WILDTYPE

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Overview

This document addresses the request for additional information re. CCC 000005 transmitted by FDA to Wildtype on 21 October 2024. For ease of reference, FDA's original questions are reproduced in black text and Wildtype's responses appear below in blue text.

Substantive Information Requests for Addition to the Disclosable Safety Narrative (DSN)

Substances used during cell culture

1. Figure 1 on page 2 of the August 30, 2024, amendment to the DSN contains a select list of media inputs that have not been previously evaluated by FDA for use in human food in the U.S. For any substance in this list that is also not naturally occurring in the U.S. food supply, please conduct analytical measurements for residual contents in the harvested cell material. In particular, we believe this applies to methyl-β-cyclodextrin.

Methyl-β-cyclodextrin concentration in the harvested cell material was quantified by an external laboratory with liquid chromatography - ultraviolet spectroscopy (LC-UV) and liquid chromatography - mass spectroscopy (LC-MS) using methods validated by for their intended use. The concentration of methyl-β-cyclodextrin in three non-consecutive lots of the harvested cell material was <0.0085 g per 100g of harvested cell material or <0.22 mg/kg bw/day, significantly lower than the 2.05 mg/kg bw/day estimated daily intake pre-wash concentration in Figure 1 of our August 30, 2024 amendment.

Analytical reports may be found in Appendix 1.[1]

2. For methyl-β-cyclodextrin, the Estimated Daily Intake (EDI) is reported as 2.05 mg/kg body weight (bw)/d. There is currently no safety data specifically for methyl-β-cyclodextrin; however, an Acceptable Daily Intake (ADI) of 5 mg/kg bw/d has been established for the read-across substance of β-cyclodextrin. FDA notes that β-cyclodextrin is used as a food additive and ingredient in various processed foods. Since methyl-β-cyclodextrin is expected to have similar toxicological properties to β-cyclodextrin, there could be additive or synergistic toxicological effects if exposure to both substances occurs simultaneously. Please provide the background EDI of β-cyclodextrin from the diet. Additionally, assess whether the combined EDI of β-cyclodextrin from the diet and methyl-β-cyclodextrin from the consumption of harvested cell material exceeds the established ADI for β-cyclodextrin. If the combined intake exceeds the ADI for β-cyclodextrin, please explain why the intake of methyl-β-cyclodextrin from the harvested cell material is still considered safe.

FDA's no questions letter to GRN 74 covers the use of β-cyclodextrin as a flavor carrier or protectant in a variety of foods. The uses in foods in this GRN are the same as those listed for FEMA # 4028. According to GRN 74, the background estimated daily intake in the diet for β-cyclodextrin at the 90th percentile is 9 mg/day (or 0.15 mg/kg bw/day based on default 60 kg body weight). Adding the analytically determined concentration of methyl-β-cyclodextrin in Wildtype's harvested cell material to the background diet exposure of β-cyclodextrin results in cumulative EDI of <0.37 mg/kg bw/day, significantly below the ADI[2] for β-cyclodextrin.

[1] Names of the receiving Wildtype employee and office addresses have been redacted from COAs for privacy.
[2] A safety factor of 100 was used to establish the ADI for β-cyclodextrin

3. Page 1 of the August 30, 2024, amendment to the DSN states, "Since submitting CCC 000005, we have removed 32 inputs and added five inputs." In response to previous questions in a request for additional information FDA sent to Wildtype on May 6, 2024, you state that certain substances are no longer used in the production process, including transferrin (page 5), D-galactose (page 9), glutathione-Na (page 9), and sodium pyruvate (page 11). Given the significant changes in material inputs, please provide, for addition to the DSN, a statement clarifying which manufacturing process was used to generate the batches of harvested cell material analyzed and submitted to the FDA in the January 24, 2024, amendment to the DSN.

The process described on pages 19-23 of our August 30, 2024 amendment was used to generate the batches of harvested cell material that were analyzed and submitted to FDA in the January 24, 2024 amendment to the DSN. Specifically, transferrin, D-galactose, glutathione-Na, and sodium pyruvate were not present in the medium used to culture the harvested cell material analyzed and submitted in our January 24, 2024 amendment.

4. On page 15 of the August 30, 2024, amendment to the DSN, the specification for folate in the harvested cell material is set at <1 mg "Folate (per 100g of cells)". The average level of folate in the harvested cell material, based on analytical data from three lots of harvested cell material, is 14.7 µg folate per 100g. For addition to the DSN, we recommend that you consider lowering the specification for folate in the harvested cell material to <100 µg folate per 100g, to align with the presented batch data.

Wildtype accepts FDA's recommendation and we have lowered our specification for folate in the harvested cell material to <100µg folate per 100g of harvested cell material.

Substances used during cell culture

5. Figure 6 on page 24 of the August 30, 2024, amendment to the DSN contains proximate specifications and batch data for three batches of harvested cell material.

a. **The categorical fat content (saturated, monounsaturated, polyunsaturated, and trans fat) is reported as percentages, but it is not clear whether these are mass percents relative to mass of harvested cell material. Please clarify.**

   The categorical fat content reported in Figure 6 on page 24 of our August 30, 2024 amendment to the DSN is reported as the mass percentage of the harvested cell material (e.g., saturated fat of 0.16% = 0.16 g / 100g of harvested cell material). The corresponding COAs in Appendix 7 of the same amendment (pages 56, 64, and 70) report the values as grams of various fats per 100 grams of harvested cell material, which we simplified as percentages in Figure 6. For clarity, Figure 1 below reports the categorical fat content values as reported in the corresponding COAs.

b. **The categorical fat content as mass percentages relative to the total fat content is reported in the analytical testing COAs provided in Appendix 7 of the amendment. For addition to the DSN, please provide a revised version of Figure 6, reporting the fat content results in the format used by the analytical testing COAs provided in Appendix 7 of the August 30, 2024, amendment.**

   Figure 1 below revises Figure 6 on page 24 of our August 30, 2024 amendment to the DSN as requested. The categorical fat content as mass percentages relative to the total fat content from the COAs is reported in Figure 1 below the fat content per 100g of harvested cell material.

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Figure 1: Revised Figure 6 from page 24 of our August 30, 2024 amendment to the DSN

Parameter	Method[3]	Specification	Lot 1: 023 2024-05-08-01	Lot 2: 024-2024-06-06-01	Lot 3: 024-2024-06-06-02
Calories (per 100g)	CFR - Atwater calculation	30 - 100 kcal	31 kcal	66 kcal	72 kcal
Total fat	AOAC 954.02 (Eurofins) AOAC 948.15 (Merieux)	0.5 - 10% (w/w)	1.13% (w/w)	1.41% (w/w)	1.75% (w/w)
Protein	AOAC 990.03(Eurofins) AOAC 991.20 (Merieux)	5 - 25% (w/w)	5.54% (w/w)	6.34% (w/w)	7.79% (w/w)
Carbohydrates	CFR 21 - Calculated	<10% (w/w)	0.75% (w/w)	6.98% (w/w)	7.63% (w/w)
Ash	AOAC 942.05 (Eurofins) AOAC 938.08 (Merieux)	<5% (w/w)	0.61% (w/w)	0.49% (w/w)	0.52% (w/w)
Moisture	AOAC 925.09 (Eurofins) AOAC 950.46A, 926.08 (Merieux)	75 - 95% (w/w)	92.4% (w/w)	84.8% (w/w)	82.9% (w/w)
Saturated fat	AOAC 996.06	<2% or 2g / 100g	0.16g / 100g 24% of total fat	0.22g / 100g 25% of total fat	0.28g / 100g 25% of total fat
Monounsaturated fat	AOAC 996.06	<5% or 5g / 100g	0.36g / 100g 54% of total fat	0.44g / 100g 51% of total fat	0.51g / 100g 46% of total fat
Polyunsaturated fat	AOAC 996.06	<5% or 5g / 100g	0.11g / 100g 17% of total fat	0.16g / 100g 18% of total fat	0.21g / 100g 24% of total fat
Trans fat	AOAC 996.06	<1% or 1g / 100g	0.04g / 100g 5% of total fat	0.05g / 100g 6% of total fat	0.06g / 100g 6% of total fat
Triglycerides	AOAC 996.06	<5% or 5g / 100g	0.69g / 100g	0.92g / 100g	1.16g / 100g
Total omega 3 isomers	AOAC 996.06	<2% or 2g / 100g	<0.01g / 100g	<0.01g / 100g	0.01g / 100g
Vitamin A	AOAC 974.29 (Eurofins) Analyst(1984)109:489 (Merieux)	<50 IU	<13.32 IU (<4 µg / 100g)	<13.32 IU (<4 µg / 100g)	<13.32 IU (<4 µg / 100g)
Vitamin B5 (per 100g)	AOAC 945.74 (Eurofins) AOAC 960.46 & Kit (Merieux)	<5 mg	0.74 mg	0.40 mg	0.33mg
Folate (per 100g)	AOAC 992.05 (Eurofins) AOAC 960.46 & Kit (Merieux)	<0.1 mg	0.007 mg	0.02 mg	0.02 mg
Vitamin B_{12} (per 100g)	AOAC 952.20 (Eurofins) AOAC 960.46 & Kit (Merieux)	<200 µg	139 µg	90 µg	101 µg
Vitamin D_2 & D_3	Huang et al. Rapid Commun, *Mass Spectrum* 2014, 28 (Eurofins) AOAC 2016.05 Mod. (Merieux)	<1,500 IU	544 IU (13.6 µg / 100g)	248 IU (6.2 µg / 100g)	291 IU (7.28 µg / 100g)

[3] All methods are validated for their intended purposes and are carried out by an external laboratory (e.g., Aemtek, Eurofins, Mérieux).

c. Categorical fat content was not provided for a conventional comparator; therefore, we were unable to complete a comparison of the harvested cell material to a conventional comparator. Please provide conventional comparator data with specific references/citations and a comparison discussion.

