CHAPTER 03 – FOODBORNE BIOLOGICAL HAZARDS

SUBJECT:	**IMPLEMENTATION DATE:**
IMPORT FOODS – GENERAL (*FY 06/07/08*)	9/5/06
PRODUCT CODES:	**COMPLETION DATE:**
All applicable food codes (except 12, 16, *40, and 41*) ***This program has completed a Good Guidance Practices clearance by CFSAN's ORP and OC/DFP/CPB in August 2006.***	*9/30/08* **PRODUCT/ASSIGNMENT CODES:** Report program activities under the following PACS: 03819A Filth 03819B Decomposition 03819C Microbiology

Note: Material that is not releasable under the Freedom of Information Act (FOIA) has been redacted/deleted from this electronic version of the program. Deletions are marked as follows: (#) denotes one or more words were deleted; (&) denotes one or more paragraphs were deleted; and (%) denotes an entire attachment was deleted.

***FIELD REPORTING REQUIREMENTS**

HARDCOPY REPORTING:

Enforcement action recommendations should be submitted directly to Division of Enforcement via the "Compliance Management System" link located on Inside FDA's IT Applications Page under CFSAN Applications (link below):
http://inside.fda.gov:9003/portal/page?_pageid=197,438944&_dad=portal&_schema=PORTAL or through paper mail or fax, as described below.

Paper or Fax submission:
Submit hard copy regulatory packages to:

Food and Drug Administration
CFSAN, Division of Enforcement
Attention: Chief, Imports Branch, HFS-606
5100 Paint Branch Parkway
College Park, MD 20740-3835
Phone: (301)436-2413
Fax: (301)436-2657*

***OASIS AND FACTS REPORTING:**

Activity	**OASIS *or* FACTS**	**OASIS Work Types *or* FACTS description**	**PAC**	**PAF**
Entry Review	OASIS	Detention without Exam Request (DER); Detention Request (DTR)	03R833	--- ---
Field Exam	OASIS	Field Exam (FEX) NOTE: Product Security and Integrity reviews are considered part of the conduct of Field Exams, report under OASIS Activity 23 – Field Exam (FEX)	------	---
Sample Collection	OASIS	Product Collection (SAM); Audit Product Collection (AUP); Additional Product Collection (ADS); Reconditioning Product Collection (RCL);	------	---
Micro Analytical Findings	FACTS	Microbiological analysis (inc rapid test kits for micro analysis); Phosphatase analysis; Penicillin analysis	03819C	MIC MIC ANT
Filth Analytical Findings	FACTS	Mold, arthropod, bird, animal foreign object, filth analysis	03819A	FIL
Decomp Analytical Findings	FACTS	Decomposition, other than fishery products	03819B	FIL
Filer Evaluation	OASIS	-----	99R833	---
Follow-up to Refusal	OASIS	Refusal Verification (RRV)	[][]R824 *	---

* Select the [][]R824 PAC appropriate to the product industry of refused shipment.

PART I - BACKGROUND

*FDA's mission is to enforce the Federal Food, Drug, and Cosmetic (FD&C) Act and other laws which are designed to protect U.S. consumers' health and safety. These laws apply equally to domestic and imported products. Imported foods must be pure, wholesome, safe to eat, and produced under sanitary conditions and they must contain informative and truthful labeling in English. Under this compliance program, all foods not covered separately under a more specific compliance program are subject to examination by FDA when they are being imported or offered for import into the United States to ensure that imported foods meet these standards.

Since, in recent years, large quantities of food products have been imported into the United States to supplement the domestically produced food supply, there has been an increased effort towards protecting the consumer from potential health hazards posed by imported foods. In addition, changes have occurred in foreign food processing technology, so that foods being offered for entry are more likely to be ready-to-eat products requiring no further processing. Raw materials offered for entry in the past received further processing in domestic plants and consequently, were subject to FDA inspection during domestic inspections.

