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Modification of the vaccine manufacturing process improves the pyrogenicity profile of inactivated influenza vaccines in young children

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ABSTRACT

Background: There were increased reports of fevers and febrile reactions in young children (particularly children aged <5 years) receiving the Seqirus/CSL Southern Hemisphere 2010 trivalent inactivated influenza vaccine (IIV3). Modifying the vaccine manufacturing process by increasing the minimum concentration of splitting agent (sodium taurodeoxycholate [TDOC]) from 0.5% w/v to 1.5% w/v for all strains resolved this issue. The current analysis compared fever rates in three pediatric studies of Seqirus IIV3 (S-IIV3) or quadrivalent inactivated influenza vaccine (S-IIV4), prepared using the modified manufacturing process, with fever rates in three pediatric studies of historical (pre-2010) IIV3 formulations. The historical IIV3 formulations, S-IIV3, and S-IIV4 had 0/3, 2/3, and 4/4 vaccine strains split at 1.5% TDOC, respectively.

Methods: For each study, fever rates (any grade and severe) were determined for the following age subgroups (as applicable), using the fever intensity grading system used in the S-IIV3/S-IIV4 studies: 6 months to <3 years; 3 to <5 years; 5 to <9 years; and 9 to <18 years.

Results: For each age subgroup, the any grade and severe fever rates were lower in the S-IIV3/S-IIV4 studies than in the historical IIV3 formulation studies, with the greatest differences in fever rates observed in the youngest age groups. In the 6 months to <3 years group, the any grade fever rate was 7.0% (severe fever: 2.5%) in one S-IIV4 study compared with 38.7% to 40.0% (severe fever: 9.6% to 17.8%) in the historical IIV3 formulation studies. In the 3 to <5 years subgroup, the any grade fever rate was 4.9% (severe fever: 1.2%) in one S-IIV4 study compared with 34.1% to 36.0% (severe fever: 6.3% to 16.5%) in the historical IIV3 formulation studies.

Conclusion: This analysis provides clinical evidence that the modified manufacturing process improved the fever profile across all pediatric age groups, in particular, in children aged <5 years.

1. Introduction

Influenza vaccines are generally considered safe; however, they may cause adverse events, some of which are more common in children than adults, such as fever and febrile seizures [1]. Although fever was very common in children with earlier whole-virus vaccines, with unacceptable rates of febrile seizures, newer split-virion influenza vaccines have greatly reduced incidences of fever and febrile seizures in children [1].

During the 2010 Southern Hemisphere (SH) influenza season, the Seqirus/CSL trivalent inactivated influenza vaccine (2010 SH IIV3) was associated with an increase in postmarketing reports of fever and febrile reactions in young children [2,3]. An increase in fever and febrile seizures was mostly observed in children aged 6 months to <5 years, leading to the suspension of its use in this age group [2,3]. There were also increased reports of fever in children aged 5 to <9 years [4]. Research conducted using in vitro modeling in a subgroup of children aged 5 years or younger who experienced 2010 SH IIV3-related febrile seizures showed differences in proinflammatory cytokine/chemokine production when peripheral blood mononuclear cells were stimulated with the 2010 SH IIV3 compared with alternative IIV3 preparations [5]. Investigations undertaken by Seqirus identified lipid-mediated delivery of RNA fragments derived from the influenza vaccine, generated under the splitting conditions used to manufacture the influenza vaccine, as likely contributing factors to the increased reports of fevers and febrile reactions [6]. As no suitable animal

Abbreviations: ACIP, Advisory Committee on Immunization Practices; CBER, Center for Biologics Evaluation and Research; CI, confidence interval; FDA, Food and Drug Administration; IIV3, trivalent inactivated influenza vaccine; NH, Northern Hemisphere; S-IIV3, Seqirus trivalent inactivated influenza vaccine; S-IIV4, Seqirus quadrivalent inactivated influenza vaccine; SH, Southern Hemisphere; TDOC, sodium taurodeoxycholate.

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model could be identified, an NF-κB HEK293 reporter assay and pediatric whole blood assays (WBAs) were used as surrogate in vitro models of proinflammatory cytokine induction to evaluate the pyrogenic potential of inactivated influenza vaccines [7]. Non-clinical studies showed that increasing the concentration of the agent used to split the virus (sodium taurodeoxycholate [TDOC]) reduced the lipid content of the vaccine and was correlated with attenuation of the proinflammatory cytokine signals associated with fever in the NF-κB HEK293 reporter assay and pediatric WBAs [6]. As a consequence of these findings, to minimize potential fever and febrile reactions, the minimum concentration of TDOC subsequently used in the manufacturing process of the Seqirus quadrivalent inactivated influenza vaccine (S-IIV4; Afluria® Quadrivalent/Afluria Quad™/Afluria Tetra™) was increased from 0.5% w/v to 1.5% w/v (thereby narrowing the approved TDOC concentration range from 0.5%–2.5% w/v to 1.5%–2.5% w/v). Analysis of vaccine lipid content and characterization of the individual inactivated strain components using the NF-κB HEK293 reporter assay have been incorporated into the manufacture of S-IIV4 to ensure that no strain components (or combinations of strain components) with pyrogenic potential are used in the manufacture of the influenza vaccine.