Figure 2 below provides conventional comparator data for categorical fat content as reported in USDA FoodData Central database for wild Coho salmon (raw). Trans fat comparator data was not available for Coho salmon in USDA's database. Trans fat contents were found on USDA's website for raw Sockeye salmon (0.019g / 100g), and raw Pink salmon (0.034g / 100g). The categorical fat content as mass percentages relative to the total fat content is reported in Figure 2 below the fat content per 100g of harvested cell material or the conventional comparator.[4]

Figure 2: Categorical fat content with reference to conventional comparator

Parameter	Method[5]	Specification	Lot 1: 023 2024-05-08-01	Lot 2: 024-2024-06-06-01	Lot 3: 024-2024-06-06-02	Conventional comparator
Total fat	AOAC 954.02 (Eurofins) AOAC 948.15 (Merieux)	0.5 - 10%	1.13% (w/w)	1.41% (w/w)	1.75% (w/w)	5.93g / 100g (or 5.93% w/w)
Saturated fat	AOAC 996.06	<2% or <2g / 100g	0.16g / 100g 24% of total fat	0.22g / 100g 25% of total fat	0.28g / 100g 25% of total fat	1.26g / 100g 21% of total fat
Monounsaturated fat	AOAC 996.06	<5% or <5g / 100g	0.36g / 100g 54% of total fat	0.44g / 100g 51% of total fat	0.51g / 100g 46% of total fat	2.13g / 100g 36% of total fat
Polyunsaturated fat	AOAC 996.06	<5% or <5g / 100g	0.11g / 100g 17% of total fat	0.16g / 100g 18% of total fat	0.21g / 100g 24% of total fat	1.99g / 100g 34% of total fat
Trans fat	AOAC 996.06	<0.1% or <0.1g / 100g	0.04g / 100g 5% of total fat	0.05g / 100g 6% of total fat	0.06g / 100g 6% of total fat	0.03g / 100g (average of pink & sockeye salmon) 0.5% of total fat

Discussion: Coho salmon cells at the point of harvest from the bioreactor generally have a lower fat content than the conventional comparator. Total fat, saturated fat, monounsaturated fat, and polyunsaturated fat levels in the harvested cell material were lower than the conventional comparator. Trans fat quantities were similar to those found in other forms of conventional salmon such as raw Sockeye or Pink salmon.

6. The analytical testing COAs provided in Appendix 7 of the August 30, 2024, amendment to the DSN contain results for the (i) fatty acid profile (as percent mass relative to total fat), (ii) amino acid profile (as percent mass relative to the mass of the harvested cell material), (iii) mineral profile, and (iv) vitamin analyses of the harvested cell material. For addition to the DSN, please provide conventional comparator data with specific references/citations and a narrative comparison discussion for the fatty acid profile, amino acid profile, and

[4] The percentage of total fat was taken from the analytical COAs (see appendix 7 from our August 2024 amendment) for the three Wildtype lots. For the conventional comparator, it was manually calculated by taking the category of fat and dividing by total fat content (e.g., saturated fat = 1.26 / 5.93 = 21%).
[5] All methods are validated for their intended purposes and are carried out by an external laboratory (e.g., Aemtek, Eurofins, Mérieux).

mineral profile as you did for vitamins in Figure 3 of the August 30, 2024, amendment. To assist you, the nutrient composition for raw, wild, coho salmon is available from the USDA FoodData Central at FoodData Central (link provided).

Figure 3 below summarizes the fatty acid, mineral, and amino acid analytical data from COAs provided in Appendix 7 of our August 30, 2024 amendment and subsequent testing of mineral and amino acids of lots 2 and 3 via-à-vis conventional comparator data provided by USDA's FoodData Central database. Amino acid and mineral testing for lots 2024-06–06-01 and 2024-06-06-02 was completed in December 2024 to support this RFI response; COAs are located in appendix 2. "SFA" denotes saturated fatty acid, "MUFA" denotes monounsaturated fatty acid, "PUFA" denotes polyunsaturated fatty acid, and "TFA" denotes trans-fatty acids. When a subcategory of fat (e.g., PUFA 20:2) from the COAs was not available in FoodData Central for raw, wild coho salmon, an average of that fat's presence in other raw Pacific salmon noted in response to question 5 (c) was used to provide a point of comparison (* is used to denote these values in the farthest right column).

Figure 3: Fatty acid, mineral, and amino acid profile with conventional comparator

Parameter	Category	Method	Cells at point of harvest			Conventional Comparator
			Lot 1: 2024-05-08-01	Lot 2: 2024-06-06-01	Lot 3:2024-06-06-02	
Iron, Fe (per 100g cells)	Mineral profile	AOAC 984.27 (mod.)	<0.25 mg	<0.24 mg	0.26 mg	0.56 mg
Magnesium, Mg (per 100g cells)	Mineral profile	AOAC 984.27 (mod.)	7.97 mg	7.17 mg	7.30 mg	31 mg
Potassium, K (per 100g cells)	Mineral profile	AOAC 984.27 (mod.)	145 mg	57.4 mg	36 mg	423 mg
Sodium, Na (per 100g cells)	Mineral profile	AOAC 984.27 (mod.)	77.1 mg	54.5 mg	28.2 mg	46 mg
Selenium, Se (per 100g cells)	Mineral profile	AOAC 2015.01Mod<2232>	0.1 ppm (w/w) or 10 µg	0.2 ppm (w/w) or 20 µg	0.2 ppm (w/w) or 20 µg	36.5 µg
SFA 4:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0.001 g 0.07% of total fat	0.002 g 0.2% of total fat	0 g
SFA 6:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0 g	0 g	0 g
SFA 8:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0 g	0 g	0 g
SFA 10:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0 g	0 g	0 g
SFA 12:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0 g	0 g	0 g
SFA 14:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.01 g 1.5% of total fat	0.011 g 1.3% of total fat	0.013 g 1.2% of total fat	0.264 g 4.5% of total fat
SFA 15:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.002 g 0.3% of total fat	0.003 g 0.3% of total fat	0.002 g 0.2% of total fat	0.014 g* 0.3%* of total fat
SFA 16:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.089 g 13.4% of total fat	0.122 g 14% of total fat	0.154 g 13.8% of total fat	0.751 g 12.7% of total fat
SFA 18:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.058 g 8.7% of total fat	0.083 g 9.5% of total fat	0.103 g 9.3% of total fat	0.207 g 3.5% of total fat
SFA 24:0 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0 g	0.003 g 0.2% of total fat	0.001 g* 0.03%* of total fat
MUFA 16:1 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.013 g 1.9% of total fat	0.014 g 1.6% of total fat	0.017 g 1.6% of total fat	0.506 g 8.5% of total fat
MUFA 17:1 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.043 g 6.5% of total fat	0.034 g 3.8% of total fat	0 g	0.015 g* 0.4%* of total fat
MUFA 18:1 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.284 g 42.8% of total fat	0.38 g 43.4% of total fat	0.472 g 42.5% of total fat	1.2 g 20% of total fat
MUFA 20:1 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.014 g 2.1% of total fat	0.014 g 1.6% of total fat	0.017 g 1.5% of total fat	0.25 g 4.2% of total fat

Figure 3 continued Parameter	Category	Method[6]	Cells at point of harvest			Conventional comparator
			Lot 1: 2024-05-08-01	Lot 2: 2024-06-06-01	Lot 3:2024-06-06-02	
MUFA 22:1 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.001 g 0.2% of total fat	0.002 g 0.2% of total fat	0.002 g 0.2% of total fat	0.146 g 2.5% of total fat
MUFA 24:1 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0 g	0.002 g 0.2% of total fat	0.024 g* 0.37%* of total fat
PUFA 18:2 inc. conj. (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.071 g 10.7% of total fat	0.099 g 11.3% of total fat	0.18 g 16.2% of total fat	0.206 g 3.5% of total fat
PUFA 18:3 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.006 g 1.0% of total fat	0.01 g 1.1% of total fat	0.014 g 1.3% of total fat	0.157 g 2.6% of total fat
PUFA 20:2 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.007 g 1.1% of total fat	0.011 g 1.3% of total fat	0.014 g 1.2% of total fat	0.017 g* 0.4%* of total fat
PUFA 20:3 5, 8, 11,14, 17 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.024 g 3.6% of total fat	0.037 g 4.2% of total fat	0.056 g 4.9% of total fat	0.03 g* 0.6%* of total fat
PUFA 20:4 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.004 g 0.7% of total fat	0.005 g 0.5% of total fat	0 g 0% of total fat	0.133 g 2.2% of total fat
PUFA 20:5 n-3 (EPA) (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0 g	0 g	0.429 g 7.2% of total fat
PUFA 22:5 n-3 (DPA) (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0 g	0 g	0.232 g 3.9% of total fat
PUFA 22:6 n-3 (DHA) (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0 g	0 g	0 g	0.656 g 11.1% of total fat
TFA 18:1 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.005 g 0.7% of total fat	0.008 g 0.9% of total fat	0.013 g 1.2% of total fat	0.009 g* 0.2%* of total fat
TFA 18:2 (per 100g cells)	Fatty acid profile	AOAC 996.06 (mod)	0.032 g 4.8% of total fat	0.042 g 4.8% of total fat	0.048 g 4.4% of total fat	0.012 g* 0.3%* of total fat
Tryptophan (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.05% or 0.05 g	0.07% or 0.07 g	0.07% or 0.07 g	0.242 g
Threonine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.21% or 0.21 g	0.24% or 0.24 g	0.25% or 0.25 g	0.948 g
Isoleucine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.22% or 0.22 g	0.25% or 0.25 g	0.25% or 0.25 g	0.996 g
Leucine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.39% or 0.39 g	0.44% or 0.44 g	0.45% or 0.45 g	1.76 g
Lysine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.38% or 0.38 g	0.46% or 0.46 g	0.46% or 0.46 g	1.98 g
Methionine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.16% or 0.16 g	0.18% or 0.18 g	0.18% or 0.18 g	0.64 g
Cysteine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.08% or 0.08 g	0.05% or 0.05 g	0.04% or 0.04 g	Not reported
Phenylalanine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.23% or 0.23g	0.24% or 0.24 g	0.25% or 0.25 g	0.844 g
Tyrosine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.17% or 0.17 g	0.19% or 0.19 g	0.19% or 0.19 g	0.73 g
Valine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.27% or 0.27 g	0.31% or 0.31 g	0.32% or 0.32 g	1.11 g
Arginine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.29% or 0.29 g	0.36% or 0.36 g	0.38% or 0.38 g	1.29 g
Histidine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.11% or 0.11 g	0.13% or 0.13 g	0.13% or 0.13 g	0.636 g
Alanine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.27% or 0.27 g	0.28% or 0.28 g	0.28% or 0.28 g	1.31 g
Aspartic acid (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.45 % or 0.45 g	0.48% or 0.48 g	0.48% or 0.48 g	2.21 g
Glutamic acid (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.68% or 0.68 g	0.68% or 0.68 g	0.68% or 0.68 g	3.23 g
Glycine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.28% or 0.28 g	0.27% or 0.27 g	0.27% or 0.27 g	1.04 g
Proline (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.26% or 0.26 g	0.28% or 0.28 g	0.27% or 0.27 g	0.764 g
Serine (% w/w or g/100g)	Amino acid profile	USDA MSS2 (1993)	0.24% or 0.24 g	0.27% or 0.27 g	0.28% or 0.28 g	0.882 g

[6] All methods are validated for their intended purposes and are carried out by an external laboratory (e.g., Aemtek, Eurofins, Mérieux).

