National Institutes of Health Standard For Nutrient and Chemical Contaminant Analyses of Laboratory Animal Diets

1. Scope:

1.1 This specification covers nutrient and chemical contaminant analyses on samples of laboratory animal diet and bedding purchased under NIH contracts.

2. REQUIREMENTS

2.1 Laboratory analyses to be performed.

Nutrient and Contaminant Analyses - Diet and bedding samples may be analyzed for all nutrients, required by the animal, and/or contaminants stipulated in the NIH specifications. The analyses will be performed in accordance with the most recent issue of Official Methods of Analyses of the Association of Official Analytical Chemists (A.O.A.C.) or other officially approved analytical methods as cited below. Methods of analyses will not be altered without a written request from the Contractor and written approval from the NIH Contracting Officer, Project Officer and Laboratory Animal Nutritionist.

Analyses Methodology

Samples shall be collected using sterile gloves and/or apparatus. Submitted diet and bedding samples shall be homogenized by the laboratory then the homogenized sub-samples shall be used for nutrient and contaminant analyses. The analyzed diet sample will be a representative sample of the diet sample submitted by the NIH. The results of each nutrient and contaminant assay will be validated using industry-wide acceptable quality assurance measures. Final approval for all quality assurance methods shall be made by the NIH Project Officer.

Diet samples shall be subjected to the following analyses in accordance with the indicated procedure.

Nutrient	Method	Description
1. Total Protein	976.05* 976.06 988.05 968.06	Kjeldahl; Dumas

2. Total Fat	920.39 922.06	Ether Extract Acid Hydrolysis
3. Crude Fiber	962.09	Digestion: Gravimetric
4. Ash	942.05	Muffle Furnace
5. Moisture	934.01	Vacuum Oven
6. Essential Fatty Acids(Linoleic and Linolenic Acids)	972.28 963.22 954.02	Acid Hydrolysis GLC
7. Calcium	935.13 965.09	Atomic Absorption
8. Phosphorous	964.06	Wet Chemical
9. Potassium	983.02	Emission Spectroscopy
10. Sodium	973.54	Emission Spectroscopy
11. Magnesium	965.09	Atomic Absorption
12. Manganese	965.09	Atomic Absorption
13. Iron	965.09	Atomic Absorption
14. Zinc	965.09 986.15	Atomic Absorption
15. Copper	965.09	Atomic Absorption
16. Molybdenum	935.14	Elmslie-Caldwell Method
17. Iodine	934.02	Knapheide-Lamb Method
18. Chloride	943.01 969.10	Titrimetric and Potentiometric Methods
19. Vitamin A	974.29 J. Agri. Food Chem., 1991	Chemical: Column Chromatography,and HPLC

Spectrophotometric;

20. Vitamin D	982.29	HPLC
21. Vitamin E	971.30	Chemical: Column Chromatography and Spectrophotometric
22. Thiamine	942.23 953.17	Chemical: Microflurometric
23. Riboflavin	970.65 981.15 940.33	Microbiological: Turbidimetric Lactobacillus casei
24. Niacin	961.14 944.13	Microbiological: Turbidimetric Lactobacillus plantarum
25. Pantothenic Acid	945.74	Microbiological: Turbidimetric Lactobacillus plantarum
26. Choline	Horowitz and Beadle, JBC 150:325, 1943	Microbiological: Turbidimetric Nerospora crassa
27. Pyridoxine	961.15	Microbiological: Turbidimetric Saccharomyces uvarum carlsbergensis
28. Total Folic Acid	944.12	Microbiological: Turbidimetric Streptococcus faecalis
29. Biotin	Difco Manual, 9th Ed., p.165 (1964) adpt. Sketts	Microbiological: Turbidimetric Lactobacillus plantarum
30. Vitamin C	984.26	Chemical: Microfluorometric
31. Vitamin B12	952.20	Microbiological:

		Turbidimetric Lactobacillus leichmanni
32. Arginine	982.30	Chemical, Chromatographic
33. Histidine	982.30	Chemical, Chromatographic
34. Isoleucine	982.30	Chemical, Chromatographic
35. Leucine	982.30	Chemical, Chromatographic
36. Lysine	982.30	Chemical, Chromatographic
37. Methionine	982.30	Chemical, Chromatographic
38. Cystine	982.30	Chemical, Chromatographic
39. Phenylalanine	982.30	Chemical, Chromatographic
40. Tyrosine	982.30	Chemical, Chromatographic
41. Threonine	982.30	Chemical, Chromatographic
42. Tryptophan	982.30	Chemical, Chromatographic
43. Valine	982.30	Chemical, Chromatographic

