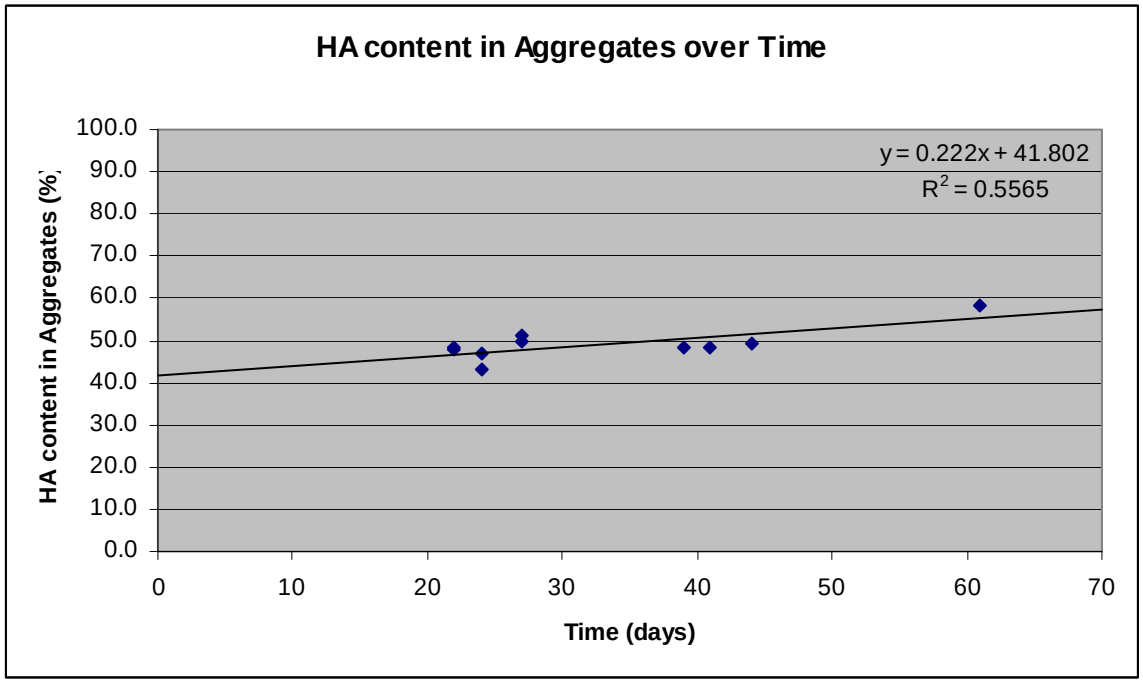


Figure 4 Percentage of Total HA Content in Aggregates over Time



1.3.2. Evolution of the AS03-Adjuvanted H1N1 Vaccine Potency over Time

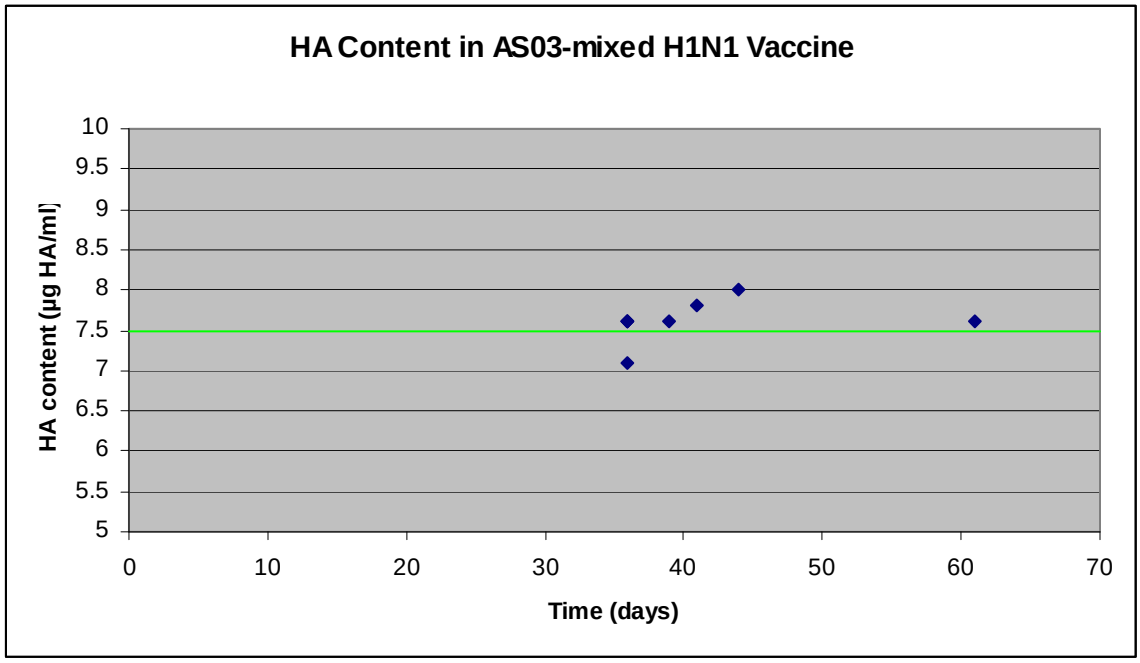
The evolution of the HA content of AS03-adjuvanted H1N1 vaccine lots over time is presented in Table 3 and is plotted in Figure 5.

Table 3 HA content by SRD in AS03-Adjuvanted H1N1 Vaccine as a Function of Time

Antigen Lot Number	Lot Age (days)	HA content by SRD (µg HA/ml)
DFLPA304A	36	7.6
DFLPA304A (24h)	36	7.1
DFLPA304A (T)	36	7.6
DFLPA304A (T) (24H)	36	7.6
AFLPA328AA	39	7.6
AFLPA324AB	41	7.8
AFLPA323B	44	8.0
DFLPA304A	61	7.6

Note: (T) identifies vials that were transported; (24H) identifies vials that were tested after 24 hours storage at 25°C after reconstitution.

Figure 5 HA content by SRD in AS03-Adjuvanted H1N1 Vaccine as a Function of Time



1.4. Discussion and Conclusions

The impact of time on the H1N1 antigen alone or in combination with the AS03-adjuvant was evaluated using a number of analytical tools.

Aggregation profile of the H1N1 antigen over time.

With respect to particle size and polydispersity index, the values observed over time were relatively broadly distributed. The particle size and polydispersity index were generally in the 130-150 nm range and 0.15-0.20 range, similar to *FluLaval*/*Fluviral* seasonal vaccine historical values (152.1±14.9nm, PDI of 0.18) and to the Arepanrix H5N1 values (136±15nm, PDI of 0.15). No clear modification of the particle size and polydispersity profiles were observed over time

Some limited effects were observed when looking at the evolution of the total proteins and total HA content present in the aggregates over time. The percentage of total protein in the aggregates tended to decrease over time at a rate of 0.21% per day whereas the percentage of HA content in the aggregates tended to increase over time at a rate of 0.22 % per day. These modifications would potentially correspond in the aggregates to a decrease of the total protein content of maximum 6.4% and to an increase of the HA content of maximum 6.6% between day 30 and day 60, i.e. the time at which clinical lots were used in the clinical trials.

With respect to clinical trials, the limited extent of the HA content increase of the aggregates, whether real or simply related to product and/or testing variability, is in any cases well within the variability of the assays used to monitor subject immune responses and are likely to have limited impact, if any, on the clinical trials outcome.

Evolution of the adjuvanted H1N1 vaccine potency over time

Table shows that there is no modification of the expected HA content of the adjuvanted H1N1 vaccine over the 61 days time course of the experiment. Values were ranging from 7.1 to 8.0 µg HA/ml i.e. around the target value of 7.5 µg HA/ml.

This observation clearly demonstrates that the presence of aggregates in the H1N1 antigen has had no impact on the potency of the adjuvanted vaccine over the course of the clinical trials.

Conclusions

This study indicated that the H1N1 antigen aggregate profile is not significantly affected between 22 and 61 days after manufacturing; although a potential trend for a limited increase in the aggregation profile over time could not be excluded. In contrast, when mixed with the adjuvant, no impact on the antigen content as measured by SRD was observed over time. These data suggests that the clinical batches at the time of the clinical studies contained similar aggregated-HA amounts, both in the antigen alone and in the mixed solution administered to the patient.

AS03-adjuvanted Quebec H1N1 Pandemic Vaccine (Arepanrix H1N1)

Status Aggregate Investigations

Date: 07 December 2009

1. EXECUTIVE SUMMARY

Several technical investigations have been carried out regarding the endogeneous protein aggregates observed in the A/California/7/2009 H1N1 antigen vials used with the Arepanrix H1N1 vaccine. Details can be found in the two reports provided in annex to this document. The present document summarizes the current results and conclusions.

Two main conclusions can be made from the current technical data available:

1. The level of Hemagglutinin (HA) content observed in the aggregates appears to be constant across the H1N1 antigen lots tested to date as demonstrated by two different techniques (around 25-30 % by filtration and around 45-50% by centrifugation). Independent of the technique used, the level does not appear to be impacted by transportation (by truck and by air), to vary over time (up to 74 days) and to be influenced by the lot manufacturing history (e.g. manufacturing in different filling sites).
2. Addition of the AS03 adjuvant reduces the level of HA present in the aggregate. When filtering (0.22 µm) AS03-adjuvanted H1N1 vaccine lots, more than 90 % of the Hemagglutinin is recovered in the vaccine filtrate, demonstrating that only a limited fraction of the antigen is present under aggregated form in the adjuvanted vaccine.

These data show that the presence of aggregates in the H1N1 antigen has limited impact, if any, on the potency of the Arepanrix vaccine lots, given the high HA content recovery upon mixing of the antigen with the AS03 adjuvant.

This conclusion is further supported by the preliminary results from the Q-Pan H1N1-001 clinical trial, which showed in adults that the AS03-adjuvanted H1N1 vaccine largely meet the 3 CHMP criteria for immunogenicity after one administration of either 3.75µg HA (the targeted formulation) or 1.9 µg HA antigen dose.