In addition to protection against natural and accidental threats to the imported food supply, since the September 11, 2001 terrorist attacks, FDA has increased its focus on protection against intentional threats to imported foods, or bioterrorism. As a result of the increased interest in food security by the Agency, and the nation as a whole, FDA's authority has been increased under the Bioterrorism Act of 2002.

With the increased authority, the Agency is in the process of shifting its approach to protecting the safety and security of imported foods. As new instructions are issued to address workplanning operations and activities, those instructions may supercede information in this compliance program. Despite potential changes in the steps FDA may take to maintain the safety and security of the imported food supply, the priorities under the current compliance program should not change. High risk foods, foods identified through assignments and Import Alerts and Bulletins, imported foods of regional significance and those which present a public health hazard should remain at the forefront of FDA's imported foods strategy.*

PART II - IMPLEMENTATION

OBJECTIVES

1.) To examine imported foods to determine if they are complying with the requirements of the FD&C Act and the regulations promulgated under this Act.

2.) *To consider appropriate regulatory remedies to preclude the future entry of food which appears to be in violation of the Act.*

***PROGRAM MANAGEMENT INSTRUCTIONS**

A. RESOURCE UTILIZATION

Resources provided in the ORA Field Workplan under the Import Foods General CP for sample collection and analysis and import field exams should be used only for this program. Resources designated in the ORA Field Workplan under the Import Foods General CP for entry review, filer evaluations, and follow-up to refusals are to be applied to this program and other food import programs. When determining how this resource pool should be expended, use the priorities listed below.*

PROGRAM PRIORITIES AND AREAS OF EMPHASIS/DE-EMPHASIS

1.) *Foods covered under assignments and Import Alerts and Bulletins

Continuing and emerging problems will be addressed through CFSAN issued assignments, Import Alerts and Import Bulletins, and may change from year to year. Refer to http://www.fda.gov/ora/import/default.htm or **#** for a listing of Import Alerts and Bulletins. Field assignments utilizing resources planned in this program will be issued by CFSAN, as warranted.

2.) High-Risk Foods

The term "High Risk Foods" is used to denote foods that may contain hazards which FDA believes may present a higher potential to cause harm from their consumption. These foods have first priority for inspectional purposes.

A number of the "High Risk" products, such as soft cheese and seafood, are covered under other Compliance Programs. These programs should be consulted when such products are encountered.

CFSAN considers the following products to be High-Risk foods covered under this program and districts should ensure sufficient coverage with resources provided in the ORA Field Workplan for the Import Foods, General Compliance Program. The laboratory in consultation with the microbiological program contact when necessary will determine the rotation of analytical coverage.

a.) Raw produce subject to pathogenic contamination

NOTE: Unless otherwise noted, the primary pathogens of concern for raw produce are *Salmonella spp.*,

Shigella, and *E. coli* O157:H7. The Field should provide routine coverage for *Salmonella spp.* when sampling for analysis, and rotate coverage of *Shigella* and *E. coli* O157:H7 to ensure uniform coverage.

i. Non-LACF vacuum packed and modified atmosphere packed fruits and vegetables, such as fresh mushrooms and salads, etc.

ii. Fruits and vegetables with structural characteristics that would make them a greater risk for harboring pathogens (i.e., rough skinned fruit such as cantaloupe).

iii. Fresh-cut fruits and vegetables, such as pre-cut lettuce, pre-cut spinach, packaged salads, etc.

iv. Green leafy vegetables, such as lettuce, spinach, cilantro, etc.

v. Tomatoes

NOTE: The primary pathogen of concern for tomatoes is *Salmonella*. The field should only provide routine coverage of tomatoes for this pathogen.

vi. Unpasteurized and non-shelf stable pasteurized fruit and vegetable juices

Juice should be covered under this program for routine sampling. All juice firms are subject to the Juice HACCP Regulation and are covered for HACCP under the Juice HACCP CP (CP 7303.847). Juice should continue to be covered under this compliance program for routine sampling because there are no import juice sample collections planned under the Juice HACCP Inspection Program at this time.

b.) Ready to eat (RTE) foods subject to pathogenic contamination including, but not limited to the products listed below.