To evaluate the influenza vaccines prepared using the modified manufacturing process, Seqirus/CSL conducted a pediatric clinical development program over three successive Northern Hemisphere (NH) influenza seasons (2013/2014 to 2015/2016). The first study [8], conducted in children aged 5 to <9 years, evaluated the Seqirus trivalent inactivated influenza vaccine (S-IIV3) in which the concentration of TDOC used to split the B strain was increased to 1.5% w/v. The H3N2 strain of this trivalent vaccine was also split at 1.5% w/v TDOC; therefore, 2/3 strains in this vaccine were split at 1.5% w/v TDOC. This age group was chosen because children of this age experienced higher than usual rates of fever with the 2010 SH IIV3, but did not experience severe febrile reactions. The second and third studies, conducted in children aged 5 to <18 years [9] and children aged 6 months to <5 years [10], respectively, evaluated S-IIV4 in which all four vaccine strains were split at 1.5% w/v TDOC. S-IIV3 and S-IIV4 were well tolerated in these three studies, with a similar safety profile to the reference/comparator vaccine [8–10], showing that the modified manufacturing process attenuated the febrile reactogenicity in young children associated with the SH 2010 IIV3.

To fully assess the effect of the modified manufacturing process on fever rates, it would be of value to compare fever rates between the three S-IIV3/S-IIV4 studies (referred to as "modified IIV3/IIV4 formulation studies") and the previously conducted studies of historical (pre-2010) IIV3 formulations (referred to as "historical IIV3 formulation studies") [11–13]. However, as different age subgroups and temperature cut-off points to define fever severity were used in these studies, it is difficult to directly compare the fever rates. Reanalyzing the data from the historical IIV3 formulation studies according to the age subgroups and fever intensity grading system used in the more recently conducted modified IIV3/IIV4 formulation studies would allow direct comparison of the fever rates between studies. In addition, pooling the fever rates for the historical IIV3 formulation studies would increase the number of subjects, providing stronger evidence for the results of the comparison.

The aim of the current analysis was to compare the rates of fever observed in children (aged 6 months to <18 years) in the three studies of S-IIV3/S-IIV4 prepared using the modified manufacturing process [8–10] with the rates of fever observed in children who had received historical IIV3 formulations [11–13]. As fever following influenza vaccination is more common in younger children than older children [1], the rates of any grade fever and severe fever were examined in four age subgroups (6 months to <3 years; 3 to <5 years; 5 to <9 years; 9 to <18 years).

2. Materials and methods

2.1. Study information

Six studies, conducted in children of various age groups, were included in this analysis (Table 1). There were three historical IIV3 formulation studies: two single-arm studies (FLU-04-05 [11]; USF-06-29 [12]) and one blinded, comparator-controlled study (USF-07-36 [13]). These three studies evaluated IIV3 formulations in which none of the vaccine strains were split at the higher TDOC concentration of 1.5% w/v (Table 1). There were three modified IIV3/IIV4 formulation studies. One blinded, comparator-controlled study (USF-10-69 [8]) evaluated S-IIV3, in which the B and A/H3N2 strains were split at 1.5% w/v TDOC (Table 1). Two blinded, comparator-controlled studies (QIV-13-02 [9]; QIV-15-03 [10]) evaluated S-IIV4, in which all four vaccine strains were split at 1.5% w/v TDOC (Table 1). These three studies (USF-10-69, QIV-13-02, QIV-15-03), in which two or all of the vaccine strains were split at 1.5% w/v TDOC, were conducted over three successive NH influenza seasons (2014/2015, 2015/2016, 2016/2017).

2.2. Vaccine characteristics

The Seqirus inactivated influenza vaccine is a purified beta-pro priolactone-inactivated, TDOC-disrupted split virion vaccine that contains seasonal influenza antigens of strains H1N1, H3N2, and B Victoria and/or B Yamagata representative of the circulating virus recommended for each hemisphere and year. Each strain is characterized by electron microscopy for appearance and degree of splitting to ensure that the majority of virus particles are split virions. The product is further qualitatively assessed by sodium dodecyl polyacrylamide electrophoresis to confirm that the protein profile is consistent with a purified influenza virus. Following the increase in the minimum concentration of TDOC for each strain from 0.5% w/v to 1.5% w/v, the product displayed identical characteristics with the exception of a lower level of lipid. This lower level of lipid within the product does not produce a signal (below the level of detection) in the NF-κB HEK293 reporter assay.