Based on the above technical data which demonstrate that the aggregates have limited impact on the adjuvanted vaccine potency and given the preliminary clinical results in adults that shows that the AS03-adjuvanted H1N1 vaccine is immunogenic, even at half the targeted antigen dose, the Company is confident that the presence of aggregates in the H1N1 antigen has no impact on the consistency and immunogenicity of the Arepanrix vaccine.

Summary results for the technical investigations and for study Q-Pan H1N1-001 are presented in section 2 and section 3 respectively

2. SUMMARY TECHNICAL INVESTIGATIONS ON H1N1 AGGREGATE LEVEL

Several investigations were conducted regarding the level of aggregates in the H1N1 vaccine. These were carried out either at the level of the H1N1 antigen alone or on the actual vaccine after mixing of the H1N1 antigen with the AS03 adjuvant. The results can be summarized as follow.

The level of aggregates in H1N1 antigen lots or in the AS03-adjuvanted vaccine lots was studied using two different techniques, both based on the same principle: removing the aggregate from the antigen first, then quantifying the residual HA or total protein content before and after treatment, thus allowing determination of the aggregate content by calculation:

1. Centrifugation (16,060 g for 30 minutes). The HA content by HPLC and the total protein content by Lowry were determined in the sample supernatant and before centrifugation. This technique aimed at separating as much as possible all aggregates, irrespective of their size
2. Filtration on 0.22 µ filter. The HA content by SRD was determined before and after filtration. This technique aimed at separating aggregates above the filter cut-off.

Different types of lots were tested: either transported or not, at different ages and having different manufacturing history (e.g. filling conducted GSK-Ste-Foy, Canada or at DSM-Greenville, USA).

Details about the investigations can be found in the two documents entitled "*Factors Impacting the Aggregates in the H1N1 Antigen Lots and in the Adjuvanted H1N1 Vaccine Lots, December 7, 2009*" and "*Kinetics of Aggregate Formation*" provided in annex to this briefing document.

A brief overview of GSK current plans regarding monitoring of aggregates is presented at the end of this section.

Investigations on the H1N1 antigen alone

The investigations regarding H1N1 antigen alone showed that the level of HA or total protein in the aggregate is constant across lots:

- Centrifugation experiments conducted on 3 antigen lots showed that the aggregates consistently contain ~ 30% of the total proteins and ~ 45-50% of the HA overall content. These levels were not modified upon transportation (~20 hours truck transportation) and were not significantly affected over time (lots were tested at various ages, ranging from 21 to 61 days).
- Filtration experiments conducted on 3 antigen lots showed that the aggregates consistently contain ~ 25-30% of the HA overall content. These levels were not affected by transportation (~10-15 hours truck transportation) and they were not significantly affected over time (lots were tested at various ages, ranging from 27 to

74 days). The levels were also the same despite different filling sites were used for their filling (GSK-Ste-Foy, Canada and DSM-Greenville, USA).

Larger amounts of HA were observed using the centrifugation method vs. the filtration method. This is likely due to the fact that the centrifugation method is aimed at collecting as much as possible aggregates irrespective of their particle sizes, whereas the filtration method only separate aggregates above the cut-off of the filter.

Although the two method yields to different numbers in terms of HA content in the H1N1 antigen lots, both methods consistently show that the same level is observed in all the lot tested in the same series, supporting the consistency of the antigen quality. In addition, both methods show that the HA content level appears to be the same, irrespective whether the lots were transported or not (~20 hours truck transportation), of their age (lots were tested up to 74 days of age) and irrespective of their manufacturing history (Lots from 2 different filling sites were tested).

All these elements indicate that the manufacturing of the H1N1 antigen lots yields to products of consistent quality, for which the level of aggregates does not significantly change over a period of at least 74 days.

Investigations on the AS03-adjuvanted vaccine

The investigations on the AS03-adjuvanted vaccine showed that mixing of the AS03 adjuvant with the H1N1 antigen result in a large reduction of the level of aggregation in the adjuvanted vaccine.

Three adjuvanted vaccine lots were filtered on 0.22µm filter to remove potential aggregated materials. Upon testing by SRD, more than 90 % of the initial HA content was consistently recovered in the filtrate. The same level was observed for transported materials (~10-15 hours truck or air transportation), for materials aged from 27 to 74 days and irrespective of the filling sites used for the antigen manufacture (GSK-Ste-Foy, Canada and DSM-Greenville, USA).

The uniformity of dosage was demonstrated for two lots of adjuvanted vaccine, manufactured with H1N1 antigen lot that have been transported (by truck or by air). Ten 0.5 ml dose have been withdrawn, all with the expected similar HA content (by SRD). This observation further provides indirect evidence that the level of aggregates in the mixed vaccine is indeed reduced.

At this stage of the investigation, it can only be hypothesized that the reduction of aggregates in the adjuvanted vaccine is the result from either aggregate dissolution or aggregate size reduction below the filter cut-off. More investigations will be needed to further understand the actual mechanism.

Overall conclusion

Overall, the experiments conducted on adjuvanted vaccine lots where aggregates have been removed by filtration, shows that the presence of aggregate in the H1N1 antigen lots

has limited impact, if any on the adjuvanted vaccine potency given the high HA content recovery consistently observed in all lot tested upon mixing with the AS03 adjuvant.

3. SUMMARY IMMUNOGENICITY RESULTS OF Q-PAN H1N1-001 STUDY

The Company has available preliminary immunogenicity results post-dose 1 from Q-Pan-001 study and believes it is of importance to share these with EMEA.

Study Q-Pan H1N1-001 is a phase I/II, observer-blind, randomized, multi-centre dose ranging trial to evaluate the safety and immunogenicity of a two-dose series of monovalent A/California/7/2009 (H1N1)v-like vaccine manufactured in Québec, Canada administered with and without AS03 adjuvant in adults aged 18 years and older. The study design is shown below in Figure 1.

Figure 1 Q-Pan H1N1-001 study design

Group	Dose 1 (Day 0)	Dose 2 (Day 21)	Subjects
A	A/Calif 3.75 µg + $AS03_A$	A/Calif 3.75 µg + $AS03_A$	210
B1	A/Calif 1.9 µg + $AS03_B$	A/Calif 1.9 µg + $AS03_B$	105
B2	A/Calif 1.9 µg + $AS03_B$	Saline placebo	105
C	A/Calif 3.75 µg + $AS03_A$	Saline placebo	210
D	A/Calif 15 µg	Saline placebo	210
E1	A/Calif 7.5 µg	A/Calif 7.5 µg	105
E2	A/Calif 7.5 µg	Saline placebo	105
F	A/Calif 3.75 µg	A/Calif 3.75 µg	210
		Total	**1,260**

This study was designed to evaluate adjuvanted and non-adjuvanted dosing regimens in adults ≥18 years old, and was randomized according to a 2:1:1:6 ratio for the following age strata: 18-40, 41-51, 52-64, ≥65 yrs. Primary objective was to show attainment of CBER criteria for group A at Day 21 and Day 42. The study is being conducted in Canada and the US.

Preliminary immunogenicity data are available in 299 subjects of a total of 1340 enrolled.

Groups were pooled for immunogenicity analysis as follows:

Group	Dose 1 (Day 0)	18-60	>60	Subjects
A + C	A/Calif 3.75 µg + AS03A	38	64	102
B1 + B2	A/Calif 1.9 µg + AS03B	13	36	49
D	A/Calif 15 µg	18	31	49
E1 + E2	A/Calif 7.5 µg	20	29	49
F	A/Calif 3.75 µg	22	28	50
	Total	111	188	299

The results are shown in the tables below. Table 1 shows the immune response in adults 18-60 years old and Table 2 shows the immune response in adults > 60 years old.

The immune response observed after administration of 3.75 microgram HA/AS03B was in line with the preliminary results obtained in study D-Pan H1N1-017 (Q-Pan arm results), and in line with data obtained so far with D-Pan H1N1 vaccine.

Interestingly, after one dose of 1.9 μHA/AS03$_B$ the immune response remained high and the 3 CHMP criteria were widely exceeded. In adults 18-60 years old a SCR of 97.4 %, a SPR of 97.4 % and a SCF of 40.2 was observed. In adults > 60 years of age receiving the full-dose Arepanrix vaccine, SCR, SPR and SCF raised up to 73.4%, 92.2% and 8.3,respectively, well exceeding the CHMP criteria set for elderly subjects, but also those set for young adults. Of note, the half-dose vaccine, when administered to subjects aged >60 years, also elicited an immune response that exceeded the CHMP criteria set for both age strata.

A similar observation can be made with the plain antigen formulation. Reducing the antigen content from 15μg to 7.5μg still leads to the induction of a high immune response in subjects aged 18-60 years, with attainment of the 3 CHMP criteria. In the older age group, however, a trend to a decrease in the immune response is observed when halving the antigen dose of the plain formulation, and the seroprotection rate criterion is not attained. These results should however be interpreted taking into account the small sample size from which results are available to date.

The Company believes that these results are of importance in the context of the discussion on the potential impact of aggregates on the immunogenicity of the vaccine. One of the points of discussion has been whether presence of aggregates could result in heterogeneity of HA content per dose (taken from the 10 dose vial of mixed vaccine). The preliminary data presented and discussed earlier in this document indicate that aggregates are solubilised following mixture with the emulsion, and therefore the concern of heterogeneity would be lifted. Still, until further confirmed, the immunogenicity data obtained in Q-Pan H1N1-001 are reassuring, as they indicate that even if a subject would receive a lower quantity of HA due to presence of aggregates this will not negatively impact their capacity to build an adequate immune response.

Of note, safety results obtained at Day 7 showed that reactogenicity profile was similar to previously observed reactogenicity profiles of D-Pan H1N1, Q-Pan H5N1 and D-Pan H5N1 vaccines.