Minerals discussion: mineral content (iron, magnesium, potassium, sodium, and selenium) in the harvested cell material as documented in the COAs is lower than or similar to the mineral content found in wild raw Coho salmon as provided in USDA's FoodData Central's database.

Fatty acid discussion: consistent with the discussion of categorical fat content in question 5, all sub-categorical fats were present at lower or similar quantities (g of fat per 100 g sample) to the conventional comparator.

Amino acid discussion: total amino acid content and individual amino acids were lower than in the conventional comparator. This is expected, as conventional comparators typically also contain protein-rich extracellular structures.

7. Figure 7 on page 25 of the August 30, 2024, amendment to the DSN lists the specifications for toxic heavy metals in the harvested cell material. Lead, mercury, and cadmium all have a specification of <20 ppb, and batch data confirm all three metals were present below the limits of detection for the analytical method. Arsenic has a specification of <100 ppb in the harvested cell material; batch data show levels of 20, 30, and 30 ppb. For addition to the DSN, please provide an EDI for arsenic based on the maximum specification of <100 ppb of arsenic in the harvested cell material. Further, for addition to the DSN, please provide a safety discussion for arsenic in the harvested cell material based on the EDI calculated using the <100 ppb specification. Alternatively, and preferably, please consider lowering the specification for arsenic in the harvested cell material to <50 ppb, or lower, for arsenic to align with the presented batch data and conform with FDA's Closer to Zero Initiative.

We accept FDA's recommendation and have reduced our specification for arsenic in the harvested cell material to <50 ppb.

Food safety management system

8. On page 20 and page 49 of August 30, 2024, amendment to the DSN, and on page 11 of the July 28, 2023, amendment, you indicate that the thermal step is one of the preventive controls at Step 1 "Receiving raw material." You also indicate that the thermal step is also one of the preventive controls at Step 6 "Cell Harvest from bioreactors" on page 50 of August 30, 2024, amendment and on page 16 of the July 28, 2023, amendment. Given the fact that the thermal step is identified as a preventive control for potential hazards introduced during the production process (including prior to harvest of the cell material), it is important for Wildtype to provide such information in its safety evaluation. We also note that the provided thermal process discussion is not adequate.

Therefore, for addition to the DSN, please provide a statement about whether you have conducted the validation study for the Sheldon dry heat oven using a target pathogen of concern (e.g., Listeria monocytogenes) and provide a discussion to confirm the thermal step is adequate to control for the presence of any biological hazards identified as adventitious agents of concern in your food safety analysis.

A validation study for the Sheldon dry heat oven was conducted by a third party laboratory (Aemtek) in November 2024 using methods validated for their intended purpose. A four strain cocktail of *Listeria monocytogenes* was used to inoculate Wildtype's salmon saku at an average level of 8.40 log cfu/g. Finished food products inoculated with *L. monocytogenes* were then loaded into the Sheldon oven and subjected to Wildtype's thermal validation process described on page 18 of our August 30, 2024 amendment. The thermal treatment yielded a minimum reduction of 7.15 log cfu/g. The log reduction in the validation study exceeds the six log reduction recommended for seafood processors in the Fish &

Fishery Products Hazard and Controls, June 2022 edition. The full study report may be found below in Appendix 3.

Given that *Listeria monocytogenes* is a pathogen of concern for Wildtype and one of the most heat resistant non-spore forming foodborne pathogens[7], the effectiveness of the thermal step in significantly reducing the presence of *L. monocytogenes* described above can be extended to other pathogens of concern described in our food safety analysis (e.g., *Salmonella spp.)* Additionally, as described on page 7 of our January 24, 2024 amendment, we test every batch of both cells at the point of harvest as well as the finished product for a wide range of pathogens of concern, providing ongoing surveillance that our thermal step is effectively mitigating biological adventitious agents of concern.

Substantive Information Requests for Addition to the Supplemental Confidential Material (SCM)

9. Appendix 1 of the August 30, 2024, amendment to the SCM contains a comprehensive list of material inputs and theoretical EDIs for each substance calculated based on the concentration of each in the medium. Analytical results for a number of these substances were found in Appendix 7 of the amendment. For addition to the SCM, we request that EDIs for media components be based on the analytical concentrations determined in the harvested cell material when such data is available. For example, in Figure 3 of the August 30, 2024, amendment, you report the analytically determined concentration of Vitamin B12 in the harvested cell material as 139 µg for 100 g of harvested cell material. For calculating the EDI based on analytical measurements, please use this mass ratio and scale for the consumption estimate, 112 g/d, as shown: 112 g/d * 139 µg/100 g = 156 µg/d Vitamin B12.

Appendix 1 in the SCM to this amendment has been updated. Those media components where analytical results were present in Appendix 7 of our August 30, 2024 have been updated to reflect the analytically determined concentration. Additionally, methyl-β-cyclodextrin has been updated with the analytically derived concentrations described in response to question 1 above. Those rows that have been updated using analytically determined concentrations have been highlighted in beige color. When there was more than one analytical result for a media component, we used the mean concentration across multiple lots multiplied by the 90th percentile daily intake of salmon (112 g/d).

[7] Pöntinen A, Aalto-Araneda M, Lindström M, Korkeala H. Heat Resistance Mediated by pLM58 Plasmid-Borne ClpL in *Listeria monocytogenes*. mSphere. 2017 Nov 1;2(6):e00364-17. doi: 10.1128/mSphere.00364-17. PMID: 29104933; PMCID: PMC5663981

Appendix 1: Certificates of Analysis for MβCD Quantification

BALAZS™ nanoanalysis
Air Liquide Electronics U.S. LP
46409 Landing Parkway, Fremont CA 94538
Telephone (510) 624-4000 Fax (510) 657-2292

[redacted]
WILD TYPE INC
[redacted]
SAN FRANCISCO CA 94107

Phone:
Fax:

Work Order: 24-12257
Revision: 0
Report Date: 15-Nov-24
Order Date: 7-Nov-24
P.O.: PF6515
Release: .

BALAZS™ TEST RESULTS

If you have any questions regarding the results, please email: Lanny Huynh at Lan.Huynh@airliquide.com

Sample: #130-2024-10-18-01 **Site:** MBCD **ID:**

Component	Units	Result Value
G0156-LC-MS-P-R Priority LC-MS Analysis		**BAL-82827-SOP**
Methyl-beta-cyclodextrin	ppm	<85

* = Analysis revealed that the element was not found at or above the detection limit.

Report Revision Note:

Report Revision 4: Customer requested results be updated from 1.43 ppm to <85ppm. 11/15/2024 FP.

This report, including any attachments has been reviewed and approved by

[redacted]
Laboratory Supervisor

WILDTYPE

BALAZS™ nanoAnalysis
Air Liquide Electronics U.S. LP
46409 Landing Parkway, Fremont CA 94538
Telephone (510) 624-4000 Fax (510) 657-2292

[redacted]
WILD TYPE INC
[redacted]
SAN FRANCISCO CA 94107

Work Order: 24-12797
Revision: 0
Report Date: 19-Nov-24
Order Date: 19-Nov-24
P.O.: PF6540
Release:

Phone:
Fax:

BALAZS™ TEST RESULTS

If you have any questions regarding the results, please email: Lanny Huynh at Lan.Huynh@airliquide.com

Sample: 023-2024-08-27-01 **Site: MBCD** **ID:**

Component	Units	Detection Limit	Result Value
G0156-LC-MS-P-R Priority LC-MS Analysis			**BAL-82827-SOP**
Methyl-beta-cyclodextrin	ppm		< 85 *

* = Analysis revealed that the element was not found at or above the detection limit.

This report, including any attachments has been reviewed and approved by

[redacted]
Chemist

WILDTYPE

BALAZS™ nanoanalysis
Air Liquide Electronics U.S. LP
46409 Landing Parkway, Fremont CA 94538
Telephone (510) 624-4000 Fax (510) 657-2292

[redacted]
WILD TYPE INC
[redacted]
SAN FRANCISCO CA 94107

Report Date: 19-Nov-24
Order Date: 19-Nov-24
Work Order: 24-12797
P.O.: PF6540

Sample: 023-2024-10-15-01 **Site: MBCD** **ID:**

Component	Units	Detection Limit	Result Value
G0156-LC-MS-P-R Priority LC-MS Analysis			**BAL-82827-SOP**
Methyl-beta-cyclodextrin	ppm		< 85

* = Analysis revealed that the element was not found at or above the detection limit.

This report, including any attachments has been reviewed and approved by

[redacted]

Chemist

Appendix 2: Certificates of Analysis for supplemental mineral & amino acid testing for two lots

Mérieux NutriSciences

CERTIFICATE OF ANALYSIS

COA No:	CCA-48860323-1
Supersedes:	CCA-48860323-0
COA Date	12/10/24
Page 1 of 4	

SILLIKER, Inc.
Salida, CA Laboratory
5262 Pirrone Court, Salida, CA 95368
Tel. 1-844-277-1680 Fax. 209-545-0245
Email: getresults6@mxns.com

TO:
[redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	11/26/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 439512883 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1.1
Sample Name: 024-2024-06-06-01

Analyte	Result	Units	Method Reference	Test Date	Loc.
Amino Acids Complete			USDA MSS2 (1993)	12/10/24	CHG
Aspartic Acid	0.48	% (w/w)			
Threonine	0.24	% (w/w)			
Serine	0.27	% (w/w)			
Glutamic Acid	0.68	% (w/w)			
Glycine	0.27	% (w/w)			
Alanine	0.28	% (w/w)			
Valine	0.31	% (w/w)			
Methionine	0.18	% (w/w)			
Isoleucine	0.25	% (w/w)			
Leucine	0.44	% (w/w)			
Tyrosine	0.19	% (w/w)			
Phenylalanine	0.24	% (w/w)			
Lysine	0.46	% (w/w)			
Histidine	0.13	% (w/w)			
Arginine	0.36	% (w/w)			
Proline	0.28	% (w/w)			
Hydroxyproline	<0.01	% (w/w)			
Cysteine	0.05	% (w/w)			
Tryptophan	0.07	% (w/w)			
ICP Sample Prep - Microwave	Microwave	-	AOAC 2011.14	12/4/24	CHG
ICP-MS Sample Prep	Acid Digest	-	AOAC2015.01Mod<2232>	12/6/24	CHG
* Iron	<0.24	mg/100g	AOAC 984.27 (mod.)	12/5/24	CHG
* Magnesium	7.17	mg/100g	AOAC 984.27 (mod.)	12/5/24	CHG
* Potassium	57.4	mg/100g	AOAC 984.27 (mod.)	12/5/24	CHG
* Selenium	0.2	ppm (w/w)	AOAC2015.01Mod<2232>	12/9/24	CHG

WILDTYPE

Mérieux NutriSciences

SILLIKER, Inc.