Contaminant	Method	Description
1. Chlorinated Hydrocarbon Pesticides	970.52 983.21 984.21	Gel Permeating Chromatography
2. Polychlorinated biphenyls (PCBs)	983.21	GLC

3. Organo-phosphate Pesticides	968.24 970.52 970.53 974.22	GLC
4. Malathion	968.24 970.52 970.53 974.22	GLC
5. Lead	972.25 986.15	Atomic Absorption Spectrophotometry
6. Arsenic	986.15	Atomic Absorption Spectrophotometry
7. Cadmium	986.15 973.34	Atomic Absorption Spectrophotometry
8. Mercury	971.21	Atomic Absorption Spectrophotometry
9. Aflatoxins	970.43 975.35 975.36 977.16	TLC Romer minicolumn method
10. Nitrate	986.31	Potentiometric
11. Deoxynivalenol	970.43 986.17 986.18	TLC; GLC
12. Fumonisin	970.43	GLC
13. Zearalenone	976.22 985.18	GLC
14. Nitrosamines	991.28	GLC
15. Chitin	945.75 970.66 969.46	Filtration
16. Salmonella	967.25 967.26 989.12 989.14 989.15 991.13	Microbiological

17. E. Coli	991.14 992.30 983.25 990.11	Microbiological
18. Selenium	974.15	Flourometric
19. Mold	937.13 982.33 974.13 984.29	Microbiological

* The numbers indicate AOAC method

2.2 Completion Time for Assays- All specified nutrient and contaminant analyses shall be completed within 10 working days after the feed samples are received by the laboratory.

2.3 Laboratory Records- All data associated with the analyses herein specified shall be retained by the laboratory for at least two years. These data shall be made available to the NIH Project Officer upon request. The laboratory shall maintain a log with a complete identification of the samples. The log shall include the following information: date each sample is received; product identification; manufacturing date; NIH stock number; name of the manufacturer, and; the assay results.

2.4 Reporting Results - Analyses shall be made in writing to the NIH Project Officer within 24 hours after all analyses are complete. Report shall include, date of the report, sample ID, laboratory ID, and analytical results including units. The results are the property of the U. S. Government. The laboratory is not authorized to make results available to anyone but the NIH Project Officer without written permission from the NIH Project Officer.

2.5 Contractor's Restrictions - To be eligible for award of a contract the contractor shall have the capabilities to perform nutrient and contaminant analyses as specified herein. The Contractor may subcontract 15% of the services upon approval of the Project Officer. The Contractor must provide evidence that the Subcontractor is capable of performing the analyses using approved analytical methods conforming to NIH standards. Any organization either directly or indirectly associated with an NIH animal feed or bedding contractor through a corporate or any other structure will not be eligible for award of a contract to perform these services.

3.0 Personnel

3.1 Each individual engaged in the conduct of or responsible for the supervision of feed and bedding analyses shall have education, training, and experience, or combination thereof, to enable that individual to perform the assigned functions. A current summary of training and experience and job description for each individual engaged in or supervising the conduct of the feed analyses shall be maintained at the testing facility. There shall be a sufficient number of personnel for the timely and proper conduct of the analyses. Personnel shall take necessary personal sanitation and health precautions designed to avoid contamination of feed and bedding. Personnel engaged in feed and bedding analyses shall wear clothing appropriate for the duties they perform. Such clothing shall be changed as often as necessary to prevent microbiological, and/or chemical contamination of feed and bedding samples.

3.2 The testing facility shall identify a scientist or other professional of appropriate education, training and experience or combination thereof as the director of feed and bedding analyses. The director has overall responsiblity for the technical conduct of the analyses, documentation and reporting of results. The director shall assure that: (a) All results including unanticipated observations are accurately recorded and verified. (b) Unforeseen circumstances that may effect the quality and integrity of the analyses are noted and corrective action is taken and documented. (c) Analytical methods are as specified in the most current NIH Std. 5 or have been approved by the Project Officer. (d) All good laboratory practices specified in the most current NIH Std 5 are followed.

4.0 Quality Assurance

4.1 A testing facility shall have a quality assurance unit which shall be responsible for monitoring feed and bedding analyses to assure management that the facilities, equipment, personnel, methods, practices, records and controls are in compliance with the specifications of this contract. The quality assurance unit shall be entirely separate from and independent of the personnel engaged in the direction and conduct of the feed and bedding analyses.