Table 1 Study Q-Pan-001: preliminary immunogenicity results (subjects 18-60 years old)

	3.75µg +AS03$_A$ n=38	1.9µg + AS03$_B$ n=13	15 µg plain n=18	7.5 µg plain n=20	3.75 µg plain n=22
GMTs (95% CI)	509.7 (308.3-842.7	375.4 (167.4-841.9)	235.3 (141.4-391.7)	298.6 (187.7-475.0)	99.6 (40.8-243.6)
SCR (95% CI)	97.4% (84.3-100.0)	92.3% (59.9-99.9)	94.4% (69.4-99.9)	95.0% (72.1-99.9)	59.1% (33.6-81.4)
SPR (95% CI)	97.4% (84.3-100)	100% (71.4-100)	100% (78.4-100)	100% (80.3-100)	68.2% (42.2-87.9)
SCF (95% CI)	40.2 (23.0-70.4)	25.9 (7.8-86.2)	20.2 (11.3-36.2)	27.9 (13.1-59.2)	11.3 (4.9-26.0)

Table 2 Study Q-Pan-001: preliminary immunogenicity results (subjects >60 years old)

	3.75µg +AS03$_A$ n=64	1.9µg + AS03$_B$ n=36	15 µg plain n=31	7.5 µg plain n=29	3.75 µg plain n=28
GMTs (95% CI)	142 (106.2-190.0)	110.8 (68.0-180.5)	113.1 (60.2-212.5)	45.1 (24.3-83.5)	39.4 (18.3-84.7)
SCR (95% CI)	73.4% (59.2-84.9)	75.0% (55.4-89.2)	59.4% (38.3-78.2)	37.9% (18.8-60.3)	25% (9.3-47.6)
SPR (95% CI)	92.2% (81.2-97.8)	83.3% (64.9-94.5)	78.1% (57.5-91.9)	55.2% (33.3-75.7)	42.9% (22.4-65.3)
SCF (95% CI)	8.3 (5.9-11.7)	11.2 (6.3-20.0)	9.4 (5.3-16.7)	4.0 (2.3-6.8)	3.0 (1.8-5.2)

AS03-adjuvanted Quebec H1N1 Pandemic Vaccine (Arepanrix H1N1)

Kinetics of Aggregates Formation

Date: 20 November 2009

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1. INTRODUCTION

Clinical batches were used in clinical trials approximately between 30 days to 60 days after their manufacturing. The kinetics of aggregate formation in the H1N1 antigen alone or in combination with the AS03 adjuvant were studied using H1N1 antigen lots of different ages spanning that period. This study indicated that the H1N1 antigen aggregate profile is not significantly affected between 22 and 61 days after manufacturing; although a potential trend for a limited increase in the aggregation profile over time could not be excluded. In contrast, when mixed with the adjuvant, no impact on the antigen content as measured by SRD was observed over time. These data suggests that the clinical batches at the time of the clinical studies contained similar aggregated-HA amounts, both in the antigen alone and in the mixed solution administered to the patient.

Details regarding the kinetics experiments are provided in this document.

2. KINETICS OF AGGREGATES FORMATION

2.1. Principle

Given that kinetics experiments starting from fresh lots would take too much time prior having the results, the kinetics of aggregates formation was studied using H1N1 antigen lots of different ages spanning the 22 to 61 days period, within which the clinical material was administered (30-60 days). The aggregation profiles of these lots at different ages were studied through a number of methods as summarized in the Table 1.

Results were plotted as a function of the age of each lot to evaluate the evolution of the aggregation over time.

Table 1 Analyses and method

Materials	Analyses	Method
H1N1 antigen	Particle size and polydispersity profiles	[redacted] instrument
	Percentage of total protein content in aggregates	H1N1 samples were centrifuged and the supernatant fractions were collected. The supernatant fractions as well as the samples prior to centrifugation were tested for total protein (by Lowry) and HA content (by HPLC). The difference between the total protein or HA contents found in the whole suspension before centrifugation and the values observed in the supernatant fractions correspond to the amount of materials present in the aggregates. The quantity of total protein or HA in the aggregate is expressed as a percentage relative to the quantity of total protein or HA present in the whole suspension before centrifugation
	Percentage of HA content in aggregates	
AS03-adjuvanted H1N1 vaccine	HA content in the mixed vaccine	Standard SRD method

2.2. Materials and Method

2.2.1. Materials

Three commercial lots (AFLPA323B, AFLPA324BA and AFLPA328AA) and the clinical lot DFLPA304A were used. Some vials of these lots were subjected to transportation. Hence some commercial lot vials were transported by truck during 20 hours; vials of the clinical lot were shipped by plane from GSK Ste-Foy (Canada) manufacturing site to GSK Belgium and back to Canada. The manufacturing dates for these lots were as follows: 30 Sep 09 (AFLPA323B), 02 Oct 09 (AFLPA324BA) and 04

Oct 09 (AFLPA328AA) and 13 Sep 09 (DFLPA304A). Lot ages were calculated from the manufacturing date to the testing date.

2.2.2. Methods

The protein content was determined using the standard Lowry method. The HA content determination in AS03-adjuvanted H1N1 vaccine is described in details in m3.2.P.3.5. Pharmaceutical Development (AS03-Adjuvanted Quebec H1N1 Influenza Vaccine) of the initial file. All other methods are briefly described below.

2.2.2.1. HA content by HPLC

This analytical method determines the hemagglutinin content based on the measurement of the HA1 subunit after separation of the proteins in a mixture by hydrophobic interaction chromatography. The quantification is obtained by comparing the area of the HA1 subunit peak at 214nm in the samples against the area generated with a reference antigen preparation.

Zorbax Poroshell 300SB-C3 Narrow-Bore column, 75 mm x 2.1 mm ID, 5µm, 300Å; and Zorbax 300SB-C3 Narrow-Bore Guard Column, 12.5 mm x 2.1 mm ID, 5µm, 300Å are used as stationary phase. Samples are pre-treated with Dithiotreitol (DTT) and boiled for 15 minutes at 95ºC prior to being analysed. A linear gradient from 23% to 95% acetonitrile, 0.1% trifluoroacetic acid w/v is the mobile phase used for the elution.

2.2.2.2. Particle Size with [redacted] Instrument

[redacted] Instruments' Zetasizer uses light scattering techniques to measure hydrodynamic size of proteins and nanoparticles. This technique is based on the principle that the intensity of light scattered is proportional to the sixth power of the diameter ($I \mu d^6$) for small molecules and particles.

The instrument offers an analytical range from approximately 0.6 to 6000 nm. It does not take into account particles larger than 6000 nm.

2.2.2.3. Pellet and Supernatant Preparation

For each lot, the volume of 4 vials was pooled in a 50 ml Falcon and mixed to ensure homogeneity. Samples were collected for analysis of the whole suspension by Lowry or by HPLC. From the pooled material, 9 ml were centrifuged (6 x 1.5 ml in eppendorf tubes) at 13 000 rpm (16,060 g) for 30 min. From each eppendorf tube, 1.4 ml of supernatant was collected and transferred to a fresh tube. Samples of the supernatant fraction were collected for analysis by Lowry or HPLC.

The speed of the centrifuge was selected with the aim to separate all potential aggregates in suspension, whether visible or sub-visible, from the soluble fraction of the H1N1 antigen drug product. This speed is not believed to be fast enough to allow pelleting soluble proteins (which require ultracentrifugation forces).

2.3. Results

2.3.1. Evolution of the Aggregation Profiles of the H1N1 Antigen over Time

Results of the particle size and polydispersity analyses as well as the determination of percentage of the total protein and total HA content in aggregates are presented in Table 2. These parameters were plotted as a function of the time and are presented in Figure 1 (Particle size), Figure 2 (Polydispersity Index), Figure 3 (Total proteins in aggregates) and Figure 4 (Total HA content in aggregates).

Table 2 Particle size, Polydispersity Index, Total Proteins and Total HA in Aggregates as a Function of Time

Lot Number	Lot Age (days)	Particle Size	Polydispersity Index	Total Proteins in Aggregates (%)[1]	Total HA in Aggregates (%)[1]
AFLPA328AA	22	133	0.1483	31.4	48.5
AFLPA328AA (T)	22	142	0.2265	25.9	47.9
AFLPA324AB	24	136	0.2094	27.7	43.1
AFLPA324AB (T)	24	139	0.1948	28.9	46.8
AFLPA323B	27	135	0.1736	26.3	51.1
AFLPA323B (T)	27	136	0.1583	29.3	49.6
DFLPA304A	31	203[2]	0.2660	ND	ND
DFLPA304A (T)	31	155	0.2900	ND	ND
AFLPA328AA	39	137	0.2289	17.6	48.4
AFLPA324AB	41	135	0.2197	32.6	48.6
AFLPA323B	44	163	0.2657	26.4	49.4
DFLPA304A	61	150	0.2401	18.9	58.2

Note:
(T) identifies vials that were transported
1. The total proteins or HA in aggregates is expressed as a percentage relative to the total amount of proteins or HA found in the whole suspension prior to centrifugation
2. Outlier value. No explanation to date for this value