Salida, CA Laboratory

5262 Pirrone Court, Salida, CA 95368

Tel. 1-844-277-1680 Fax. 209-545-0245

Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-48860323-1
Supersedes:	CCA-48860323-0
COA Date	12/10/24
Page 2 of 4	

TO:

[redacted]

Quality and Food Safety Manager

Wildtype

[redacted]

Received From:	San Francisco, CA
Received Date:	11/26/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 439512883 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1.1

Sample Name: 024-2024-06-06-01

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Sodium	54.5	mg/100g	AOAC 984.27 (mod.)	12/5/24	CHG

WILDTYPE

Mérieux NutriSciences

SILLIKER, Inc.

Salida, CA Laboratory

5262 Pirrone Court, Salida, CA 95368

Tel. 1-844-277-1680 Fax. 209-545-0245

Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-48860323-1
Supersedes:	CCA-48860323-0
COA Date	12/10/24
Page 3 of 4	

TO:

[redacted]

Quality and Food Safety Manager

Wildtype

[redacted]

Received From:	San Francisco, CA
Received Date:	11/26/24
P.O.# / ID:	PF4852

Location of Test: (except where noted) Salida, CA

Analytical Results

Laboratory ID: 439512888 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1.1

Sample Name: 024-2024-06-06-02

Analyte	Result	Units	Method Reference	Test Date	Loc.
Amino Acids Complete			USDA MSS2 (1993)	12/10/24	CHG
Aspartic Acid	0.48	% (w/w)			
Threonine	0.25	% (w/w)			
Serine	0.28	% (w/w)			
Glutamic Acid	0.68	% (w/w)			
Glycine	0.27	% (w/w)			
Alanine	0.28	% (w/w)			
Valine	0.32	% (w/w)			
Methionine	0.18	% (w/w)			
Isoleucine	0.25	% (w/w)			
Leucine	0.45	% (w/w)			
Tyrosine	0.19	% (w/w)			
Phenylalanine	0.25	% (w/w)			
Lysine	0.46	% (w/w)			
Histidine	0.13	% (w/w)			
Arginine	0.38	% (w/w)			
Proline	0.27	% (w/w)			
Hydroxyproline	<0.01	% (w/w)			
Cysteine	0.04	% (w/w)			
Tryptophan	0.07	% (w/w)			
ICP Sample Prep - Microwave	Microwave	-	AOAC 2011.14	12/4/24	CHG
ICP-MS Sample Prep	Acid Digest	-	AOAC2015.01Mod<2232>	12/6/24	CHG
* Iron	0.26	mg/100g	AOAC 984.27 (mod.)	12/5/24	CHG
* Magnesium	7.30	mg/100g	AOAC 984.27 (mod.)	12/5/24	CHG
* Potassium	36.0	mg/100g	AOAC 984.27 (mod.)	12/5/24	CHG
* Selenium	0.2	ppm (w/w)	AOAC2015.01Mod<2232>	12/9/24	CHG

WILDTYPE

Mérieux NutriSciences

CERTIFICATE OF ANALYSIS

SILLIKER, Inc.

Salida, CA Laboratory

5262 Pirrone Court, Salida, CA 95368

Tel. 1-844-277-1680 Fax. 209-545-0245

Email: getresults6@mxns.com

COA No:	CCA-48860323-1
Supersedes:	CCA-48860323-0
COA Date	12/10/24
Page 4 of 4	

TO:

[redacted]

Quality and Food Safety Manager

Wildtype

[redacted]

Received From:	San Francisco, CA
Received Date:	11/26/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 439512888 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1.1

Sample Name: 024-2024-06-06-02

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Sodium	28.2	mg/100g	AOAC 984.27 (mod.)	12/5/24	CHG

[redacted]

Julienne Mortensen Laboratory Director

Noted Test Locations: CHG-Silliker, Inc. Crete, IL Laboratory, 3600 Eagle Nest Drive, North Building, Crete, IL 60417

I Customer supplied information * ISO17025 Accredited Analysis † Indicates reason for COA amendent when applicable

Appendix 3: Thermal Process Validation Study: Sheldon Oven

AEMTEK

STUDY REPORT FOR

Wildtype Inc.

Thermal Process Validation Study of Wildtype Saku Salmon

Project Title:	**Thermal Process Validation Study of Wildtype Saku Salmon**
AEMTEK Project ID:	24102336 (Sheldon Dry Heat Oven)
Reported Issued To:	Wildtype Inc.
Product Tested:	Salmon Product – Saku
Analysis Performed by:	AEMTEK Research Laboratory
Report Written by:	Justin Chow
Report Reviewed by:	Heidi Wright
Date of Reporting:	November 14, 2024

EXECUTIVE SUMMARY

The objective of this process validation study was to provide scientific evidence suppporting a 6-Log reduction claim for Wildtype Inc.'s thermal treatment using the Sheldon Dry Heat Oven. A four-strain cocktail of *Listeria monocytogenes* was used to inoculate Wildtype's Saku Salmon. The confirmed average inoculation level was 8.40 log CFU/g. The inoculated products were heat-treated at a setting of 176°F/80°C for 120 minutes.

The thermal treatment yielded an average and minimum reduction of 7.15 Log CFU/g in the inoculated product. Based on the validation results, **the heat treatment of Saku Salmon with Sheldon Dry Heat Oven at 176°F for 120 minutes achieved the 6-log reduction requirement.**

Risk Assessment Statement:

It is recommended that the production team does not exceed the production load used in this process validation study to ensure all internal temperature reach the minimum processing time of 15 seconds at 145°F/63°C.

AEMTEK, INC.
466 Kato Terrace, Fremont, CA 94539
Phone: 510-979-1979 Fax: 510-668-1980
Email: lab@aemtek.com
www.aemtek.com

STARS Sampling Testing Analytics Research Solutions

INTRODUCTION

Wildtype Inc. requested an onsite process validation study to evaluate the reduction of *Listeria monocytogenes* during their thermal treatment via the Sheldon Dry Heat Oven for one product, Saku Salmon. This study was conducted to ensure target thermal lethality was achieved for each production batch in the oven, which was a replacement to the water-bath pasteurization method validated in May 2023. A kill step validation needed to be performed to ensure the process complies with seafood processors under 21 CFR Part 123 for a 6-Log reduction of *Listeria monocytogenes*.

MATERIALS AND METHODS

Validation Methodology

WildType Inc. determined, set up, and recorded the processing parameters, operated and monitored the treatment, and recorded treatment data. AEMTEK provided inoculated products for treatment and collected treated samples for testing.

Challenge Microorganism and Inoculum Solution

Based on Fish and Fishery Products Hazards and Controls Guidance, *Listeria monocytogenes* was selected as the target inoculation organism in this study as it is the main non-spore forming pathogen of concern for seafood pasteurization. AEMTEK's standard operating procedures were followed to prepare the inoculum at the desired concentration. The *Listeria monocytogenes* cocktail included the following strains: ATCC 49594, ATCC 19115, ATCC 51414, ATCC 43257.

An 8oz sample of Saku Salmon was syringe injected with 1cc of the four-strain *Listeria monocytogenes* cocktail into three center spots (**Figure 1**). The inoculated product was held overnight under refrigerated conditions and transported to WildType Inc.'s processing facility. AEMTEK microbiologists assisted in performing onsite heat treatments and collected samples for enumeration.

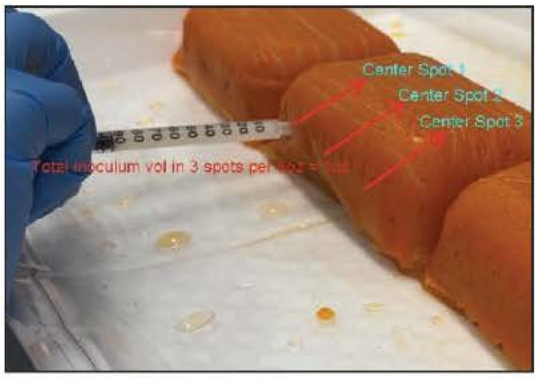


Figure 1. Saku Salmon inoculation with *Listeria monocytogenes* cocktail.

Validation Study Design and Process Parameters

- One commercial Oven: Sheldon Dry Heat Oven (**Figure 2**)
- Time/temperature setup: 176°F/80°C for 120 minutes
- Products: 8oz Saku Salmon
- Treated Samples: 14 replicates of inoculated and treated samples for each process
- Production Samples: 36 replicates of uninoculated and treated samples for each process.
- Positive Controls: 10 replicates of inoculated and untreated samples for each product
- Negative Controls: 3 replicates of uninoculated and untreated samples for each product
- Data Logger: 8 units (WildType), 1 unit (AEMTEK)

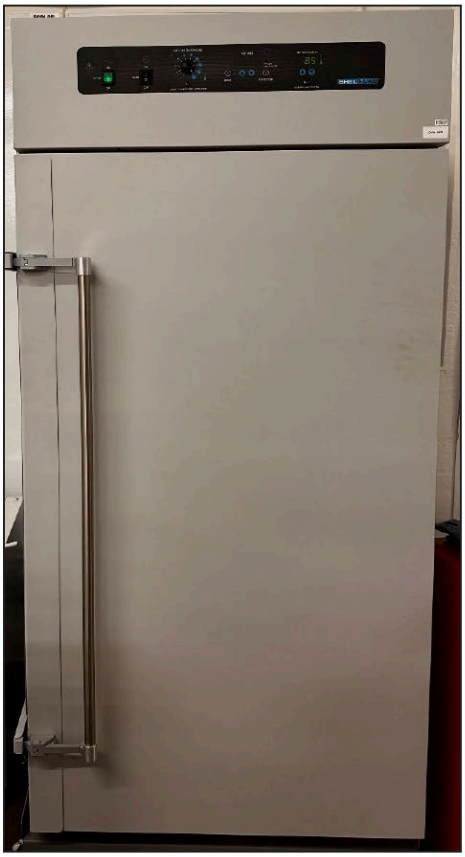


Figure 2. Sheldon Dry Heat Oven used for validation at WildType Inc.'s production facility.