4.2 The quality assurance unit shall: (a) Maintain copies of all protocols pertaining to the methods required to analyze the feed and bedding. (b) Inspect each analytical procedure at intervals adequate to assure the integrity of the analyses and maintain written and properly signed records of each periodic inspection showing the date of inspection, the

person performing the inspection, findings and problems, and action taken to resolve existing problems. (c) Submit to the director of feed and bedding analyses written status reports noting problems and corrective actions taken. (d) Determine that no deviation from approved analytical methods were made without proper authorization and documentation. (e) Prepare biannual inspection reports for the NIH specifying the dates inspections were performed and findings.

4.3 The NIH Project Officer or designated NIH representative shall have access to the written procedures established for the inspection and may request laboratory management to certify that inspections are being implemented, performed, documented and followed-up in accordance with this specification.

5.0 Facility

5.1 The testing facility shall be of suitable size and construction to facilitate the proper conduct of feed and bedding analyses. As necessary to prevent contamination or mixups, there shall be separate areas for receipt, storage and analyses of feed and bedding.

5.2 The facility shall be kept in a sanitary condition at all times.

6.0 Laboratory Operation- Standard Operating Procedures

6.1 The laboratory shall have standard operating procedures (SOP) in writing setting forth analytical methods that the NIH is satisfied are adequate to insure the quality and integrity of the data generated in the course of nutrient and contaminant analyses of the laboratory animal feed and bedding. All deviations in analytical methods from the SOPs shall be authorized by the director of laboratory animal feed and bedding analyses and shall be documented in the raw data. Significant changes in SOPs shall be properly authorized in writing by the director of feed and bedding analyses. The NIH Project Officer shall be notified within 24 hours of any changes in SOPs. The NIH Project Officer will make the final decision concerning any changes to analytical methodology.

6.2 SOPs shall be established for, but not limited to, the following:
(1) Receipt, identification, storage handling, and method of sampling of the laboratory animal feed and bedding.
(2) Laboratory tests.
(3) Data handling, storage, and retreival.
(4) Maintenance and calibration of equipment.

6.3 Each laboratory area shall have immediately available laboratory manuals and SOPs relative to the laboratory procedures being performed. Published literature may be used to supplement SOPs.

6.4 A historical file of SOPs, and all revisions thereof, including the dates of such revisions, shall be maintained. The Contractor shall submit SOPs to the Project Officer upon request.

7.0 Equipment

7.1 Equipment used in the performance of the nutrient and contaminant analyses of laboratory animal feed and bedding and equipment used for facility environmental control shall be of appropriate design and adequate capacity to function according to the protocol. The equipment shall be suitably located for operation, inspection, cleaning and maintenance.

7.2 Maintenance and Calibration of Equipment

(a) Equipment shall be adequately inspected, cleaned, and maintained. Equipment used for the assessment of data shall be adequately tested, calibrated and/or standardized.
(b) Written standard operating procedures, SOP, required under 6.2(4) shall set forth in sufficient detail the methods, materials, and schedules to be used in routine inspection, cleaning, maintenance, testing, calibration, and/or standardization of equipment, and shall specify, when appropriate, remedial action to be taken in the event of failure or malfunction of equipment. The written SOP shall designate the person responsible for the performance of each operation.
(c) Written records shall be maintained for all inspection, maintenance, testing, calibrating and/or standardizing operations. These records containing the date of the operation, shall describe whether the maintenance operations were routine and followed the written SOP. Written records shall be kept of nonroutine repairs performed on equipment as a result of failure or malfunction. Such records shall document the nature of the defect, how and when the defect was discovered, and any remedial action taken in response to the defect.

8.0 Reagents and Solutions- All reagents and solutions in the laboratory areas shall be labeled to indicate identity, titer or concentration storage requirements, and expiration date. Deteriorated or outdated reagents and solutions shall not be used.

9.0 Verification of Results - The Government reserves the right to verify results of all analyses. This may be accomplished by inspection of data accumulated by the contractors, by submitting coded samples collected from products delivered to NIH, or by conducting the analyses on duplicate product samples at other laboratories.

10.0 Inspection- The testing facilities (Contractor and subcontractor) shall permit authorized employees of the National Institutes of Health, at reasonable times and in a reasonable manner to inspect the facility, all records(and in the case of records also to copy), and specimens required to be maintained regarding analyses within the scope of this contract. A pre-award inspection of all laboratory facilities to be used in providing these services will be made. The Government also reserves the right to make either announced or unannounced inspections at any time during the contract period.