Figure 1 Average Particle Size over Time

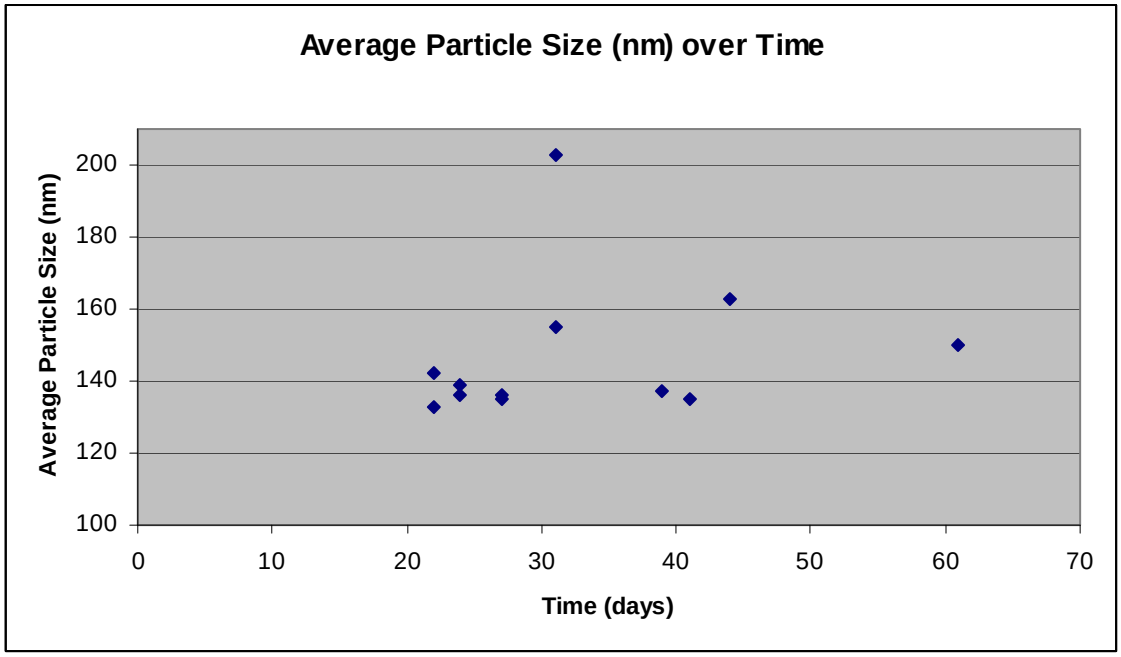


Figure 2 Polydispersity Index over Time

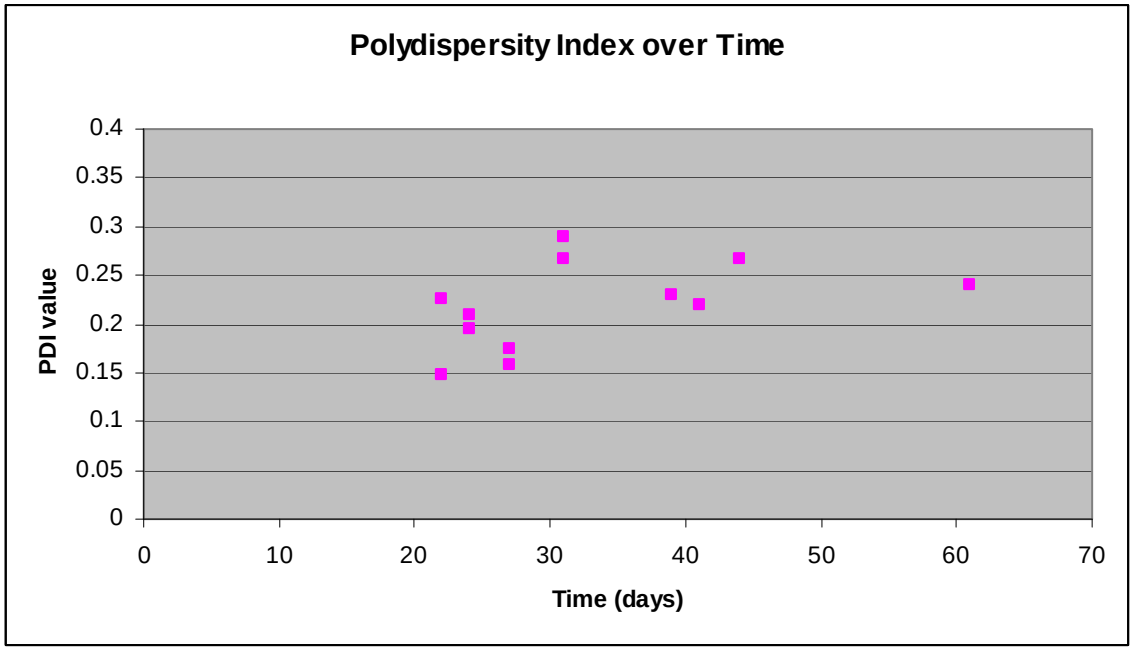


Figure 3 Percentage of Total Protein Content in Aggregates over Time

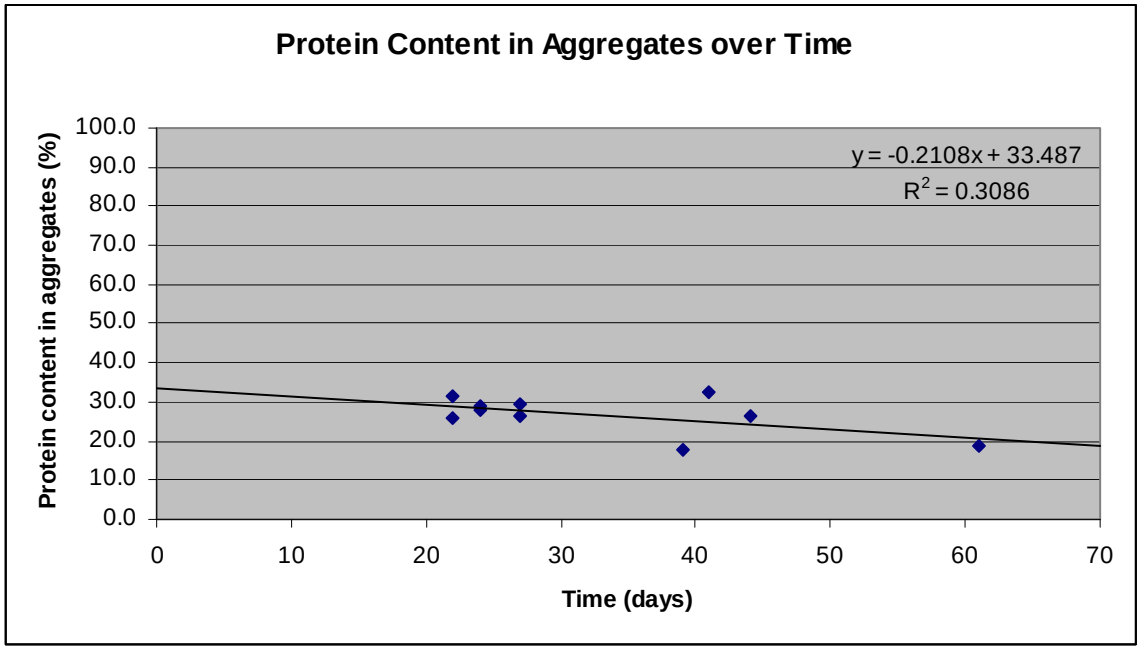
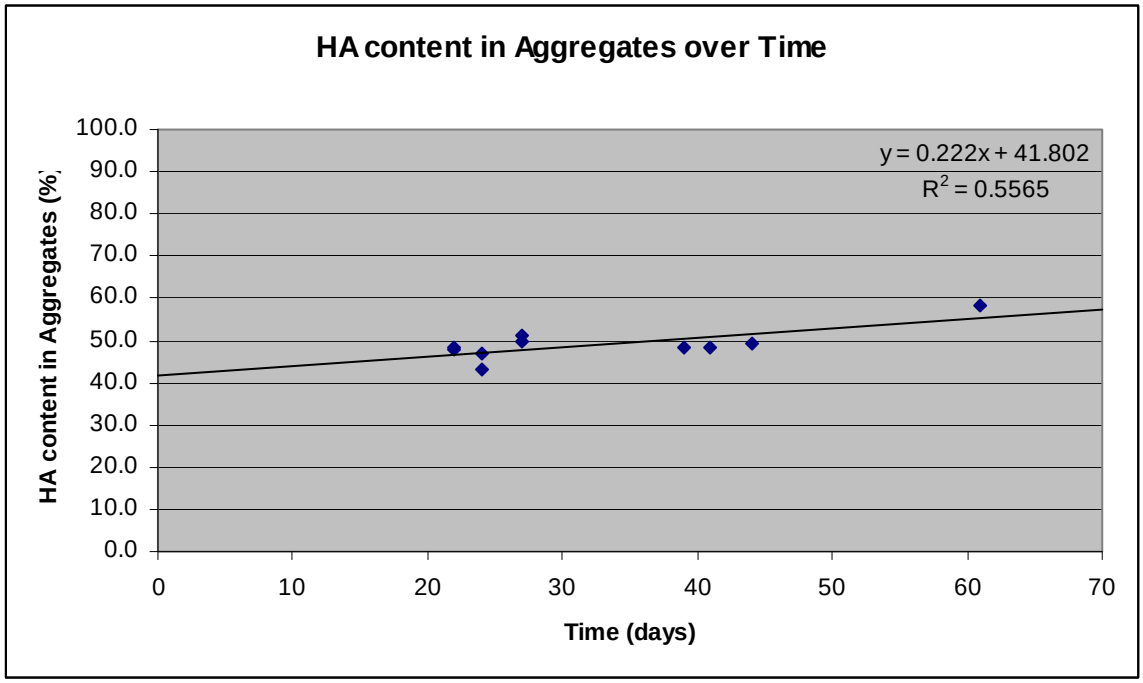