NOTE: The primary pathogen of concern for RTE foods is *Listeria monocytogenes*. The field should provide routine coverage for this pathogen.

i. Prepared salads (e.g., deli salads such as macaroni and potato salads)

ii. Ready to eat sandwiches

This type of product may not be imported in significant quantities; however, it is a high risk product and should be prioritized if imported.

c.) Baked Goods, Custard or Cream-filled (Egg Containing)

NOTE: The primary pathogens of concern are *L. monocytogenes*, *Salmonella spp.*, and *S. aureus*. The Field should provide routine coverage for *Listeria monocytogenes* when sampling for analysis, and rotate coverage of *Salmonella spp.* and *S. aureus* to ensure uniform coverage.

d.) BSE

BSE coverage should be limited to FDA entry review activities. Entry review should include an assessment of product ingredients to determine whether they include ruminant material subject to the APHIS prohibition on such material from BSE at-risk countries. These products could include meat containing soups, stews, gravies and sauces. Refer to Import Bulletin #99-B14 for guidance.

*For more information as identifiers of High-Risk foods refer to the high-risk food instruction as updated at http://intranet.cfsan.fda.gov/OC/pages/hot/hi-risk.pdf.

3.) Other Priority Foods

a.) Ice Cream Products

NOTE: The pathogens of concern for ice cream products are *Listeria monocytogenes*, *Salmonella spp.*, and *S. aureus*. Rotate analytical coverage of ice cream products for these pathogens to ensure uniform coverage.

b.) Dried Milk Products

NOTE: The primary pathogens of concern are ETEC, EHEC O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, and *Salmonella spp*. Base sampling of dried milk products on a history of contamination with these pathogens and generic *E. coli* which is an indicator of process failure or post-process contamination.

c.) Tahini, Sesame Seed and Halva Candy

NOTE: The primary pathogen of concern is *Salmonella spp*. The field should provide routine coverage for this pathogen.

d.) Bush Meat

Refer to IA #17B-09, "CDC EMBARGO ON AFRICAN RODENTS" for guidance.

4.) Imported Foods of Regional Significance

a.) Foods of significance to particular Import Districts

Certain foods, such as ethnic or specialty foods, may have a larger presence in particular import districts. Priority should be given to higher volume products. Coverage of these foods should be consistent with the other priorities of this program.

b.) Foods covered under Memoranda of Understanding (MOU)

Districts should be aware of food products covered under International Agreements or Memoranda of Understanding (MOU). Refer to http://www.fda.gov/oia/ for a complete and current list of these agreements.

5.) Foods with a high frequency of contamination with filth which presents a public health hazard, including the following:

a.) Food products, the intended use or further processing of which will not remove or neutralize potential pathogen hazards from filth which are vectors of pathogenic organisms. This includes products which will not be further processed or washed by consumers before being consumed, such as:

i. Whole Spices
ii. Dried fruit and nut products
iii. Packaged salads

Districts should use this as a list of examples, rather than a comprehensive list of products for coverage.

b.) Bakery dry mixes (biscuit, bread, cake, pancake, pastry, etc.) which are susceptible to contamination with allergenic mites. (If districts need assistance in the identification of allergenic mites, contact the CFSAN Filth Contact; information is also available in reference 7 of this compliance program.)

c.) Products with a history of physically hazardous foreign objects or visually objectionable contamination.

Physically hazardous foreign objects include objects which are not a natural or expected component of the product, which would cause a choking or other type of physical hazard, such as:

Glass fragments
Stones
Metal fragments
Pit fragments in pitted olives
Shell in nut products

Visually objectionable contaminants include:

i. Whole insects
ii. Rodent droppings
iii. Cigarette butts
iv. Chewing gum

See the Compliance Policy Guides (CPGs), (http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/defau

lt.htm), for more examples. This type of filth may occur in a variety of products. Various manufacturers or industry practices, rather than products, may have a history of violations of this type. Districts should refer to their own detention records for a listing of manufacturers with a history of filth violations.