2.3. Vaccinations

In the six studies included in this analysis, children received one or two doses of influenza vaccine (delivered intramuscularly), depending on their age and/or vaccination history [8–13]. In general, children aged 6 months to <9 years received two doses of vaccine, unless they had previously received the influenza vaccine, and children aged 9 to <18 years received one dose of vaccine. In Study USF-07-36 (conducted during the NH 2009/2010 influenza season), children aged 6 months to <9 years received one dose of vaccine during the study if they had received two doses of influenza vaccine during the NH 2008/2009 influenza season or at least one dose of influenza vaccine during an influenza season before the 2008/2009 influenza season; all other children aged 6 months to <9 years received two doses of vaccine [13]. In Study USF-10-69 (conducted during the NH 2014/2015 influenza season), children (aged 5 to <9 years) received one dose of vaccine if they had received two or more seasonal influenza vaccinations since July 2010; all other children were to receive two doses of vaccine [8]. In Studies QIV-13-02 and QIV-15-03, children received one or two doses of vaccine according to the most recent US Advisory Committee on Immunization Practices (ACIP) guidelines for seasonal influenza vaccination [14,15].

Table 1
Summary of studies.

Study/ClinicalTrials.gov identifier	Study design	Influenza season	Study population	Age subgroups per protocol	Vaccine strains	% TDOC
Historical IIV3 formulation studies						
FLU-04-05	Single-arm	SH 2005	6months to <9years	6months to <3years;	2005:	
NCT00700193 [11]		(primary)		3 to <9years	A/New Caledonia/20/1999 (H1N1)	0.9
		SH 2006			A/Wellington/1/2004 (H3N2)	0.9
		(booster)			B/Jiangsu/10/2003 (Yamagata lineage)	0.5
					2006:	
					A/New Caledonia/20/1999 (H1N1)	0.9
					A/New York/55/2004 (H3N2)	0.9
					B/Malaysia/2506/2004 (Victoria lineage)	0.5
USF-0629	Single-arm	SH 2009	6months to<18years	6months to<3years;	A/Brisbane/59/2007 (H1N1)	0.9
NCT00825162 [12]				3 to<9years;	A/Brisbane/10/2007 (H3N2)	1.1
				9 to<18years	B/Florida/4/2006 (Yamagata lineage)	0.5
USF-0736	Blinded, comparator-controlled	NH 2009/2010	6months to<18years	6months to<3years;	A/Brisbane/59/2007-like virus (H1N1)	0.9
NCT00959049 [13]				3 to<9years;	A/Brisbane/10/2007-like virus (H3N2)	1.1
				9 to<18years	B/Brisbane/60/2008-like virus (Victoria lineage)	0.5
Modified IIV3/IIV4 formulation studies						
USF-1069 (S-IIV3)	Blinded, comparator-controlled	NH 2014/2015	5 to<9years	5 to<9years	A/California/7/2009-like virus (H1N1)	0.9
NCT02212106 [8]					A/Texas/50/2012-like virus (H3N2)	1.5
					B/Massachusetts/2/2012-like virus	1.5
QIV-1302 (S-IIV4)	Blinded, comparator-controlled	NH 2015/2016	5 to<18years	5 to<9years;	A/California/7/2009-like virus (H1N1)	1.5
NCT02545543 [9]				9 to<18years	A/Switzerland/9715293/2013-like virus (H3N2)	1.5
					B/Phuket/3073/2013-like virus (Yamagata lineage)	1.5
					B/Brisbane/60/2008-like virus (Victoria lineage)	1.5
QIV-1503 (S-IIV4)	Blinded, comparator-controlled	NH 2016/2017	6months to<5years	6months to<3years;	A/California/7/2009-like virus (H1N1)	1.5
NCT02914275 [10]				3 to<5years	A/Hong Kong/4801/2014-like virus (H3N2)	1.5
					B/Phuket/3073/2013-like virus (Yamagata lineage)	1.5
					B/Brisbane/60/2008-like virus (Victoria lineage)	1.5

Abbreviations: IIV3=trivalent inactivated influenza vaccine; NH=Northern Hemisphere; S-IIV3=Seqirus trivalent inactivated influenza vaccine; S-IIV4=Seqirus quadrivalent inactivated influenza vaccine; SH=Southern Hemisphere; TDOC=sodium taurodeoxycholate.

2.4. Age subgroups

The age subgroups defined in the study protocols for the six studies differed: for example, the historical IIV3 formulation studies analyzed age subgroups of 3 to <9 years, whereas the modified IIV3/IIV4 formulation studies analyzed smaller age subgroups of 3 to <5 years or 5 to <9 years (Table 1). In the current analysis, the fever data from the three historical IIV3 formulation studies were reanalyzed according to the age subgroups used in the modified IIV3/IIV4 formulation studies, allowing direct comparison across studies between smaller, matching age subgroups.