Figure 4 Percentage of Total HA Content in Aggregates over Time



2.3.2. Evolution of the AS03-Adjuvanted H1N1 Vaccine Potency over Time

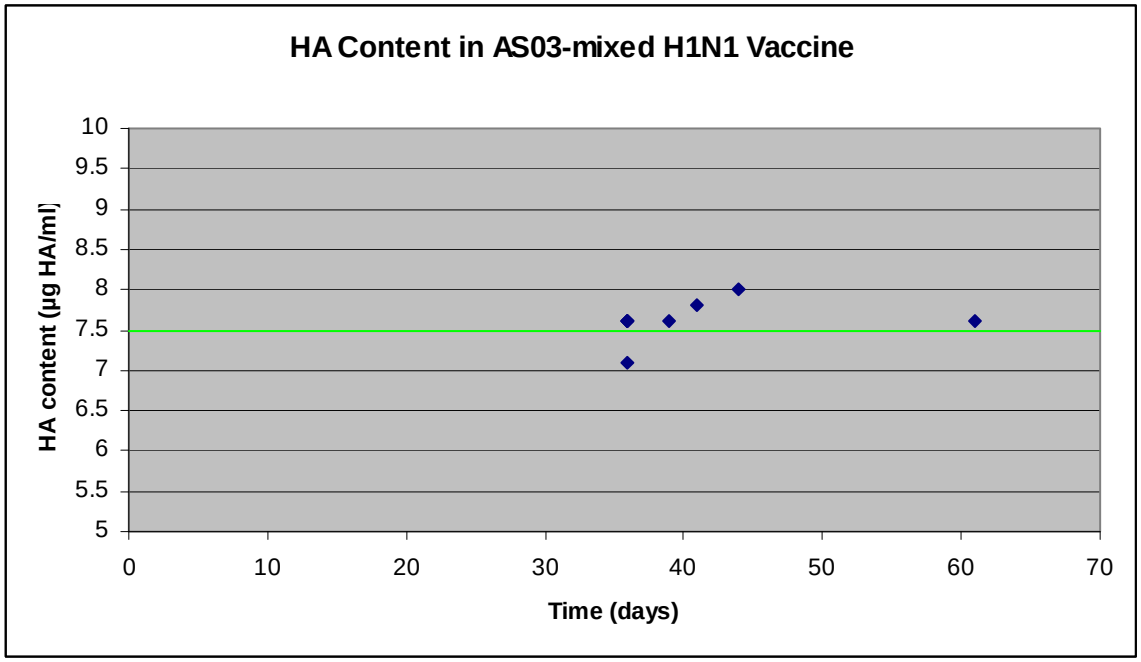
The evolution of the HA content of AS03-adjuvanted H1N1 vaccine lots over time is presented in Table 3 and is plotted in Figure 5.

Table 3 HA content by SRD in AS03-Adjuvanted H1N1 Vaccine as a Function of Time

Antigen Lot Number	Lot Age (days)	HA content by SRD (µg HA/ml)
DFLPA304A	36	7.6
DFLPA304A (24h)	36	7.1
DFLPA304A (T)	36	7.6
DFLPA304A (T) (24H)	36	7.6
AFLPA328AA	39	7.6
AFLPA324AB	41	7.8
AFLPA323B	44	8.0
DFLPA304A	61	7.6

Note: (T) identifies vials that were transported; (24H) identifies vials that were tested after 24 hours storage at 25°C after reconstitution.

Figure 5 HA content by SRD in AS03-Adjuvanted H1N1 Vaccine as a Function of Time



2.4. Discussion and Conclusions

The impact of time on the H1N1 antigen alone or in combination with the AS03-adjuvant was evaluated using a number of analytical tools.

Aggregation profile of the H1N1 antigen over time.

With respect to particle size and polydispersity index, the values observed over time were relatively broadly distributed. The particle size and polydispersity index were generally in the 130-150 nm range and 0.15-0.20 range, similar to *FluLaval*/*Fluviral* seasonal vaccine historical values (152.1±14.9nm, PDI of 0.18) and to the Arepanrix H5N1 values (136±15nm, PDI of 0.15). No clear modification of the particle size and polydispersity profiles were observed over time

Some limited effects were observed when looking at the evolution of the total proteins and total HA content present in the aggregates over time. The percentage of total protein in the aggregates tended to decrease over time at a rate of 0.21% per day whereas the percentage of HA content in the aggregates tended to increase over time at a rate of 0.22 % per day. These modifications would potentially correspond in the aggregates to a decrease of the total protein content of maximum 6.4% and to an increase of the HA content of maximum 6.6% between day 30 and day 60, i.e. the time at which clinical lots were used in the clinical trials.

With respect to clinical trials, the limited extent of the HA content increase of the aggregates, whether real or simply related to product and/or testing variability, is in any cases well within the variability of the assays used to monitor subject immune responses and are likely to have limited impact, if any, on the clinical trials outcome.

Evolution of the adjuvanted H1N1 vaccine potency over time

Table 3 shows that there is no modification of the expected HA content of the adjuvanted H1N1 vaccine over the 61 days time course of the experiment. Values were ranging from 7.1 to 8.0 µg HA/ml i.e. around the target value of 7.5 µg HA/ml.

This observation clearly demonstrates that the presence of aggregates in the H1N1 antigen has had no impact on the potency of the adjuvanted vaccine over the course of the clinical trials.

Conclusions

This study indicated that the H1N1 antigen aggregate profile is not significantly affected between 22 and 61 days after manufacturing; although a potential trend for a limited increase in the aggregation profile over time could not be excluded. In contrast, when mixed with the adjuvant, no impact on the antigen content as measured by SRD was observed over time. These data suggests that the clinical batches at the time of the clinical studies contained similar aggregated-HA amounts, both in the antigen alone and in the mixed solution administered to the patient.

APPENDIX 2 /AS03-adjuvanted Quebec H1N1 Pandemic Vaccine
(Arepanrix H1N1)

Factors Impacting the Aggregates in the H1N1 Antigen Lots and in the Adjuvanted H1N1 Vaccine Lots

Date: 07 December 2009

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1. INTRODUCTION

This document presents an overview of the investigations conducted by GSK regarding factors potentially impacting the aggregation level in H1N1 antigen lots and in the adjuvanted H1N1 vaccine lots were investigated. The following factors were studied: transportation (by air or by truck), aging (a range of lots at different age were tested) and manufacturing history (i.e. production at different manufacturing sites).

Three sets of experiments were conducted to evaluate factors potentially influencing the aggregation level of the Arepanrix vaccine:

- Evaluation of aggregates HA content in H1N1 antigen lots by centrifugation.
- Evaluation of aggregates HA content in H1N1 antigen lots and in adjuvanted H1N1 vaccine lots by filtration
- HA content and dose uniformity evaluation of adjuvanted H1N1 vaccine lots

Results of these experiments show the following:

1. The level of Hemagglutinin (HA) content observed in the aggregates appears to be constant across the H1N1 antigen lot tested to date as demonstrated by two different techniques (around 25-30 % by filtration and around 45-50% by centrifugation). Independent of the technique used, the level does not appear to be impacted by transportation (by truck and by air), to vary over time (up to 74 days) and to be influenced by the lot manufacturing history (e.g. manufacturing in different filling sites).
2. Addition of the AS03 adjuvant reduces the level of HA present in the aggregate. When filtering (0.22 µm) AS03-adjuvanted H1N1 vaccine lots, more than 90 % of the Hemagglutinin is recovered in the vaccine filtrate, demonstrating that only a limited fraction of the antigen is present under aggregated form in the adjuvanted vaccine.

These studies showed that transportation, aging (up to 74 days) or the manufacturing history (e.g. filling of the antigen at different sites) has limited impact if any, on the AS03-adjuvanted Arepanrix vaccine potency. It also provided first evidence that the AS03 adjuvant is able to reduce the amount of aggregate in the final mixed vaccine.

2. MATERIALS AND METHODS

2.1. Materials.

H1N1 Antigen Lots. Vials from one clinical lot (DFLPA304A) and from commercial lots (AFLPA327A, AFLPA323BB, AFLPA324BA and AFLPA328AA) were used in the study. Lots DFLPA304A, AFLPA323BB, AFLPA324BA and AFLPA328AA were manufactured and filled at GSK Ste-Foy (Canada) whereas lot AFLPA327A was filled at DSM Greenville (USA). Their manufacturing dates were Sep 15, 2009 (DFLPA304A), Sep 30, 2009 (AFLPA323BB), Oct 02, 09 (AFLPA324BA), Oct 04, 09 (AFLPA328AA) and Nov 1, 2009 (AFLPA327A). The transportation history of the vials used in the study was as follow: (1) Japan vials (n=5): vials from lot DFLPA304A were shipped by air to Japan and back to GSK Ste-Foy, Canada, (2) vials from lot DFLPA304A from GSK Ste-Foy retention (no transportation), (3) vials from lot DFLPA304A that were shipped by air to GSK, Rixensart, Belgium and back to GSK-Ste-Foy, Canada, (4) vials from lot AFLPA323BB, AFLPA324BA and AFLPA328AA from GSK Ste-Foy retention (no transportation), (5) vials from lot AFLPA323BB, AFLPA324BA and AFLPA328AA that were shippped to GSK-Mississauga, Canada and back to GSK-Ste-Foy, Canada (~ 20 hours by truck) and (6) vials from lot AFLPA327A, which were manufactured at DSM Greenville (USA) and shipped to GSK Ste-Foy (Canada) (~10-15 hours by truck).

Adjuvanted vaccine lots were reconstituted by mixing in a 1:1 ratio the adjuvant with the antigen.

Sample preparation by centrifugation. For each lot analyses, the volume of 4 vials was pooled in 50mL Falcon tubes and mixed to ensure homogeneity. Samples were collected for analysis of the whole suspension by SRD. From the pooled material, 9mL were centrifuged (6 x 1.5mL in Eppendorf tubes) at 13,000 rpm (16,060 g) for 30 min. From each Eppendorf tube, 1.4mL of supernatant was collected and transferred to a fresh tube. Samples of the supernatant fraction were collected for analyses by SRD.

2.2. Methods

Determination of the HA and total protein content in aggregates by centrifugation. A quantitative analysis of the amount of antigen and total protein in the H1N1 antigen lots before and after transportation, in either soluble or whole suspension was conducted. Two methods were used for the HA content determination: (1) High Performance Liquid Chromatography (HPLC), which measures the total amount of HA antigen, irrespective of its configuration; and (2) the standard Single Radial Immunodiffusion (SRID), which assess the amount of HA antigen using specific anti-H1N1 antibodies. Total protein content was determined using the standard Lowry method.
Attempts were made to evaluate the amount of total protein and HA content present in the aggregates. Samples of vaccine materials were centrifuged and the supernatant fractions were collected. The supernatant fractions as well as the samples prior to centrifugation were tested for total protein and HA content. The difference between the total protein or HA contents found in the whole suspension before centrifugation and the values observed in the supernatant fractions correspond to the amount of materials present in the aggregates.