Sheldon Dry Heat Oven Validation Process Information

The heating process records and temperature measurements were collected onsite with Wildtype Inc's Quality Assurance and Production team. The production load was 50 pieces of 8oz Saku Salmon per batch. The products and data loggers were placed into predetermined locations to cover temperature distribution thoroughly within the equipment (**Figure 3**).

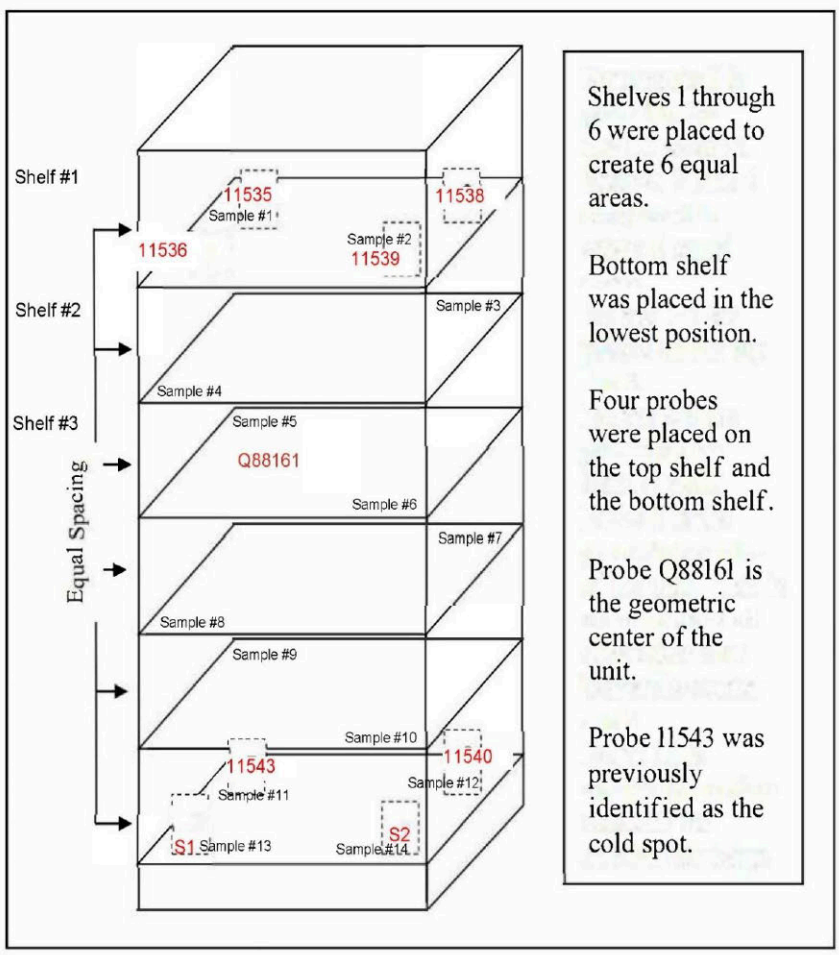


Figure 3. Sheldon Dry Heat Oven sample and data logger placement.

Production Record of Sheldon Dry Heat Oven Validation

Validation Date:	11/06/2024
Client:	WildType Inc. 2325 3rd street, Unit 209 San Francisco, CA, 94107, USA
Production load:	50 units of 8oz Saku Salmon
Product/Probe Placement and Start Time:	10:19 AM
Oven reached 176°F/80°C:	10:54 AM
Heat Treatment End Time:	12:19 PM
Post Validation Microbiological Testing	3:00PM

Microbiological Testing

Positive controls, treated, and untreated samples were analyzed following standard microbiological testing protocols using an enumeration plating method of Trypticase Soy Agar with Oxford agar overlay. Culture plates were incubated aerobically at 35°C for 48 hours before counting *Listeria monocytogenes* colonies. Serial dilutions were performed to obtain plates with colonies that were within the countable range.

Uninoculated and untreated product samples were analyzed for aerobic plate counts to obtain information about naturally occurring bacteria. Plate count results were expressed in log10 format and data analysis was performed to evaluate inoculated microbial population change.

RESULTS

Product aW and pH of Saku Salmon

The pH and water activity of the production batch were provided by Wildtype, Inc. for the process validation, records are displayed under **Table 1.**

Table 1: Water activity and pH of Saku Salmon

Product	aW	pH
Saku Salmon	1.01	7.5 to 7.9

Validation Run Product Internal Temperature Records

The validation run records for the study set based on the data loggers provided by AEMTEK and Wild Type are shown in **Figure 4 and 5** and summarized in **Table 2** below. The temperatures for both data loggers showed production temperatures were higher than target temperatures.

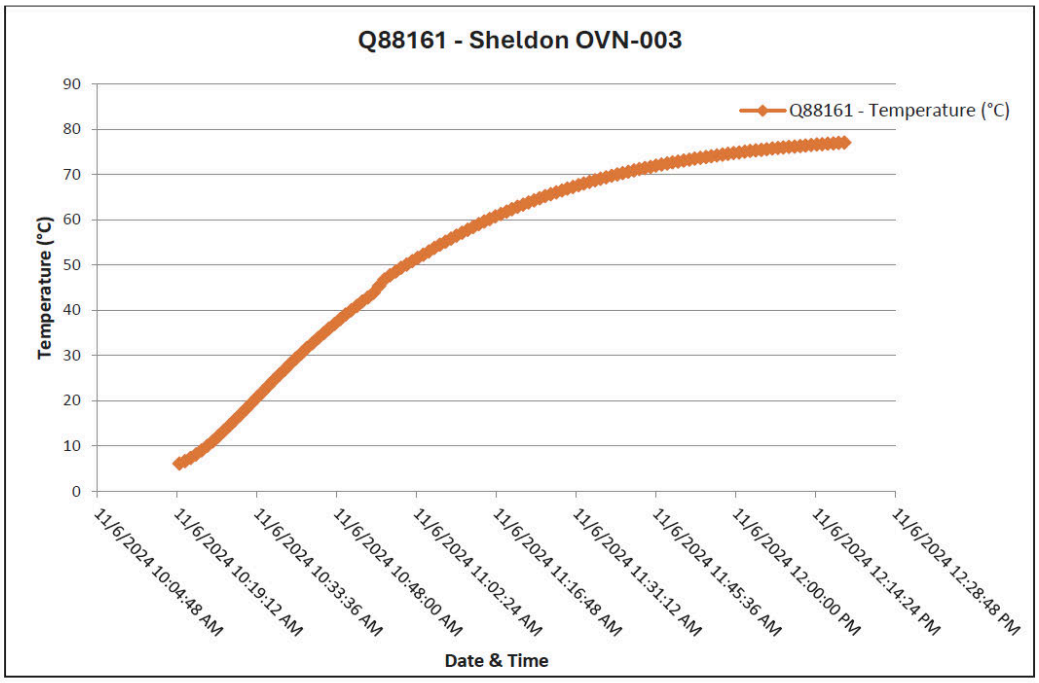
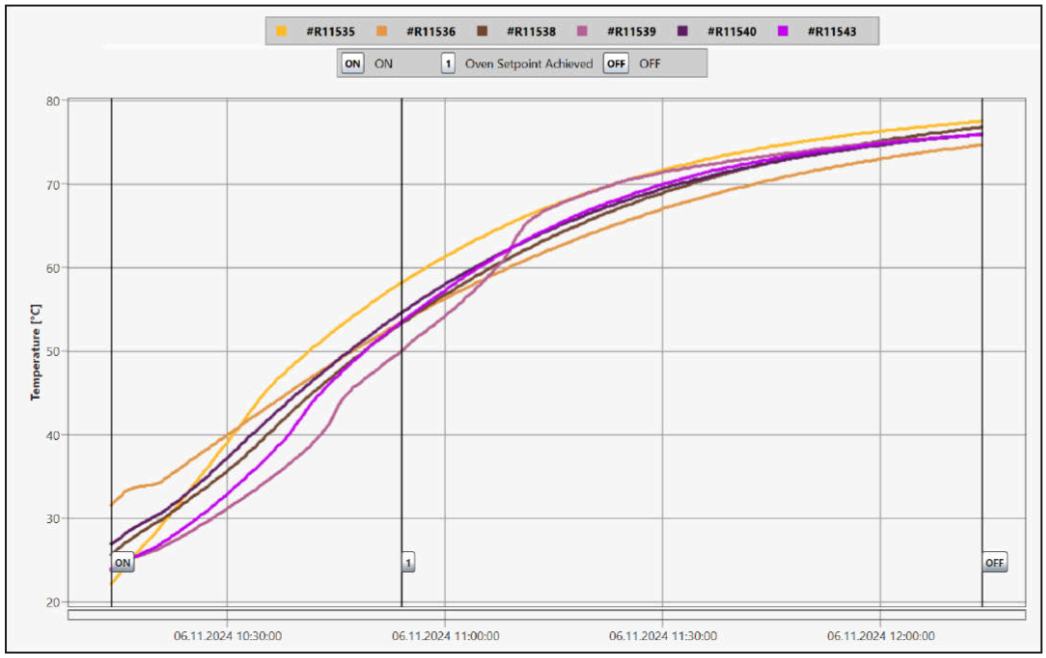


Figure 4: Product internal temperature chart by AEMTEK, Inc. data loggers



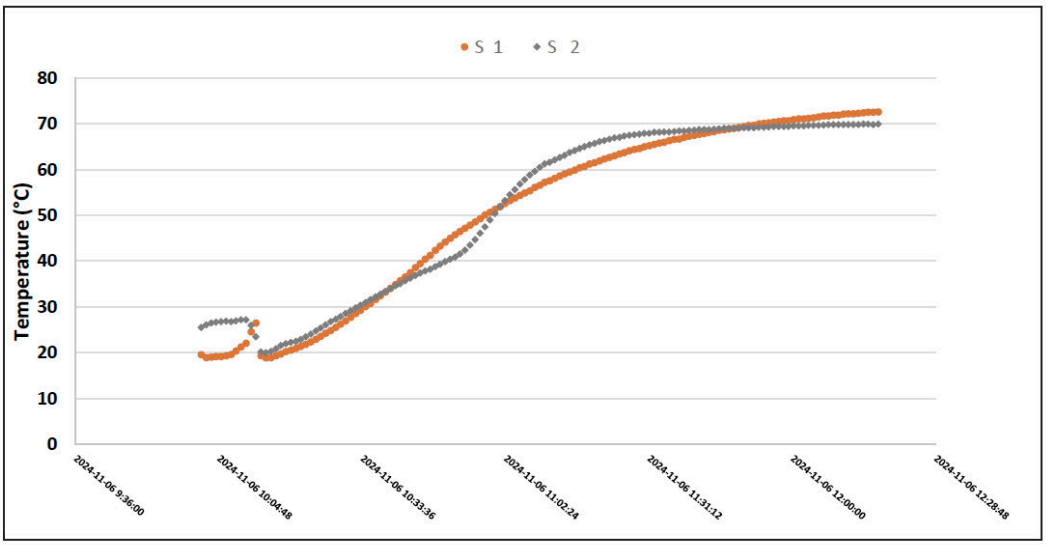


Figure 5: Product internal temperature chart by Wildtype Inc. data loggers.