2.5. Assessment of fever

The fever gradings used in the historical IIV3 formulation studies were different from, and less conservative than, the gradings used in the modified IIV3/IIV4 formulation studies (Supplementary Table S1). In the current analysis, the fever data from the three historical IIV3 formulation studies were reanalyzed according to the fever grading systems used in the modified IIV3/IIV4 formulation studies, which were based on the Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) guidance [16]. For children aged 6 months to <5 years, any grade fever was defined as axillary temperature ≥ 99.5°F/≥37.5 °C and severe fever was defined as axillary temperature ≥ 101.3°F/≥38.5 °C. For children aged 5 to <18 years, any grade fever was defined as oral temperature ≥ 100.4°F/≥38.0 °C and severe fever was defined as oral temperature ≥ 102.2°F/≥39.0 °C.

2.6. Statistical analysis

The rates of any grade fever and severe fever (frequency and associated 95% confidence intervals [CIs]) are presented for four age subgroups: (i) 6 months to <3 years; (ii) 3 to <5 years; (iii) 5 to <9 years; and (iv) 9 to <18 years. For each of these age subgroups, the fever rate in the S-IIV4 studies was compared with the pooled fever rate for the historical IIV3 formulation studies using Fisher's exact test. Analyses were conducted with SAS Version 9.3 (SAS Institute, Inc., Cary, NC) and JMP Version 13 (SAS Institute, Inc., Cary, NC).

3. Results

3.1. Fever rates in the 6 months to < 3 years age group

In the 6 months to <3 years age group, fever rates were lower in one S-IIV4 study (any grade: 7.0%; severe: 2.5%) than in the three historical IIV3 formulation studies (any grade: range, 38.7% to 40.0%; severe: range, 9.6% to 17.8%) (Table 2). In addition, the rates of any grade fever (Fig. 1) and severe fever (Fig. 2) in this age group were lower in the S-IIV4 study than in the pooled analysis of the historical IIV3 formulation studies ($p < 0.001$ for both).

3.2. Fever rates in the 3 to <5 years age group

In the 3 to <5 years age group, fever rates were lower in one S-IIV4 study (any grade: 4.9%; severe: 1.2%) than in the three historical IIV3 formulation studies (any grade: range, 34.1% to 36.0%; severe: range, 6.3% to 16.5%) (Table 2). In addition, the rates of any grade fever (Fig. 1) and severe fever (Fig. 2) in this age group were lower in the S-IIV4 study than in the pooled analysis of the historical IIV3 formulation studies ($p < 0.001$ for both).

3.3. Fever rates in the 5 to <9 years age group

In the 5 to <9 years age group, fever rates were lower in the S-IIV3 study (any grade: 8.2%; severe: 2.1%) and one study of S-IIV4

Table 2
Summary of fever rates in children aged 6 months to < 3 years and 3 to < 5 years.

Age group/study	Type of vaccine/ Number of strains split at 1.5% w/v TDOC	N	Any fever n % (95% CI)	Severe fever n % (95% CI)
6 months to < 3 years				
FLU-04–05 [11]	IIV3[a]	146	58	14
	0 of 3		39.7 (31.7, 48.2)	9.6 (5.3, 15.6)
USF-06–29 [12]	IIV3[a]	701	271	106
	0 of 3		38.7 (35.0, 42.4)	15.1 (12.6, 18.0)
USF-07–36 [13]	IIV3[a]	230	92	41
	0 of 3		40.0 (33.6, 46.6)	17.8 (13.1, 23.4)
Pooled analysis	IIV3[a]	1077	421	161
	0 of 3		39.1 (36.2, 42.1)	14.9 (12.9, 17.2)
QIV-15–03 [10]	S-IIV4[b]	669	47	17
	4 of 4		7.0 (5.2, 9.2)	2.5 (1.5, 4.0)
3 to < 5 years				
FLU-04–05 [11]	IIV3[a]	64	23	4
	0 of 3		36.0 (24.3, 48.9)	6.3 (1.7, 15.2)
USF-06–29 [12]	IIV3[a]	362	126	46
	0 of 3		34.8 (29.9, 40.0)	12.7 (9.5, 16.6)
USF-07–36 [13]	IIV3[a]	91	31	15
	0 of 3		34.1 (24.5, 44.8)	16.5 (9.5, 25.7)
Pooled analysis	IIV3[a]	517	180	65
	0 of 3		34.8 (30.7, 39.1)	12.6 (9.8, 15.7)
QIV-15–03 [10]	S-IIV4[b]	949	46	11
	4 of 4		4.9 (3.6, 6.4)	1.2 (0.6, 2.1)

Abbreviations: CI = confidence interval; IIV3 = trivalent inactivated influenza vaccine; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine; TDOC = sodium taurodeoxycholate.
[a] Historical IIV3 formulation.
[b] Modified IIV4 formulation.