Determination of the HA content in aggregates by filtration. The aggregation level in the AS03-adjuvanted vaccine and in the antigen alone was evaluated by filtration. Each test sample was filtered through a 0.22µm filter in order to remove aggregated materials. The HA content before and after filtration as assayed by SRD was measured to assess the amount of HA in the aggregated materials. A percentage of HA recovery was determined for each material tested. This percentage corresponds to the amount of antigen recovered in the filtrate as compared to the initial amount prior to filtration. It thus gives an estimate of the amount of antigen present in the aggregates. The impact of both transportation and the age of the lots on the recovery percentages were investigated. These analyses were conducted on the AS03-adjuvanted mixed vaccine, which is ultimately the product injected to subjects as well on the antigen alone.

Uniformity of doses in adjuvanted vaccine. The test used to indicate antigen uniformity was HA content by SRD assay. Using a syringe, the entire content of a multi-dose vial containing the AS03 adjuvant was removed and injected into the antigen vial to produce approximately 6.25 ml of final vaccine (approximately 7.5 µg HA/ml). The reconstituted vaccine was then hand shaken briefly. Ten doses of 0.5 ml were then withdrawn from the reconstituted vials and each was tested for HA content by SRD. Calculation of acceptance value (AV) demonstrating dose uniformity was done as

described in the Ph. Eur Monograph 2.9.40 using the following formula:

$AV = | M - X | + ks$

Where :M = Reference value (case 2, when T>101.5 and if X<98.5, then M=98.5, if X≥98.5, then M=X); X = Mean of individual contents of the dosage units, expressed as a percentage of the label claim; k = Acceptability constant (for n=10 dosage units, k=2.4) and s = Sample standard deviation

HA content by SRD. The SRD testing assays used to test the antigen or the AS03-adjuvanted vaccine are the same as those used for antigen release or for AS03-adjuvanted characterization. Both are described in the Arepanrix file and are validated.

HA content by HPLC. This analytical method determines the hemagglutinin content based the measurement of the HA1 subunit after separation of the proteins in a mixture by hydrophobic interaction chromatography. The quantification is obtained by comparing the area of the HA1 subunit peak at 214nm in the samples against the area generated with a reference antigen preparation. Zorbax Poroshell 300SB-C3 Narrow-Bore column, 75 mm x 2.1 mm ID, 5µm, 300Å; and Zorbax 300SB-C3 Narrow-Bore Guard Column, 12.5 mm x 2.1 mm ID, 5µm, 300Å are used as stationary phase. Samples are pre-treated with Dithiotreitol (DTT) and boiled for 15 minutes at 95ºC prior to being analyzed. A linear gradient from 23 to 95% acetonitrile, 0.1% trifluoroacetic acid w/v is the mobile phase used for the elution.

Total Protein Content. The Lowry standard method was used for total protein content measurement.

3. RESULTS AND DISCUSSION

3.1. Determination of the HA content in aggregates (by centrifugation)

The total protein and HA contents were determined in Lots AFLPA323B, AFLPA324BA and AFLPA328AA, whether transported or not (~20 hours by truck). In addition, the composition of the aggregates in terms of protein and HA content was also assessed. Lot samples were centrifuged to separate the aggregates from the soluble part of the antigen suspension. The amount of total protein and HA content in the aggregates was calculated by comparing the amount of materials in the antigen suspension before and after centrifugation of the aggregates.

Table 1 and Table 2 respectively present testing results for total protein content by Lowry and for HA content by HPLC in the whole suspension prior centrifugation, in the soluble fraction (supernatant) and in the aggregates (pellet, by calculation).

Table 1 Total Protein content (µg/ml) by Lowry

Lot number	Transportation	Whole suspension (µg/ml)	Soluble fraction (Supernatant) (µg/ml)	Aggregate (by calculation) (µg/ml)[1]	Aggregate /whole suspension Ratio (%)[2]
AFLPA323B	No	49.8	36.7	13.1	26.3
	Yes	54.0	38.2	15.8	29.3
AFLPA324BA	No	44.8	32.4	12.4	27.7
	Yes	47.0	33.4	13.6	28.9
AFLPA328AA	No	52.3	35.9	16.4	31.4
	Yes	52.1	38.6	13.5	25.9
Average (SD)	No	49.0 (3.8)	35.0 (2.3)	14.0 (2.1)	28.5 (2.6)
	Yes	51.0 (3.6)	36.7 (2.9)	14.3 (1.3)	28.0 (1.9)

1. Aggregate content = whole suspension content – soluble fraction content
2. Aggregate/whole suspension ratio = (aggregate content/whole suspension content) x 100

Table 1 shows that the aggregates contain around 28 % of the total proteins present in the whole antigen suspension. The same percentage is observed before and after transportation.

Table 2 HA content (µg HA/ml) by HPLC

Lot number	Transportation	Whole suspension (µg/ml)	Soluble fraction (Supernatant) (µg/ml)	Aggregate (by calculation) (µg/ml)[1]	Aggregate /whole suspension Ratio (%)[2]
AFLPA323B	No	13.7	6.7	7.0	51.1
	Yes	13.5	6.8	6.7	49.6
AFLPA324BA	No	11.6	6.6	5.0	43.1
	Yes	10.9	5.8	5.1	46.8
AFLPA328AA	No	13.0	6.7	6.3	48.5
	Yes	14.0	7.3	6.7	47.9
Average (SD)	No	12.8 (1.1)	6.7 (0.1)	6.1 (1.0)	47.6 (4.1)
	Yes	12.8 (1.7)	6.6 (0.8)	6.2 (0.9)	48.1 (1.4)

1. Aggregate content = whole suspension content – soluble fraction content
2. Aggregate/whole suspension ratio = (aggregate content/whole suspension content) x 100

Table 2 shows that the aggregates contain around 48% of the HA present in the whole antigen suspension (as determined by HPLC). The same percentage is observed before and after transportation.

Attempts were also made to determine the HA content in the aggregates by SRD. However, in all the commercial lots tested (before and after transportation), values in the supernatant were all below 10 µg HA/ml, which is the limit of validated range of the assay, preventing an accurate calculation of the HA content by SRD in the aggregates. Given that the antigen is formulated at 15 µg HA/ml, this would suggest that a minimum 30 % of the overall HA as measured by SRD is present in the aggregates. This is in line with the HA content by HPLC measurements as described above.

The results confirmed that the aggregates indeed are composed of proteins and H1N1 hemagglutinin antigen, which respectively represent ~ 30% of the total proteins and ~ 45-50% of the HA content of the whole antigen suspension. The analyses also show that under the experimental conditions (centrifugation at 16,060g and ~20 hours truck transportation), these percentages are not affected by the transportation.

With respect to the antigen content in the whole suspension, the analysis of the total HA content by HPLC shows that the same amount of HA is found irrespective whether the materials is transported or not.

3.2. Determination of the HA content in aggregates (by filtration)

The HA content testing results before and after filtration for adjuvanted vaccine lots and for antigen lots are presented in Table 3 and in Table 4, respectively.

Table 3 AS03-adjuvanted vaccine: HA content before and after 0.22µm filtration

Sample ID	Materials	HA Content (µg/mL)		Recovery (%)[3]	Lot Age[4] (Days)
		before filtration (SD)	after filtration (SD)		
DFLPA304A[1]	Clinical lot: vials sent to Japan and returned to Canada	7.3	6.8	93.2	74
DFLPA304A[1]	Clinical lot: vials from Ste-Foy retention (no transportation)	9.4	8.6	91.5	74
AFLPA327A[2]	Commercial lot: vials filled at DSM (US) and transported to Canada	9.1	8.3	91.2	59
AFLPA323BB[1]	Commercial lot: vials from Ste-Foy retention (no transportation)	7.6	7.6	100.0	27

1. Filled at GSK – Sainte-Foy
2. Filled at DSM
3. Recovery percentage = (HA content after filtration/ HA content before filtration)x 100
4. Age of the lot at time of testing

Table 4 Antigen Alone: HA content before and after 0.22µm filtration

Sample ID	Materials	HA Content (µg/mL)		Recovery (%)[3]	Lot Age[4] (Days)
		before filtration (SD)	after filtration (SD)		
DFLPA304A[1]	Clinical lot: vials from Ste-Foy retention (no transportation)	14.9 (1.415)	11.8 (2.057)	79.2	74
AFLPA327A[2]	Commercial lot: vials filled at DSM (US) and transported to Canada	20.7 (1.926)	15.6 (1.510)	75.4	59
AFLPA323BB[1]	Commercial lot: vials from Ste-Foy retention (no transportation)	13.6 (1.510)	10 (1.381)	73.5	27

1. Filled at GSK – Sainte-Foy

2. Filled at DSM
3. Recovery percentage = (HA content after filtration/ HA content before filtration)x 100
4. Age of the lot at time of testing

Analysis of AS03-Adjuvanted Vaccine lots

The HA recovery values after filtration were consistently similar for all vials (between 90 to 100%), irrespective whether the lots were transported or not and irrespective of their age (age range: 27 to 74 days). These results clearly demonstrate that the transportation of the antigen component of the Arepanrix vaccine has limited effect, if any, on the potency of the final reconstituted vaccine. It also gives an indication of the stability of the vaccine response over a time (up to 74 days). Finally, no differences are seen irrespective of the antigen filling site as well. Hence overall, these data demonstrate the product quality of the adjuvanted vaccine.

The consistent high recovery values after filtration (above 90%) observed with all four materials also indicate that only a limited amount of aggregates are present in the vaccine following mixing of the antigen with the adjuvant. Hence upon mixing with the adjuvant, the aggregation in the reconstituted vaccine represent less than 10 % of the overall HA content. This extent is expected to have no clinical impact on the vaccine immunogenicity.
Although further investigations on the adjuvant effect on aggregation is needed, it could be hypothesized that the presence of detergent in the adjuvant might re-dissolve or reduce the size of the aggregates to such an extent that they are below 0.22 µ in size (cut-off of the filter).