Table 2: Real-Time Temperature Tracking Summary with Data Loggers

Logger	#R11535	#R11536	#R11538	#R11539	#R11540
Label	Sample #1	N/A	N/A	Sample #2	Sample #12
Time to Reach 63°C (min.)	49	62	59	55	56
Time at or above 63°C (min.)	71	58	61	65	64
Time In Oven (min.)	120	120	120	120	120
Logger	#R11543	#S1	#S2	#Q88161	
Label	Sample #11	Sample #13	Sample #14	Center	
Time to Reach 63°C (min.)	56	70	59	61	
Time at or above 63°C (min.)	64	50	61	59	
Time In Oven (min.)	120	120	120	120	

Enumeration Results of *Listeria monocytogenes* and Log Reduction Calculation

Enumeration results of *Listeria monocytogenes* and respective log CFU/g values are presented in **Table 3 and 4** below. The log reduction was calculated by subtracting the log CFU/g in the treated inoculated samples from the average log CFU/g in the untreated inoculated samples.

The inoculation level of all samples was within the target level at average of 8.40 log CFU/g. The heat treatment for 120 minutes of Saku Salmon resulted in a minimum of 7.15 log reduction of the inoculated *L. monocytogenes* population respectively. (Limit of Detection = 1.00 log CFU/g)

Table 3. *Listeria monocytogenes* Enumeration Results of Positive controls

Travel Positive Control	Average CFU/g	Average Log10 CFU/g	Average Log10 CFU/g
1	2.12E+08	8.33	8.40
2	2.83E+08	8.45	
3	2.75E+08	8.44	
4	2.14E+08	8.33	
5	2.78E+08	8.44	
6	2.32E+08	8.37	
7	2.97E+08	8.47	
8	3.95E+08	8.60	
9	1.41E+08	8.15	
10	2.48E+08	8.39	

Table 4. *Listeria monocytogenes* Enumeration Results of Heat-Treated Samples

Process Condition	Sample Replicates	Average CFU/g	Average Log10 CFU/g	Log reduction
176°F/80°C 120 minutes	1	<10	<1.0	7.40
	2	<10	<1.0	7.40
	3	<10	<1.0	7.40
	4	<10	<1.0	7.40
	5	<10	<1.0	7.40
	6	<10	<1.0	7.40
	7	<10	<1.0	7.40
	8	<10	<1.0	7.40
	9	<10	<1.0	7.40
	10	<10	<1.0	7.40
	11	<10	<1.0	7.40
	12	<10	<1.0	7.40
	13	<10	<1.0	7.40
	14	<10	<1.0	7.40

Table 6. Aerobic Plate Count Results of uninoculated Saku Salmon

Product	Negative Control	Average CFU/g	Average Log 10 (CFU/g)
Untreated Control	1	20	1.11
	2	10	
	3	10	
Heat-Treated	4	<10	<1.00
	5	<10	
	6	<10	

CONCLUSIONS

The heat treatment of Saku Salmon at 176°F/80°C for 120 minutes yielded a minimum reduction of 7.15 log CFU/g for the inoculated *Listeria monocytogenes* population. Based on these validation study results, the heat treatment process achieved the 6- log reduction requirement at a production load of 50 pieces per batch using the Sheldon Dry Heat Oven.

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Bucur FI, Grigore-Gurgu L, Crauwels P, Riedel CU, Nicolau AI. Resistance of *Listeria monocytogenes* to Stress Conditions Encountered in Food and Food Processing Environments. Front Microbiol. 2018 Nov 13;9:2700. doi: 10.3389/fmicb.2018.02700. PMID: 30555426; PMCID: PMC6282059.

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J.E. Gaze, G.D. Brown, D.E. Gaskell, J.G. Banks, Heat resistance of Listeria monocytogenes in homogenates of chicken, beef steak and carrot, Food Microbiology, Volume 6, Issue 4, 1989, Pages 251-259, ISSN 0740-0020, https://doi.org/10.1016/S0740-0020(89)80006-1

Martin B Cole, Kenneth W Davies, Gary Munro, Caroline D Holyoak, Derrick C Kilsby, A vitalistic model to describe the thermal inactivation of *Listeria monocytogenes*, *Journal of Industrial Microbiology*, Volume 12, Issue 3-5, 1 September 1993, Pages 232–239, https://doi.org/10.1007/BF01584195

M.Ellin Doyle, Alejandro S. Mazzotta, Tim Wang, Dana W. Wiseman, Virginia N. Scott, Heat Resistance of Listeria monocytogenes, Journal of Food Protection, Volume 64, Issue 3, 2001, Pages 410-429, ISSN 0362-028X, https://doi.org/10.4315/0362-028X-64.3.410

McDermott A, Whyte P, Brunton N, Bolton DJ. Thermal Inactivation of Listeria monocytogenes in Crab Meat. J Food Prot. 2018 Dec;81(12):2003-2006. doi: 10.4315/0362-028X.JFP-18-276. PMID: 30476441.

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US Food and Drug Administration (2022) Bacteriological Analytical Manual (BAM) Chapter 10 for environmental surface sponges and swabs.

US Food and Drug Administration Fish and Fishery Products Hazards and Controls Guidance (4th edition, June 2022). SKU: SGR 129

Shi, Y., Tang, J., Yue, T., Rasco, B., & Wang, S. (2015). Pasteurizing Cold Smoked Salmon (*Oncorhynchus nerka*): Thermal Inactivation Kinetics of *Listeria monocytogenes* and *Listeria innocua*. *Journal of Aquatic Food Product Technology*, *24*(7), 712–722. https://doi.org/10.1080/10498850.2013.808303

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Received: 27 March 2025
Responded: 2 April 2025

Overview

This document responds to the request for clarification regarding CCC 000005 transmitted by FDA to Wildtype on 27 March 2025. For ease of reference, FDA's original questions are reproduced in black text and Wildtype's responses appear below in blue text.

Requests for Clarification

1. In the January 24, 2024, amendment you state (page 6):

"For the harvested cell material, we will follow the same testing frequency for the first six months of commercial production. After six months, if there is no material discrepancy between test results for the harvested cell material and test results for finished food products, then we would consider testing of the finished food products to be sufficient to detect contamination events that were present at the point of harvest. Following the six-month period, Wildtype will routinely test the harvested cell material for all of the potential adventitious agents listed below at least quarterly to validate efficacy of controls. If this frequency is changed, we will submit a supplement to FDA."

In the August 30, 2024, amendment you state (page 23):

"No changes have been made to the testing strategy outlined in our January 24, 2024 amendment beyond the addition of an *E. coli* O157 panel to our standard testing scope as described in our response to question 10 above. No other material changes have occurred."

Based on the statements provided in the January 24, 2024, amendment it is unclear if, after the stated 6-month monitoring period, you intend to cease testing every batch of the harvested cell material for the presence of adventitious agents. We note that this would be an unacceptable strategy as ongoing batch testing is an important element to ensure safety of the harvested cell material, as this is, for example, listed as a critical control point in your HACCP plan in Appendix 6 on page 54 of the August 30, 2024, amendment to the DSN. Further, the testing frequency listed in Figure 4 on page 17 of the August 30, 2024, is "Every batch." The absence of detectable adventitious agents in the harvested cell material after a six-month testing period is not an appropriate reason to discontinue batch testing.

We would expect that this testing frequency would continue beyond six months, for every batch of harvested cell material produced. For the administrative record, please confirm that you will commit to testing each batch of harvested cell material for the presence of adventitious agents beyond six months. As a reminder, the scope of the consultation ends during harvest, and the subject of the consultation is the harvested cell material not the final food product. We acknowledge your commitment that, when modifications may be made to the specifications for the harvested cell material, you will notify FDA in a supplement.

Wildtype commits to testing each batch of harvested cell material for the presence of adventitious agents beyond six months. If there is evidence to support a change to testing specifications of the harvested cell material, we will notify FDA in a supplement.

2. On page 5 of the January 24, 2024, amendment, you state "We will test every batch of the harvested cell material as defined by FDA (cells harvested from bioreactors prior to freezing) for the presence of toxic heavy metal contaminants for a period of six months." It is our understanding that you intend to

test for the presence of toxic heavy metals (i.e., lead, cadmium, arsenic, mercury) for every batch of harvested cell material produced, and we would expect that this testing frequency would continue beyond six months, for every batch of harvested cell material produced. For the administrative record, please confirm that you will commit to testing each batch of harvested cell material for the presence of toxic heavy metals (i.e., lead, cadmium, arsenic, mercury) beyond six months. As a reminder, the scope of the consultation ends during harvest, and the subject of the consultation is the harvested cell material not the final food product.

Wildtype commits to testing each batch of harvested cell material for the presence of toxic heavy metals (i.e. lead, cadmium, arsenic, mercury) beyond six months. If there is evidence to support a change to testing specifications of the harvested cell material, we will notify FDA in a supplement.

3. On page 10 of the March 27, 2022, submission for the DSN, you state that isolated mesenchymal cells are "... characterized with respect to general shape (cellular morphology), proliferative capacity, genetic stability over the course of multiple generations, and gene expression patterns." For addition to the DSN, please list the method used to assess "genetic stability" (e.g., karyotyping, whole genome sequencing (WGS) of cells from multiple passages). Further, please discuss assay results which would indicate genetic instability and the controls, if any, that are in place if altered genetic stability of the cell line is detected.

Wildtype assesses genetic stability using exome sequencing and gene expression data for a selected panel of genes. Baseline exome sequencing and gene expression data were obtained for Wildtype's production cell line in 2020; this serves as the control. Re-sequencing and gene expression analysis of Wildtype's production cell line in subsequent years from multiple passages has revealed no changes in DNA sequence or gene expression levels.