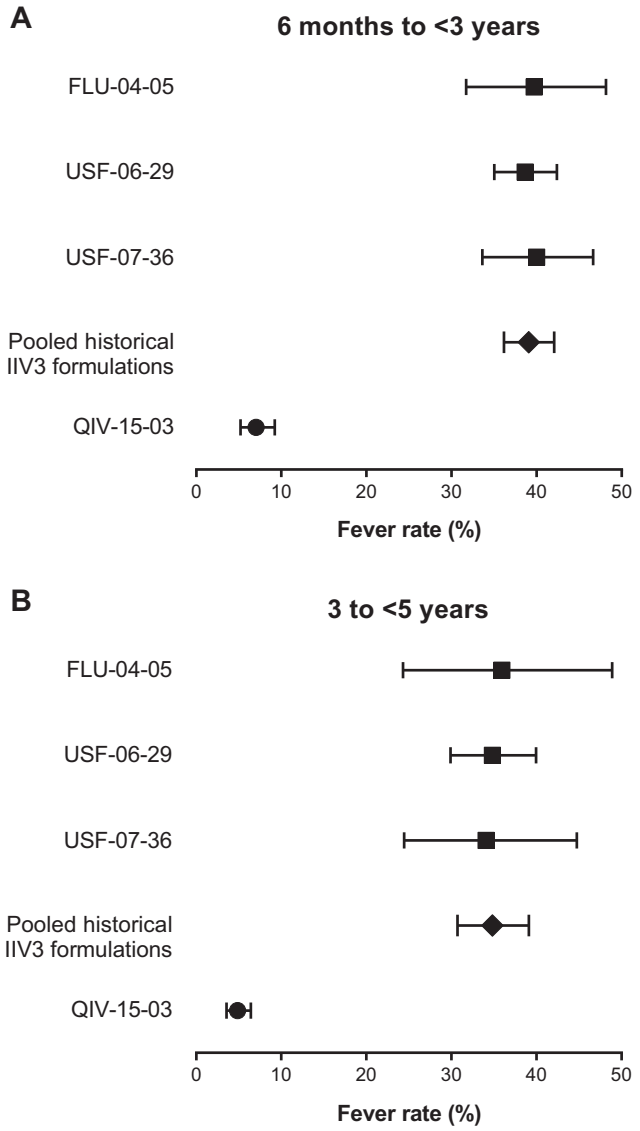

Fig. 1. Any grade fever rates. Data are the rate of any grade fever (95% CI) for the 6 months to <3 years age group (A) and the 3 to <5 years age group (B) of the FLU-04-05, USF-06–29, and USF-07-36 studies individually and pooled (=pooled historical IIV3 formulations), and the QIV-15–03 study (=S-IIV4). Any grade fever was defined as axillary temperature ≥ 99.5°F/≥37.5 °C. Abbreviations: CI = confidence interval; IIV3 = trivalent inactivated influenza vaccine; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine.

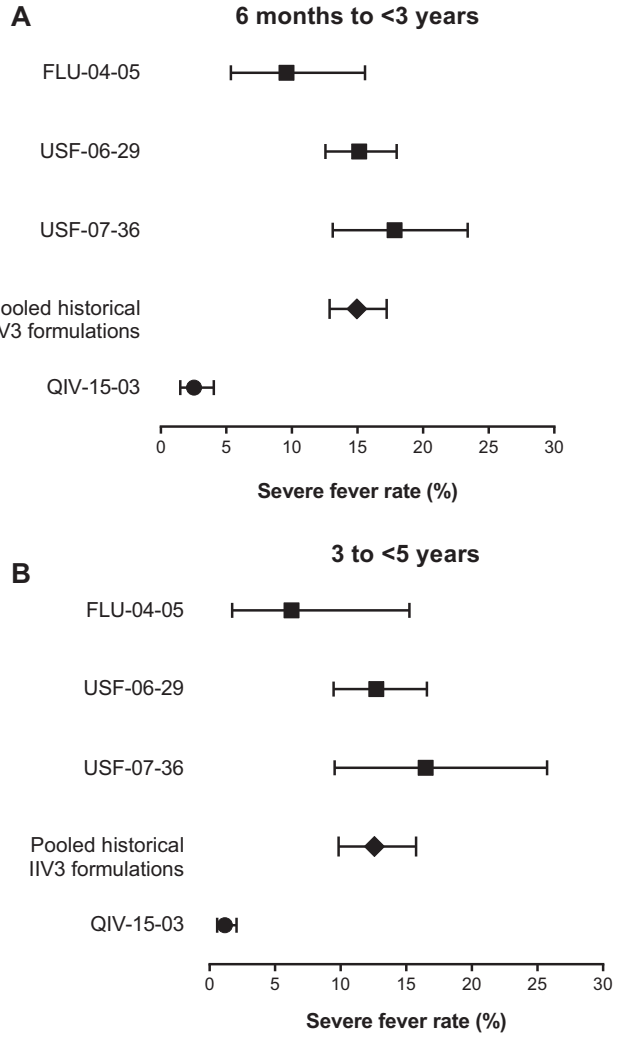

Fig. 2. Severe fever rates. Data are the rate of severe fever (95% CI) for the 6 months to <3 years age group (A) and the 3 to <5 years age group (B) of the FLU-04-05, USF-06-29, and USF-07-36 studies individually and pooled (=pooled historical IIV3 formulations), and the QIV-15-03 study (=S-IIV4). Severe fever was defined as axillary temperature ≥ 101.3°F/≥38.5 °C. Abbreviations: CI = confidence interval; IIV3 = trivalent inactivated influenza vaccine; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine.