Analysis of Antigen Drug Product Lots

Similar and consistent percentages of HA recovery ranging from 73.5 % to 79.2% were observed for the three antigen lots studied. These values are lower than those observed in the corresponding AS03-adjuvanted vaccine lots (> 90%), indicating that 20 to 25 % of the total HA of antigen vial is present as aggregates.

However, like for the AS03-adjuvanted vaccine, the HA recovery values in the antigen lots remain similar irrespective whether the lots were transported or not and irrespective of their age (age range: 27 to 74 days). These results collected with the antigen confirm the results observed with the AS03-adjuvanted vaccine i.e. that the transportation does not appear to have an impact on the aggregation level of the antigen component of the

Arepanrix vaccine. It also gives an indication of the stability of the antigen over a time (up to 74 days). Finally, no differences are seen irrespective of the antigen filling site as well. Hence overall, these data demonstrate that despite the fact that aggregates are observed in the H1N1 antigen product, these are consistently present at the same level in all three antigen lot demonstrating the ability of the manufacturing process to consistently yielding product of the same quality.

3.3. HA Content and Uniformity of doses in AS03-adjuvanted Vaccine Lots

3.3.1. HA content by SRD in Adjuvanted Vaccine Lot

Results of the SRD testing of the clinical lot mixed with AS03 are presented in Table 5. Data on the H1N1 antigen lot prior to mixing is also shown

Table 5 HA content: DFLPA304A Clinical Lot, Before and After Transportation, Prior and After Mixing with AS03 Adjuvant

Test material Antigen	HA content (SRD) [1] (µg HA/ml) DFLPA304A (no transportation)	HA content (SRD) [1] (µg HA/ml) DFLPA304A (after transportation[5])
Prior to mixing – Final Container	16.4 (14.9-17.9) [2] (100%)[4]	14.7 (13.5-15.9) [3] (90%)[4]
Antigen +Adjuvant	DFLPA304 (no transportation) + AS03	DFLPA304 (after transportation) + AS03
Following immediate mixing	7.6	7.6
24 hours at 30°C after mixing	7.1	7.6

1. Testing performed with CBER antigen reagent and NIBSC antibodies reagent
2. Testing done at release (Sep 15, 2009)
3. Re-test done on Oct 19, 2009
4. Recovery = (SRD value/initial SRD value)*100; initial SRD value (non-transported)
5. The Clinical lot was packaged in GSK-Rixensart clinical plant and sent back to GSK-Ste-Foy, Canada plant fr testing (air transportation)

The analysis of HA content by SRD that makes use of specific anti-H1N1 antibodies show that there is limited impact (maximum 10%) in the HA content between the H1N1 antigen lot transported vs. non-transported.

The SRD analysis of the reconstituted AS03-adjuvanted vaccine show that the HA content in the adjuvanted H1N1 antigen formulation before or after transportation is reaching the expected range of HA value resulting from a 1:1 dilution of the antigen. Specifically, the resulting HA content values in the adjuvanted H1N1 vaccine were in the same range irrespective whether the antigen vials were transported or not, indicating that the potential observed HA content drop at the level of the antigen vial is not reflected in the AS03-mixed solutions. This would suggest that the presence of aggregates in antigen vials has no impact when the antigen is mixed with the adjuvant.

3.3.2. Uniformity of Dose

The uniformity of doses was evaluated using a vial from clinical lot DFLPA304A that was sent to Japan and returned to Canada and a vial from the Commercial lot AFLPA327A that was filled in DSM-Greenville (USA) and sent back to Canada.

Analysis of dose uniformity was conducted according to the Ph. Eur monograph 2.9.40 - Uniformity of dosage units, which stipulate that the requirements for dosage uniformity are met when the acceptance value of the first 10 dosage units is less than or equal to 15.

The results of the dose uniformity evaluation are presented in Table 6. They show that the content in each dose is homogenous for each of the 10 dose, and this for the two lots tested. The relative standard deviation across doses was 1.5% (Japan transported lot) and 5.2% (DSM lot).

Uniformity of dosage units acceptance value (AV) was 6.6 (Japan-transported lot) and 0.6 (DSM lot). Therefore the criterion of the test (less than or equal to 15) has been met, and uniformity of dosage has been demonstrated for both the Japan-transported lot or the DSM lot.

This confirms that transportation of antigen vials has no effect on the uniformity of content in the adjuvanted H1N1 multidose presentation.

Together with the percentage of HA recovery observed in the same reconstituted lots (over 90%), these data are indicative that there is limited if any residual aggregates in the AS03-adjuvanted vaccine.

Table 6 Uniformity of dose of H1N1 adjuvanted vaccine following extemporaneously mixing

Test performed	Lot number (Antigen)	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	Dose 10	Mean (n=10)	SD	RSD	AV ≤ 15
HA concentrat. (µg/mL)	DFLPA304A (Japan)[1]	6.8	7.0	7.0	6.8	6.8	7.0	6.8	7.0	7.0	6.8	6.9	0.11	1.5	N/Ap
HA content (µg)*		3.40	3.50	3.50	3.40	3.40	3.50	3.40	3.50	3.50	3.40	3.45	0.05	1.5	6.6
HA concentrat. (µg/mL)	AFLPA327A (DSM)[2]	9.3	10.0	9.6	9.6	8.7	10.3	9.7	9.7	9.3	10.4	9.7	0.5	5.2	N/Ap
HA content (µg)*		4.65	5	4.8	4.8	4.35	5.15	4.85	4.85	4.65	5.2	4.8	0.3	5.2	0.6

* Results expressed as content per delivered dose

1. Clinical lot vial filled at GSK – Sainte-Foy (Canada), shipped to Japan and returned to GSK -Ste-Foy (Canada)
2. Commercial lot vial filled at DSM – Greenville (US), shipped to GSK Ste-Foy (Canada)

4. OVERALL CONCLUSION

Factors potentially impacting the level of aggregates in H1N1 antigen lots and in adjuvanted H1N1 vaccine lots were investigated.

Investigations on the H1N1 antigen alone

The investigations regarding H1N1 antigen alone showed that the level of HA or total protein in the aggregates is constant across lots:

- Centrifugation experiments conducted on 3 antigen lots showed that the aggregates consistently contain ~ 30% of the total proteins and ~ 45-50% of the HA overall content. These levels were not modified upon transportation (~20 hours truck transportation).
- Filtration experiments conducted on 3 antigen lots showed that the aggregates consistently contain ~ 25-30% of the HA overall content. These levels were not affected by transportation (~10-15 hours truck transportation) and they were not significantly affected over time (lots were tested at various ages, ranging from 27 to 74 days). The levels were also the same despite different filling sites were used for their filling (GSK-Ste-Foy, Canada and DSM-Greenville, USA).

Larger amount of HA was observed using the centrifugation method vs. the filtration method. This is likely due to the fact that the centrifugation method is aimed at collecting as much as possible aggregates irrespective of their particle sizes, whereas the filtration method only separate aggregates above the cut-off of the filter.

Although the two method yields to different numbers in terms of HA content in the H1N1 antigen lots, both methods consistently show that the same level is observed in all the lot tested in the same series, supporting the consistency of the antigen quality. In addition, both methods show that the HA content level appears to be the same, irrespective whether the lots were transported or not (~20 hours truck transportation), of their age (lots were tested to 74 days of age) and irrespective of their manufacturing history (Lots from 2 different filling sites were tested).

All these elements indicate that the manufacturing of the H1N1 antigen lots yields to products of consistent quality, for which the level of aggregates does not significantly change over a period of at least 74 days.

Investigations on the AS03-adjuvanted vaccine

The investigations on the AS03-adjuvanted vaccine showed that mixing of the AS03 adjuvant with the H1N1 antigen result in a large reduction of the level of aggregate in the adjuvanted vaccine.

Three adjuvanted vaccine lots were filtered on 0.22µm filter to remove aggregated materials. Upon testing by SRD, more than 90 % of the initial HA content was consistently recovered in the filtrate. The same level was observed for transported materials (~10-15 hours truck or air transportation), for materials aged from 27 to 74 days

and irrespective of the filling sites used for the antigen manufacture (GSK-Ste-Foy, Canada and DSM-Greenville, USA).

The uniformity of dosage was demonstrated for two lots of adjuvanted vaccine, manufactured with H1N1 antigen lot that have been transported (by truck or by air). Ten 0.5 ml dose have been withdrawn, all with the expected similar HA content (by SRD). This observation further provides indirect evidence that the level of aggregates in the mixed vaccine is indeed reduced.

At this stage of the investigation, it can only be hypothesized that the reduction of aggregates in the adjuvanted vaccine is to result from either aggregate dissolution or aggregate size reduction below the filter cut-off. More investigations will be needed to further understand the actual mechanism.

Overall conclusion

Overall, the experiments conducted on adjuvanted vaccine lots where aggregates have been removed by filtration, shows that the presence of aggregate in the H1N1 antigen lots has limited impact, if any on the adjuvanted vaccine potency given the high HA content recovery consistently observed in all lot tested upon mixing with the AS03 adjuvant.