The company predominantly relies upon phenotypic monitoring to assess culture stability, which is discussed at length in response to Question 13 (p. 17 and 18) of the January 17, 2023 amendment. In the event that phenotypic changes were observed, exome sequencing and gene expression analysis would be repeated. The presence of *de novo* changes in DNA sequence or the observation of statistically significant changes in gene expression levels would result in culture termination and initiation of the seed train from another frozen cell bank.

4. On page 12 of the March 27, 2022, submission to the DSN, you state, "Wildtype has also completed DNA and RNA sequencing analyses of its cell lines for a complete characterization of all expressed genes." For addition to the DSN, please information about this statement as follows:

1. State the type of DNA sequencing assay (e.g., targeted sequencing, exome sequencing, WGS) that was performed during cell line characterization. For the administrative record, please explain whether the results of the DNA sequencing analyses were used to support your conclusion regarding cell line species identity and genetic stability.

2. Describe the results and interpretation of the cell line RNA sequencing, including information about whether differential gene expression analyses were used to verify the differentiation potential of cells prior to cell banking.

1. Cell line species identity is discussed in response to questions 5 and 6 below. With respect to genetic stability, Wildtype has performed DNA sequencing of its production cell line using exome sequencing. Neither exome sequencing nor RNA sequencing of expressed genes (as noted above) have revealed the presence of *de novo* changes in DNA sequence or gene expression levels across multiple passages.

These consistent findings, along with the phenotypic characteristics described in our response to question 3 above, as well as consistent nutritional composition analyses of non-sequential harvested cell material lots (submitted in prior RFI responses) span multiple cell line passages and collectively support the company's assessment of the cell line's genetic stability.

2. Both RNA sequencing and quantitative PCR (qPCR) have been used to characterize gene expression. The gene expression profile of Wildtype's production cell line has demonstrated expression of myogenic genes (such as MyoD, Myf5, MyoG, Pax7) and adipogenic genes (such as C/EBPβ) that are elevated when compared to a control cell line. Taken together, these results imply that this cell line is of the mesenchymal lineage and served to verify the differentiation potential of cells prior to cell banking. These gene expression patterns were documented as baseline characteristics for the cell line. Consistent gene expression patterns have been confirmed in several subsequent RNA sequencing analyses over the course of 4 years, demonstrating stability of gene expression networks over multiple passages.

5. On pages 1-2 of the July 28, 2023, amendment to the DSN you state, "… before submitting a vial to Wildtype's master cell bank, species confirmation via genetic barcoding or confirmation by cytochrome C oxidase I polymerase chain reaction (PCR) amplification must be performed on DNA extracted from Wildtype cell line candidates." For addition to the DSN, please clarify whether the DNA barcoding system used by Wildtype to confirm the species identity of the master cell bank uses the *cytochrome oxidase subunit 1* mitochondrial gene or a different target region of the genome.

The DNA barcoding system used by Wildtype to confirm the species identity of the master cell bank uses the *cytochrome c oxidase subunit 1* mitochondrial gene to confirm species identity.

6. For addition to the DSN, please provide information about the identity of the harvested cell material with respect to species and cell type as follows:

1. A statement clarifying whether you perform analytical testing (e.g., PCR of the *cytochrome oxidase subunit 1* gene and Sanger sequencing) to confirm the species identity of the harvested cell material. If such testing is performed, please name the assay and provide a summary of the test results.

2. A statement about the identity of the cultured cells at harvest (i.e., cell types present in the harvested cell material). Please explain whether you perform analytical testing (e.g.,quantitative reverse transcription PCR (RT-qPCR) for gene expression analysis, immunofluorescence staining of proteins expressed in specific cell types) to characterize the harvested cell material with respect to cell type or differentiation state. If such testing is performed, please name the assay, and provide a summary of the test results. If such testing is not performed, please provide a statement addressing whether it is an issue if Wildtype does not verify the cell type or differentiation state of the cells at harvest.

1. As noted in the disclosable safety narrative, species identity of Wildtype's production cell line is confirmed by regular testing of harvested cell material in the company's GMP facility using PCR and Sanger sequencing of the *cytochrome c oxidase subunit 1* mitochondrial gene. Test results have invariably confirmed that the species identity is *Oncorhynchus kisutch* (Pacific coho salmon).

2. Wildtype does not routinely perform genetic or immunohistochemical testing to characterize the gene expression profile of the harvested cell material. The company confirms cell type and differentiation state by phenotypic inspection (i.e. cell growth rate, size, and morphology) during

sampling and the harvest process. Specifically, cell growth rate is quantified during each production run as viable cell density over time, and compared to historical trends (see pg. 17 of our January 17, 2023 amendment). Cell size is also quantified during sampling, with a typical diameter range of 11-18µm. Wildtype's production cells are uniform, spherical, and do not display membrane protrusions or irregularities observed in other cell lines.

Significant deviations in growth rate (±35% or more from the average expected growth rate), cell diameter (±25%), or cell shape (observable differences) would lead to a deviation and root cause analysis as described in Wildtype's quality documents. To date, all cell material harvests produced in our GMP facility have demonstrated consistent phenotypic characteristics including growth rate, size, and shape.

Given the baseline expression of mesenchymal lineage genes (as described in the response to Question 4 above), the theoretical potential for differentiation into these cell types exists. This is unlikely for several reasons. First, differentiation is typically associated with proliferative arrest, which would be self-limiting in cell culture. Differentiation would result in a slowed growth rate that would be readily detected, as noted above. Second, differentiation is a complex process that depends upon defined sequences of gene expression changes, a conducive extracellular substrate, and often a nutrient media that promotes differentiation; none of these are part of Wildtype's production process, and the company has not observed spontaneously differentiated cells in any of its harvested cell material to date. Finally, even if the company's production cells were to undergo spontaneous differentiation, all possible differentiation lineages for this mesenchymal cell line (i.e. fat, muscle, connective tissue) are regularly found in conventional salmon.

7. On page 10 of the March 27, 2022, submission to the DSN, you state, "Given that the target cell characteristics in the final product are those of muscle, fat, or connective tissue, isolated cells are first selected by attachment proclivities (i.e., affinity for structural proteins such as laminin, fibronectin, gelatin, etc.) and ability to thrive in various nutrient formulations. These attachment affinities and nutritional requirements predispose cells to have the capability of becoming muscle, fat, and connective tissues." For addition to the supplemental, confidential material (SCM), please provide a discussion regarding whether you expect these structural proteins, which coat the surfaces of the cell culture vessels, to be present in the banked cells. For addition to the SCM, please provide additional information regarding the structural proteins used during cell line development, including the identity of the proteins, species of origin of the amino acid sequence of the proteins, and the estimated daily intake (EDI) of the proteins in the harvested cell material. Please note, the EDI for substances used solely during the cell line development phase of the process may be theoretical estimates based on dilution arguments.

Animal-derived proteins such as those described above have been used to select cell lines based upon attachment proclivities, but the Coho salmon cell line currently used in production was created and selected under conditions that did not involve the use of animal-derived structural proteins or other animal-derived selection agents. In other words, Wildtype's production cell line was cultured directly in uncoated tissue culture vessels from the time of isolation. Therefore, these proteins are not present in banked cells. If a cell line developed with animal-derived affinity selection components is used in future production, the company will notify FDA in a supplement.

8. On page 6 of the June 3, 2022, submission to the SCM you list multiple animal-derived inputs, including trypsin, insulin, and "USDA Approved Origin Fetal Bovine Serum," as substances used during the production process. On page 4 of the July 23, 2023, amendment to the DSN, you informed FDA that "Since submission of CCC 000005, optimization of Wildtype's cell culture media formulation has resulted

in the discontinuation of insulin and fetal bovine serum," but does not address trypsin. For addition to the SCM, please clarify whether you employed animal-derived reagents (e.g., fetal bovine serum, bovine serum albumin, trypsin) to establish the cell lines used to manufacture the harvested cell material. If animal-derived reagents are employed during cell line establishment, please note that such substances must conform with 21 CFR 189.5, prohibited cattle materials, and provide a statement, for addition to the DSN, affirming that all applicable material inputs used are in conformance with the regulation.

Animal-derived reagents (fetal bovine serum, bovine serum albumin, and porcine trypsin) were used early in the cell line establishment stage and have not been used in subsequent stages of our process. Wildtype confirms that we do not use materials prohibited under 21 CFR 189.5, prohibited cattle materials, in any part of our process. All applicable material inputs used are in conformance with the regulation.

As discussed in the disclosable safety narrative (e.g., pgs 25 and 51) as well as subsequent amendments (e.g., August 30, 2024 amendment, pgs 20, 49), Wildtype maintains a number of supply chain preventive controls, including a supplier approval program. While not used in our production process, Wildtype has additional controls in place for animal-derived products used in the cell line establishment stage, including requiring vendors to provide statements that these products have tested negative for relevant pathogens. None of these animal-derived inputs were created using recombinant technologies. As the certificates of analysis included below in Appendix 1 demonstrate, animal-derived components are tested extensively for potential pathogens and are certified to not contain pathogens, including those causing Bovine Spongiform Encephalopathy (BSE).

Appendix 1: Certificates of Analysis for animal-derived products

VWR Seradigm LIFE SCIENCE

Certificate of Analysis and Origin

Catalog Number: 89510-182, 89510-186
Heat Inactivated: 89510-184, 89510-188
When additional processing is requested, original catalog # will appear on product along with heat inactivation sticker. VWR product # will be amended to reflect processing.