(any grade: 4.5%; severe: 1.2%) than in the three historical IIV3 formulation studies (any grade: range, 11.1% to 17.2%; severe: 3.7% to 4.9%; $p < 0.001$ for the S-IIV4 study compared with the pooled historical IIV3 studies for both any grade and severe fever) (Table 3).

3.4. Fever rates in the 9 to <18 years age group

In the 9 to <18 years age group, fever rates were lower in one S-IIV4 study (any grade: 2.0%; severe: 0.5%) than in two historical IIV3 formulation studies (any grade: 5.0% and 6.3%; severe: 1.0% and 3.2%; $p < 0.001$ for the S-IIV4 study compared with the pooled historical IIV3 studies for any grade fever) (Table 3).

3.5. Fever rates in vaccination-naïve and non-naïve children aged < 5 years

In vaccination-naïve children in the 6 months to <3 years and 3 to <5 years age groups, fever rates (any grade and severe) were higher after the first dose of vaccine than after the second dose in two historical IIV3 formulation studies and one S-IIV4 study (Table 4). In the USF-06-29 study, the fever rates in vaccination-naïve children after the first dose of vaccine were higher than those in non-naïve children after the single dose of vaccine in the 6 months to <3 years age group (30.4% vs 13.6%) and the 3 to <5 years age group (31.9% vs 11.9%) (Table 4). In contrast, in the USF-07-36 study, the fever rates were similar in vaccination-naïve children after the first dose of vaccine and non-naïve children after the single dose of vaccine in the 6 months to <3 years age group (36.6% vs 37.2%) and the 3 to <5 years age group (26.7% vs 32.8%) (Table 4).

Table 3
Summary of fever rates in children aged 5 to < 9 years and 9 to < 18 years.

Age group/study	Type of vaccine/ Number of strains split at 1.5% w/v TDOC	N	Any fever n % (95% CI)	Severe fever n (95% CI)
5 to <9 years				
FLU-04-05 [11]	IIV3[a] 0 of 3	81	9 11.1 (5.2, 20.1)	3 3.7 (0.8, 10.4)
USF-06-29 [12]	IIV3[a] 0 of 3	513	88 17.2 (14.0, 20.7)	22 4.3 (2.7, 6.4)
USF-07-36 [13]	IIV3[a] 0 of 3	162	26 16.1 (10.8, 22.6)	8 4.9 (2.2, 9.5)
Pooled analysis	IIV3[a] 0 of 3	756	123 16.3 (13.7, 19.1)	33 4.4 (3.0, 6.1)
USF-10-69 [8]	S-IIV3[b] 2 of 3	292	24 8.2 (5.3, 12.0)	6 2.1 (0.8, 4.4)
QIV-13-02 [9]	S-IIV4[b] 4 of 4	829	37 4.5 (3.2, 6.1)	10 1.2 (0.6, 2.2)
9 to <18 years				
USF-06-29 [12]	IIV3[a] 0 of 3	398	20 5.0 (3.1, 7.7)	4 1.0 (0.3, 2.6)
USF-07-36 [13]	IIV3[a] 0 of 3	253	16 6.3 (3.7, 10.1)	8 3.2 (1.4, 6.1)
Pooled analysis	IIV3[a] 0 of 3	651	36 5.5 (3.9, 7.6)	12 1.8 (1.0, 3.2)
QIV-13-02 [9]	S-IIV4[b] 4 of 4	791	16 2.0 (1.2, 3.3)	4 0.5 (0.1, 1.3)

Abbreviations: CI = confidence interval; IIV3 = trivalent inactivated influenza vaccine; S-IIV3 = Seqirus trivalent inactivated influenza vaccine; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine; TDOC = sodium taurodeoxycholate.
[a] Historical IIV3 formulation.
[b] Modified IIV3/IIV4 formulation.

Table 4
Summary of fever rates in vaccination-naïve and non-naïve children aged < 5 years.

