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3 **Method Name:** Determination of Phospholipids in Infant and Adult/ Pediatric
4 Nutritional Formula

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7 **Approved by:** Stakeholder Program for Infant Formula and Adult Nutritionals

8 **Final version date:**

9 **Effective date:**

10
11 **Intended Use:** Reference method for dispute resolution.

12
13 **1. Applicability:**

14 Quantitative determination of nutritionally relevant total and individual classes of
15 phospholipids (PL) including phosphatidylcholine (PC), phosphatidylethanolamine (PE),
16 phosphatidylinositol (PI), phosphatidylserine (PS), and sphingomyelin (SM), in infant
17 formula and adult nutritionals.

18
19 **2. Analytical Technique:**

20 Any analytical technique that meets the following Method Performance Requirements is
21 acceptable.

22
23 **3. Definitions:**

24 **Total Phospholipids**

25 For the purposes of this SMPR, total phospholipids for nutritional purposes are calculated as
26 the sum of PC, PE, PI, PS, SM.

27
28 **Accuracy¹**

29 The closeness of agreement between the average of an infinite number of replicate
30 measured quantity values and a reference quantity value.

31
32 **Adult/Pediatric Formula**

33 Nutritionally complete, specially formulated food, which may constitute the sole source of
34 nourishment, made from any combination of milk, soy, rice, whey, hydrolyzed protein,
35 starch, and amino acids, with and without intact protein.

36
37 **Infant formula**

38 Breast-milk substitute specially manufactured to satisfy, by itself, the nutritional
39 requirements of infants during the first months of life up to the introduction of appropriate
40 complementary feeding², made from any combination of milk, soy, rice, whey, hydrolyzed
41 protein, starch, and amino acids, with and without intact protein.

42

¹ Corresponds to the VIM definition for “trueness”.

² Codex Standard 72 – 1981.

43 **Limit of Quantitation (LOQ)**

44 The minimum concentration or mass of analyte in each matrix that can be reported as a
45 quantitative result.

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47 **Recovery**

48 The fraction or percentage of spiked analyte that is recovered when the test sample is
49 analyzed using the entire method.

50
51 **Repeatability**

52 Variation arising when all efforts are made to keep conditions constant by using the same
53 instrument and operator and repeating during a short time period. Expressed as the
54 repeatability standard deviation (SD_r); or % repeatability relative standard deviation
55 (% RSD_r).

56
57 **Reproducibility**

58 The standard deviation or relative standard deviation calculated from inter-laboratory data.
59 Expressed as the reproducibility relative standard deviation (SD_R); or % reproducibility
60 relative standard deviation (% RSD_R).

61

62 **4. Method Performance Requirements:**

	PC	PE	PI	PS	SM	Total
Analytical range* (mg/100g RFP)	0.4–50	0.4–44	0.4–44	0.4–44	0.4–44	0.4–226
Limit of Quantitation* (LOQ)	0.3	0.3	0.3	0.3	0.3	N/A
Recovery (%)	90–110	90–110	85–115	85–115	90–110	90–110
Repeatability (RSD, %)	7	7	7	7	7	7
Reproducibility (RSD_R , %)	14	14	14	14	14	14
* Concentrations apply to: a) “ready-to-feed” liquids “as is”; b) re-constituted powders (25 g into 200 g of water); c) liquid concentrates diluted 1:1 by weight. $1 \text{ mg}/100\text{g RFP} \approx 1\text{mg}/100\text{g dry weight} \times 25\text{mL}/(25 + 200 \text{ mL}).$						

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64 **5. System suitability tests and/or analytical quality control:**

65 Suitable methods will include blank check samples, and check standards at the lowest point
66 and midrange point of the analytical range.

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68 **6. Reference Material(s):**

69 No infant formula certified reference materials available.

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71 **7. Standard Material(s):**

72 As part of validation studies, the source of PL standard needs to be provided and its
73 composition specified to address how the standard relates to forms measured in samples.

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- 75 **8. Validation Guidance:**
76 Recommended level of validation: *Official Methods of Analysis*SM.
77
78 **9. Maximum Time-To-Result:**
79 No maximum time.

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