Material description:	**Fetal Bovine Serum**
Grade:	**USDA Approved Origin**
Lot Number:	**262B18**
Total Volume:	**485.0 Liters**
Date of Manufacture:	**19 September 2018**
Expiration Date:	**October 2023**
Date Released:	**01 November 2018**
Origin:	**Collected in Mexico and Processed in USA**
Filtration:	**Triple 0.1µm Sterile Filtered**
Storage:	**-10° to -20°C**

Certified Analysis

Test/Method	Unit of Measure	Specification	Result
Endotoxin (USP 85)	EU/mL	≤30	1.82
Hemoglobin (Fleming & Woolf)	mg/dL	≤30	17.21
Sterility (Current USP and EP 2.6.1 for Bacteria & Fungi)	N/A	No Growth	No Growth
Mycoplasma (Barile & Kern; Large Volume, Direct Culture)	N/A	Not Detected	Not Detected
pH (USP 791)	N/A	Test & Report	7.23
Osmolality (USP 785)	mOsm/KgH20	Test & Report	311
Virus Testing (9 CFR 113.53c)			
Bluetongue	N/A	Tested	Not Detected
Bovine Adenovirus	N/A	Tested	Not Detected
Bovine Parvovirus	N/A	Tested	Not Detected
Bovine Respiratory Syncytial Virus	N/A	Tested	Not Detected
Bovine Viral Diarrhea Virus	N/A	Tested	Not Detected
Rabies	N/A	Tested	Not Detected
Reovirus	N/A	Tested	Not Detected
Cytopathogenic Agents (IBR)	N/A	Tested	Not Detected
Hemadsorbing Agents (PI3)	N/A	Tested	Not Detected

Biochemical Assay

Test/Method	Unit of Measure	Result	Test/Method	Unit of Measure	Result
Albumin	g/dL	2.2	Phosphorus	mg/dL	10.3
Alkaline Phosphatase	U/L	196	Potassium	mEq/L	>10.0
ALT (SGPT)	U/L	8	Protein, Total	g/dL	3.5
AST (SGOT)	U/L	82	Sodium	mEq/L	134
Bilirubin – Total	mg/dL	0.3	Triglycerides	mg/dL	68
Calcium	mg/dL	13.6	Urea Nitrogen (BUN)	mg/dL	16
Chloride	mEq/L	98	Uric Acid	mg/dL	2.8
Cholesterol - Total	mg/dL	28	**Electrophoretic Profile**		
Creatinine	mg/dL	2.7	Alpha 1 & 2	g/dL	1.2
GGT	U/L	4	Beta 1 & 2	g/dL	0.2
Glucose	mg/dL	117	Gamma 1	N/A	0.1
HDL Cholesterol	mg/dL	8			
Iron, Total	ug/dL	170			
LDL Cholesterol	mg/dL	18			

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VWR International bvba/sprl, Haasrode Research Park Zone 2020, Geldenaaksebaan 464, 3001 Leuven, Belgium
http://www.vwr.com/seradigm Technical phone: 866-508-7315 Technical email: SeradigmTechnicalSupport@vwr.com

WILDTYPE

VWR Seradigm LIFE SCIENCE

Certificate of Analysis and Origin

Statements

Statement of Origin: This product was manufactured from fetal bovine whole blood collected exclusively from USDA approved countries of origin. All fetal bovine serum used in this product is derived from fetuses collected from cows that are of Mexico origin and have passed ante- and post-mortem inspection. All collection and processing activities are performed under strict guidance of standard operating procedures.

Statement of Intended Use: This product is intended for further manufacturing or research use. This product is not intended for human or therapeutic use. Not for human or animal consumption.

ISIA Certified Traceability: All raw serum is certified by the International Serum Industry Association (ISIA) to be sourced in accordance with their strict traceability guidelines (www.serumindustry.org).

ISIA Compliant Documentation: This document complies with all documentation standards issued by the ISIA regarding the definition, quality control, country of origin and certified analysis of fetal bovine serum (www.serumindustry.org).

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) STATEMENT
Seradigm certifies that this product does not contain, and is not derived from, specified risk material as defined in Commission Decision 97/534/EC. The Commission Decision defines specified risk material of bovine origin as: the skull, including the brain and eyes, tonsils and spinal cord of bovine animals aged over 12 months.

Bovine spongiform encephalopathy cannot be removed using collection or filtration methods. No assays are available to detect prions in blood products, there preventing any inactivation processes from being performed that would guarantee bovine blood to be prion-free. The European Pharmacopeia (Ph.Eur. 2002, 5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products) and the World Health Organization both assign fetal bovine serum a Category iV "no detectable infectivity" classification, a designation of least amount of risk.

OIE Resolution No. 20, issued May 2013, upgraded the United States' risk status classification for BSE to "negligible risk".

Signed on behalf of VWR:

John Manley
Quality Manager

WILDTYPE

Mediatech, Inc.
A Corning Subsidiary

Certificate of Analysis

Description:	**0.05% Trypsin, 0.53 mM EDTA, 1X** [-] sodium bicarbonate	**Catalog #:**	25-052-CI
		Lot #:	06221005
		Expiration Date:	2022-03
Storage:	**-25 to - 15°C**	**Country of Manufacture:**	USA

Notes:

Mediatech, Inc. products are prepared by a validated aseptic sterile filtration process to ensure that all products comply with the industry's Sterility Assurance Level (SAL) of at least 10^{-3} (i.e. demonstrating that a product produced in this manner has no more than 1 random contaminant per 1000 units).

This product was tested in accordance with currently approved Mediatech, Inc. specifications and procedures. Testing procedures are maintained in compliance with the current versions of the USP and/or EP, where applicable.

For Research or Further Manufacturing Use Only Not for use in Diagnostic Procedures. Utilization of this product apart from the labeled intended use may be a violation of local and/or Federal Law.

TEST PARAMETERS	SPECIFICATION	RESULT
pH	7.6 ± 0.4	7.6
Osmolality	275 ± 20 mOsm/Kg H_2O	275 mOsm/Kg H_2O
Sterility	Pass	Pass
Trypsin Activity VERO MRC-5	Pass	Pass
Mycoplasma	Tested Negative (trypsin raw material lots)	Negative
Porcine Parvovirus and Circovirus (9CFR testing)	Not detected (trypsin precursor lots)	Negative
E-beam Irradiation	Minimum of 25 kGy (trypsin precursor lots)	Pass

For further information, contact Corning Scientific Support at 800-492-1110 or ScientificSupport@corning.com.

Following signatures indicate the above material has met all quality specifications and has been reviewed by a Quality representative.

Written By/Date: VR 06 OCT 2021 Reviewed By/Date: [redacted] 06 OCT 2021

CORNING

Mediatech, Inc.
A Corning Subsidiary
9345 Discovery Boulevard
Manassas,VA 20109
1-800-492-1110
www.corning.com

Received: 11 April 2025
Responded: 11 April 2025

Overview

This document responds to the request for clarification regarding CCC 000005 transmitted by FDA to Wildtype on 11 April 2025. For ease of reference, FDA's original questions are reproduced in black text and Wildtype's responses appear below in blue text.

Requests for Clarification

1. On page 7 of the April 2, 2025, amendment to the DSN, you provide a certificate of analysis (COA) for fetal bovine serum which includes the disclaimer "Statement of Intended Use: This product is intended for further manufacturing or research use. This product is not intended for human or therapeutic use. Not for human or animal consumption." For the administrative record, please provide a statement acknowledging this language and justify why it may or may not be relevant considering the additional work you have done to characterize your cell line and control any potential hazards introduced during your manufacturing process.

We acknowledge this language and confirm that Wildtype has complied with applicable requirements. All animal-derived products were used during the cell line establishment phase and were discontinued years ago, ensuring these products are not found in the harvested cell material. Additional controls are in place to mitigate potential hazards. First, animal-derived components were either received sterile from the vendor or sterilized via filtration prior to use in cell culture. Second, as discussed in our 2 April 2025 amendment, Wildtype maintains a number of supply chain preventive controls, including a supplier approval program, ensuring components are free from relevant animal-borne pathogens prior to use. For example, COAs are verified prior to release to ensure vendors complete standard testing and quality control. Third, as discussed throughout the disclosable safety narrative, aseptic technique, regular sterile-filtration in cell culture, and characterization of our cell lines (discussed in the 2 April amendment) adequately manage the risk of contamination from animal-derived components used during our cell-line establishment phase.

2. On page 5 of the April 2, 2025, amendment to the DSN, you state, "Animal-derived reagents (fetal bovine serum, bovine serum albumin, and porcine trypsin) were used early in the cell line establishment stage and have not been used in subsequent stages of our process." On pages 6-8 you provide COAs for fetal bovine serum and porcine trypsin. For addition to the DSN, please provide a COA for bovine serum albumin.

A COA for bovine serum albumin is attached below in Appendix 1. As noted in the COA, relevant pathogenic testing including for Vesicular Stomatitis Virus (VSV) and Bluetongue (BT) virus was carried out prior to shipment, as well as heat treatment at 65° C for 3 hours.

3. The COA for porcine trypsin on page 8 of the April 2, 2025, amendment to the DSN lists "Porcine Parvovirus and Circovirus (9CFR testing)" under "Test Parameters." For the administrative record, please clarify whether the reference to "9CFR testing" refers to 9 CFR § 113.53 (d) "Requirements for ingredients of animal origin used for production of biologics."

We confirmed verbally with the vendor on 11 April 2025 that the reference to "9CFR testing" refers to 9 CFR § 113.53 (d) "Requirements for ingredients of animal origin used for production of biologics."

Appendix 1: Certificates of Analysis for animal-derived products

Sigma-Aldrich.

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Bovine Serum Albumin - heat shock fraction, protease free, pH 7, ≥98%

Product Number:	**A3294**
Batch Number:	**SLCN4832**
Brand:	SIGMA
CAS Number:	9048-46-8
MDL Number:	MFCD00130384
Formula Weight:	66,000 g/mol
Storage Temperature:	Store at 2 - 8 °C
Quality Release Date:	03 JUN 2022
Recommended Retest Date:	JUN 2027

Test	Specification	Result
Appearance (Color)	White to Light Yellow to Light Brown	Faint Beige
Appearance (Form)	Powder	Powder
Solubility (Color)	Very Faint Green-Yellow to Green-Yellow to Yellow	Faint Yellow
Solubility (Turbidity) 40 mg/mL, H2O	Clear to Slightly Hazy	Clear
Identity	Bovine Origin	Conforms
Agarose Electrophoresis	≥ 98 %	100 %
Nitrogen	14.5 - 16.5 %	15.8 %
pH 1% in 0.15 M NaCl	6.5 - 7.5	6.8
Loss on Drying	≤ 5 %	1 %
VSV and BT Virus	None Detected	None Detected
Inactivation Process pH not more than 5.0 for at least 2 hours; temperature not less than 65 deg C for at least 3 hours	Conforms	Conforms
Purification Method Heat Shock Fractionation	Conforms	Conforms
Protease by FITC	None Detected	None Detected

WILDTYPE

Sigma-Aldrich.

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Number: A3294
Batch Number: SLCN4832

Test	Specification	Result
Chapter 4(D) - Heat Product meets European Union requirements for treated technical blood products. Form of treatment heat treated at 65 deg C for 3 hours.	----------	----------

Brian Dulle, Supervisor
Quality Assurance
St. Louis, Missouri US