Age group/study	Type of vaccine/ Number of strains split at 1.5% w/v TDOC	Non-naïve (1 dose)		Vaccination-naïve (2 doses)			
				Dose 1		Dose 2	
		N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
		Any fever					
6 months to <3 years							
USF-06-29	IIV3[a] 0 of 3	59	13.6 (6.0, 25.0)	639	30.4 (26.8, 34.1)	601	18.5 (15.4, 21.8)
USF-07-36	IIV3[a] 0 of 3	129	37.2 (28.9, 46.2)	101	36.6 (27.3, 46.8)	101	14.9 (8.6, 23.3)
QIV-15-03	S-IIV4[b] 4 of 4	267	4.1 (2.1, 7.3)	396	5.8 (3.7, 8.6)	350	4.6 (2.6, 7.3)
3 to <5 years							
USF-06-29	IIV3[a] 0 of 3	67	11.9 (5.3, 22.2)	295	31.9 (26.6, 37.5)	280	13.6 (9.8, 18.2)
USF-07-36	IIV3[a] 0 of 3	61	32.8 (21.3, 46.0)	30	26.7 (12.3, 45.9)	30	13.3 (3.8, 30.7)
QIV-15-03	S-IIV4[b] 4 of 4	714	3.9 (2.6, 5.6)	232	5.2 (2.7, 8.9)	201	3.5 (1.4, 7.0)
		Severe fever					
6 months to <3 years							
USF-06-29	IIV3[a] 0 of 3	59	1.7 (0, 9.1)	639	12.4 (9.9, 15.2)	601	5.0 (3.4, 7.1)
USF-07-36	IIV3[a] 0 of 3	129	17.1 (11.0, 24.7)	101	14.9 (8.6, 23.3)	101	4.0 (1.1, 9.8)
QIV-15-03	S-IIV4[b] 4 of 4	267	1.1 (0.2, 3.3)	396	2.0 (0.9, 3.9)	350	1.7 (0.6, 3.7)
3 to <5 years							
USF-06-29	IIV3[a] 0 of 3	67	4.5 (0.9, 12.5)	295	10.5 (7.3, 14.6)	280	4.6 (2.5, 7.8)
USF-07-36	IIV3[a] 0 of 3	61	16.4 (8.2, 28.1)	30	13.3 (3.8, 30.7)	30	3.3 (0.1, 17.2)
QIV-15-03	S-IIV4[b] 4 of 4	714	1.0 (0.4, 2.0)	232	1.7 (0.5, 4.4)	201	0.5 (0, 2.7)

Abbreviations: CI = confidence interval; IIV3 = trivalent inactivated influenza vaccine; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine; TDOC = sodium taurodeoxycholate.
[a] Historical IIV3 formulation.
[b] Modified IIV4 formulation.

4. Discussion

This analysis of the fever profile of influenza vaccines from studies of historical IIV3 formulations and from current studies of S-IIV3 and S-IIV4 prepared using the modified manufacturing process showed that the S-IIV4 formulations were associated with less pyrogenicity than the historical IIV3 formulations, especially in children < 5 years of age. Both any grade fever and severe fever rates were lower for the modified S-IIV4 formulations than the historical IIV3 formulations in the comparative pediatric age subgroups. While this analysis focused on the important clinical events of fever and severe grade fever in children, other solicited systemic adverse events that were observed at high frequency in the historical IIV3 formulation studies [11–13] such as irritability, loss of appetite, and malaise were also generally reported at a lower frequency in the S-IIV4 studies [9,10]. The modified vaccine manufacturing process and quality control strategy to monitor pyrogenicity was sufficient to address the issue of increased rates of fever and febrile reactions in young children receiving the Seqirus/CSL 2010 SH IIV3. This analysis indicates that modifying the Seqirus/CSL influenza vaccine manufacturing process such that all four vaccine strains of the S-IIV4 are split using a higher concentration of the splitting agent results in considerably lower rates of fever in children. Ongoing monitoring for a pyrogenic signal following the introduction of a new vaccine strain(s) ensures consistent quality control to minimize further safety events.

Fever is a common systemic reaction to vaccination in children, especially in younger children [1], as observed in the current analysis. For each vaccine, the highest rates of fever were seen in the 6 months to <3 years subgroup and the fever rate decreased with increasing age. The current analysis demonstrated that, compared with the historical IIV3 formulations, there were consistently lower rates of any grade and severe fever across the four age subgroups with the S-IIV4 formulations prepared using the modified manufacturing process. Of note, in the 5 to <9 years subgroup, the fever rate observed with S-IIV3 (in which the B and A/H3N2 strains were split at 1.5% w/v TDOC) was intermediate (8.2%) between the fever rate observed with S-IIV4 (all four vaccine strains split at 1.5% w/v TDOC; 4.5%) and the historical IIV3 formulations (11.1% to 17.2%). This suggests that increasing the concentration of the splitting agent alters the pyrogenicity of the vaccine, and that splitting all strains of the vaccine at the higher concentration had the greatest effect. Although strain differences between seasonal influenza vaccines may contribute to a change in reactogenicity, the magnitude and consistency of reduction in any grade and severe fever with S-IIV3 and S-IIV4 are unlikely to be completely due to strain differences between the vaccines.