Arepanrix
H1N1 Pandemic vaccine

QC preliminary data on retained samples
Dec 10, 2009

Investigation strategy

- Five lots are used for investigation purpose (Combo)
  - A80CA007A: antigen AFLPA313AA
    - Adverse Event reporting rate higher than usually seen in the seasonal vaccine
  - A80CA005A: antigen AFLPA312AA
    - Adverse Event reporting rate lower than 007
  - A80CA009A: antigen AFLPA319BA* and/or AFLPA319BB
  - A80CA013A considered as a Control lot: antigen AFLPA325AA
    - Adverse Event reporting rate almost nil
  - A80CA014A considered as a Control lot: antigen AFLPA304CA
    - Adverse Event reporting rate very low

*EM analyses conducted by Dr G. DeSerres and Dr. Ward

Investigation testing plan

Materials	Tests	Retain Samples		Field Samples	
		Testing	Status	Testing	Status
H1N1 Antigen	Western Blot	X	Complete	X	Stand-by
	Particle size by DLS	X	Complete	X	Stand-by
	Particle count by FMI	X	Complete	X	Stand-by
	Phospholipid content	on monovalent bulk		Not Planned	
	SDS-PAGE (silver)	X	Complete	X	Stand-by
	HA content by SRD	X	Complete	X	Stand-by
	HA content by HPLC	X	Complete	X	Stand-by
	Electron Microscopy	X	Ongoing	To be Confirmed	Stand-by
	Particle size by SLS	X	Ongoing	To be confirmed	Stand-by
	GST	X	Ongoing	X	Stand-by
AS03 Adjuvant	α-tocopherol content	X	Ongoing	X	Stand-by
	Squalene content	X	Ongoing	X	Stand-by
	α-tocopherol quinone content	X	Ongoing	X	Stand-by
	Endotoxin content	X	Ongoing	X	Stand-by
	GST	Not Planned		X	Stand-by

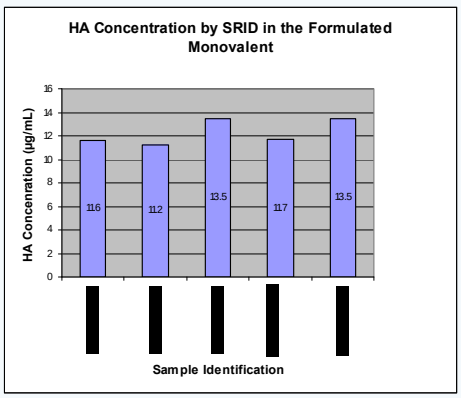
Investigation testing plan

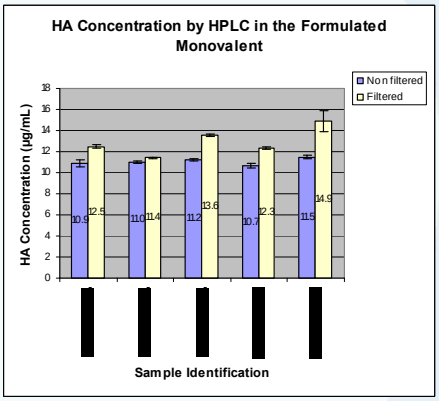
Materials	Tests	Retain Samples		Field Samples	
		Testing	Status	Testing	Status
Adjuvanted H1N1 Vaccine	GST	X	Ongoing	X	Stand-by
	HA content by SRD	X	Complete	X	Stand-by
	HA content by HPLC	X	Complete	X	Stand-by

Preliminary results

HA Concentration

- All investigated antigen lots show similar HA content by SRID and similar HA content by HPLC before and after filtration

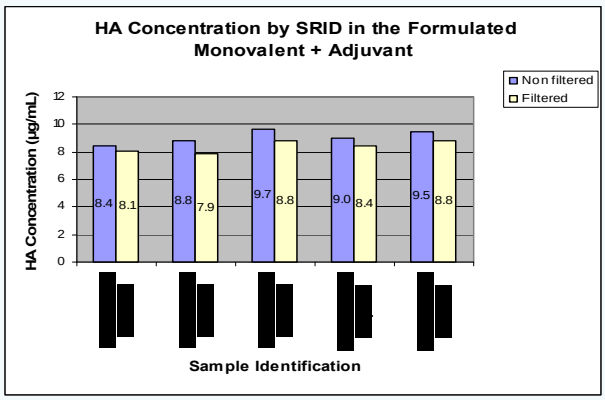




Preliminary results

HA Concentration

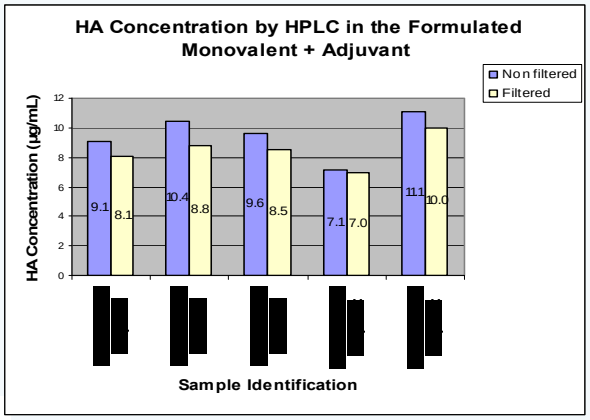
- All investigated combo lots (antigen + adjuvant) show similar HA content by SRID before and after filtration (0,22um)



Preliminary results

HA Concentration

- All investigated combo lots (antigen + adjuvant) show similar HA content by HPLC before and after filtration (0,22um)



Preliminary results

Recovery after filtration

- The HA recovery after filtration of the antigen alone or mixed with adjuvant is overall similar for all lots, although lot 313AA (combo 007) may be on the lower end of the values

Lot Identification	% Recovery After Filtration on 0.2 micron		
	Antigen alone by HPLC	Antigen + Adjuvant by HPLC	Antigen + Adjuvant by SRID
AFLPA319BA	115	89	96
AFLPA313AA	104	85	90
AFLPA312AA	121	89	91
AFLPA325AA	115	99	93
AFLPA304CA	130	90	93

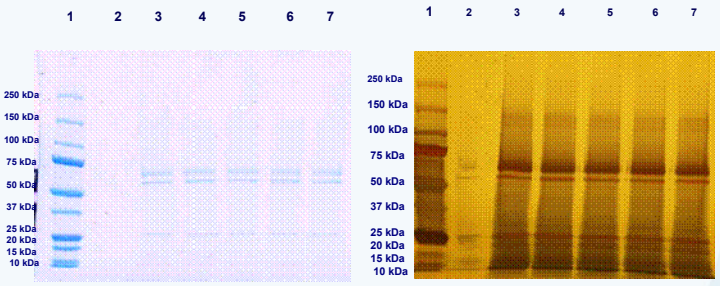
Preliminary results

Protein profile SDS-Page

- Similar Protein profile in all antigen final containers lots investigated

Légende :

1.Standard
2.Tampon
3.304C_SF_NF
4.312AA_SF_NF
5.313AA_SF_NF
6.319B_SF_NF
7.325A_SF_NF



Preliminary results

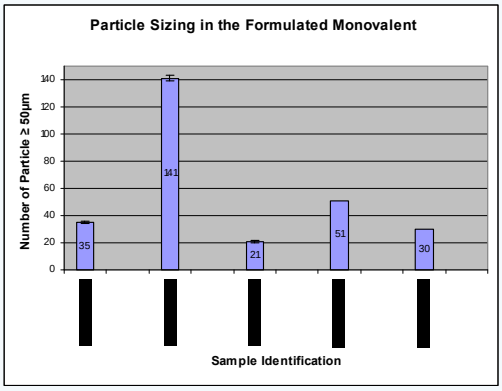
Particle sizing

- Particle sizing was done by two different methods :
  - Dynamic Light Scattering (DLS): detection range = 0.6 – 6000 nm
  - Flow Microscopy Imaging (FMI): detection range > 2000 nm

Lot Identification	Particle sizing					
	DLS		FMI			
	Z ave (nm)	Polydispersity (PDI)	Total	Mean (µm)	Max	≥ 50µm
AFLPA319BA	134	0.1502	9346	5.9	178.00	35
AFLPA313AA	139	0.2390	14081	7.0	269.75	141
AFLPA312AA	135	0.1567	7494	5.1	121.00	21
AFLPA325AA	138	0.1714	5365	7.1	592.25	51
AFLPA304CA	132	0.1602	11437	5.6	230.25	30

Preliminary results

Particle sizing



Preliminary results

Particle sizing

- Dynamic Light Scattering (DLS):
  - No significant difference in average particle size but higher polydispersity index (PDI) in lot 313 suggesting more heterogeneity in particle distribution
- Flow Microscopy Imaging (FMI):
  - Mean average particle size similar in all lots (5.6 – 7.1 µm)
  - Lot 313 shows 3 to 4.5 fold higher counts of visible particles (> 50 µm) compared to control lots 325AA and 304CA
  - The max particle size in control lot 325AA is 2-fold larger than in lot 313

Preliminary results

Conclusion

- The investigated lots show similar HA content in antigen alone or mixed with adjuvant, before and after 0.22 µm filtration
  - HA recovery after filtration (SRD) in adjuvanted vaccine is at least 90% showing presence of aggregates in antigen lots has limited impact on vaccine potency
- The investigated lots show similar protein profile by SDS-Page
- Particle size analyses show:
  - All antigen lots have similar average particle size by both DLS and FMI methods
  - Retention sample of antigen lot AFLPA313AA (combo 007) presents 3 to 4.5-fold higher counts of visible particles (> 50 µm) compared to control lot AFLPA325AA (combo 013) and to lot AFLPA304CA (combo 014)
  - FMI test on will be conducted on additional lots (15 lots overall) to accumulate further history of particle size variability

Manufacturing and QC investigations

- Technical data compilation of the 5 investigated antigen lots was conducted:
  - Review included:
    - Quality attributes
      - Total protein content
      - Ratio HA content/total protein content.
      - Endotoxin level
      - Ovalbumin content
      - DOC concentration
    - Manufacturing attributes
      - Raw materials
      - Holding times
      - Splitting efficiency
  - Results: All antigen lots presented similar quality and manufacturing profiles

Conclusions

- Only one lot of Arepanrix (lot A80CA007A) appears to have a signal regarding increased reporting rate of anaphylaxis
- QA manufacturing investigation of lot A80CA007A (both antigen and adjuvant): nothing unexpected to report
- Technical data compilation on antigen: nothing unexpected from lot to lot
- Antigen analytical investigation on retained sample:
  - The only element needing more investigation is the amount of visible aggregates (> 50 µm) in the antigen lot 313AA used in combo 007A
- Awaiting samples coming from the field for further analyses