In addition to increased reports of fevers in children aged 6 months to <5 years receiving the Seqirus/CSL 2010 SH IIV3, there were increased reports of febrile seizures [2,3]. Febrile seizures are convulsions associated with fever, which most commonly occur with fevers of 102°F/38.9 °C or higher in children aged 6 months to <5 years, mostly in those aged 14 to 18 months [17]. Therefore, children aged 6 months to <5 years with fever classified as severe (axillary temperature $\geq$ 101.3°F/$\geq$38.5 °C) are at risk of febrile seizures. In the current analysis, the rates of severe fever in children aged 6 months to <5 years receiving S-IIV4 were low (2.5% and 1.2% in the 6 months to <3 years and 3 to <5 years age groups, respectively). Importantly, no vaccine-related febrile seizures were observed in the S-IIV4 study conducted in children aged 6 months to <5 years [10]. In the three historical IIV3 formulation studies, two children (a 3-year-old [11] and a 7-month-old [12]) experienced vaccine-related febrile seizures (a rate of 0.07% across the three studies [18]). The numbers of febrile seizures reported in these studies are too small to accurately compare between the influenza vaccines prepared using the modified manufacturing process and the historical IIV3 formulations.

As children aged 6 months to <9 years require a second dose of influenza vaccine to generate an adequate immune response, they require two vaccine doses (administered $\geq$ 4 weeks apart) during their first vaccination season for optimal protection [19,20]. The current analysis showed that fever rates were higher after the first dose of vaccine than the second dose with both S-IIV4 and the historical IIV3 formulations in vaccination-naïve children aged < 5 - years. However, in contrast to the historical IIV3 formulations, the difference in fever rates after the first and second doses in vaccination-naïve children was small for S-IIV4. It is possible that the higher fever rates in non-naïve children in the USF-07-36 study than those in the USF-06-29 study in the 6 months to <3 years subgroup (37.2% vs 13.6%) and 3 to <5 years subgroup (32.8% vs 11.9%) are related to the availability of the vaccine in the country in which the study was conducted. The USF-06-29 study was conducted in Australia, where Seqirus/CSL IIV3 had been licensed for many years. In contrast, the USF-07-36 study was conducted in the US, where Seqirus/CSL IIV3 was not licensed at the time and thus the children in this study would be "naïve" to Seqirus/CSL IIV3. This suggests that Seqirus/CSL IIV3 differed from other split-virion influenza vaccines in the US market.

Strengths of this analysis include the analysis of fever rates in four pediatric age subgroups, the generally large numbers of subjects in the age subgroups, and the ability to pool fever rates from the three historical IIV3 formulation studies. Access to subject-level data meant that the fever data from the historical IIV3 formulation studies could be reanalyzed according to the age subgroups and fever gradings used in the S-IIV3/S-IIV4 studies, thereby allowing comparison of matching subgroups between studies. Limitations of this analysis include different study designs (eg, variations in vaccine dose according to vaccine history, single arm vs active comparator-controlled), the use of historical controls, and the analysis of study data from the studies of historical IIV3 formulations being post hoc in nature (and, therefore, the *p* values should be interpreted with caution). In addition, except for the 5 to <9 years age group, there was only one study of the modified vaccine formulation in each age group; however, the studies of S-IIV3/S-IIV4 were large, with the number of subjects in the subgroups from these studies ranging from 292 (USF-10-69) to 949 (QIV-15-03). Analysis of fever rates in vaccination-naïve and non-naïve children aged 5 to <9 years in the studies of historical IIV3 formulations was not conducted because there were no corresponding analyses of fever rates in vaccination-naïve and non-naïve children in the S-IIV4 study conducted in this age group (QIV-13-02). The studies being conducted in different populations in different seasons with different vaccine strains was a limitation, but also a strength, as it allows generalizability of results for future influenza seasons.

In conclusion, the rates of fever observed for S-IIV3 and S-IIV4, manufactured using the modified manufacturing process, were considerably lower than the rates of fever observed for the historical IIV3 formulations in the comparative age groups. The reduced rates of fever in children aged 6 months to <5 years receiving S-IIV4 indicate that increasing the concentration of splitting agent has resolved the issue of the increased incidence of fever and febrile reactions in young children that was previously observed with the Seqirus/CSL 2010 SH IIV3. These results provide support for the safe use of S-IIV4 in this population. As the studies evaluating S-IIV3 and S-IIV4 were conducted over three successive NH influenza seasons, during which A/H3N2 and B strain changes occurred, it is likely that these results can be generalized to future influenza vaccines containing different vaccine strains.

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Role of the sponsor

Seqirus Pty Ltd was involved in the study design, data collection, and data analysis of the studies described in this manuscript, the analyses conducted for this manuscript, and the preparation of the manuscript.

Role of contributors

All authors were involved in the study design and participated in the interpretation of study results, and in the drafting, critical revision, and approval of the final version of the manuscript. DCS was involved in conceiving the analysis/manuscript, AGJ and FRA were involved in the data collection, and AGJ was involved in the statistical analysis.

Conflicts of interest

DCS, AGJ, and FRA are employees of Seqirus Pty Ltd. DCS and FRA own shares in CSL Pty Ltd. Seqirus Pty Ltd is a subsidiary of the CSL group.

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Appendix A. Supplementary material

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