6.) Foods that may contain allergens

The Agency has initiated an allergen program to focus on eight foods that are most frequently implicated in serious allergic responses. The eight foods are peanuts, soybeans, fish (finfish), crustacea, tree nuts, milk, eggs, and wheat. Coverage of allergenic foods in imports will be directed by CFSAN field assignment.

7.) Foods subject to decomposition which presents a public health hazard

Decomposition in non-seafood products includes, but is not limited to, moldy product, rot and other types of spoilage. Many types of decomposition are categorized as potential health hazards requiring higher priority food safety coverage. Examples include whole spices and many processed fruits and vegetables. See *The Food Defect Action Levels (DALs)* http://vm.cfsan.fda.gov/~dms/dalbook.html. Investigators are encouraged to familiarize themselves with this document to gain an understanding of the kinds of products and defects which are of significance.

8.) Other areas of emphasis will appear on the Office of Compliance Website as they are identified. The field should de-emphasize coverage of products that are not consistent with the above priorities.*

INTERACTION WITH OTHER PROGRAMS AND ASSIGNMENTS

1.) *This compliance program involves Import Food operations for PPS 03, Foodborne Biological Hazards, only. The table below lists a number of products not covered under this compliance program and indicates the appropriate compliance program under which time should be reported for each product. Unless the table indicates otherwise, do not report time covering any of the following products under the Import Foods, General Compliance Program.

Product	Compliance Program
Imported Acidified and Low-Acid Canned Foods	CP 7303.003 - Import Acidified and Low-Acid Canned Foods CP
Imported Cheese and Cheese products	CP 7303.037 - Domestic and Imported Cheese CP
Imported Seafood Products	CP 7303.844 – Imported Seafood Products CP
Imported Juice and Juice Products for routine sampling	CP 7303.819 – Import Foods, General CP (Juice should continue to be covered under this compliance program for routine sampling because there are no import juice

	sample collections planned under the Juice HACCP Inspection Program (CP 7303.847), at this time. Juice will be covered under the Juice HACCP CP for HACCP considerations, only.)
Pesticides in Imported Foods	CP 7304.004 – Pesticides and Industrial Chemicals in Domestic and Imported Foods CP
Imported Food Economic Activities	While there is no current field economic program or field resources provided for economic issues, significant violations that may be detected while examining products may be reported under PAC 21004.
Nutrient Content, Nutritional Labeling and General Food Labeling of Imported Foods	CP 7321.005 – Dom and Imp NLEA, Nutrient Sample Analysis and General Food Labeling Req'ts CP
Imported Infant Formula	CP 7321.006 - Infant Formula CP (If the infant formula or any other FDA regulated product is suspected to be counterfeit, the district should report the incident to OCI in accordance with the instruction in the Investigations Operations Manual (IOM) Subchapter 8.8.)

2.) Food Defense: This compliance program indicates an emphasis on food defense. However, as this food defense work is integrated throughout most import operations as routine coverage, personnel should not report time for this work under the PAC for food defense; rather time should be reported under the principal program covered. For example, all import field exams under this compliance program should include a food defense component (refer to IOM 5.4.1.4 and 6.4.3) and should be reported as an Import Foods, General Field Exam. Field Exams conducted specifically for food defense should be reported under the Food Defense PAC (03R845). Enhanced food defense coverage will be issued throughout the year in the form of assignments or other instructions and will specify separate directions for reporting time for such non-routine food defense work.*

INTERACTION WITH OTHER AGENCIES

1.) A number of food products and potential hazards with which they are associated require coordination with other Federal and State Agencies. The table below lists these food products and hazards and the appropriate Agency with whom Districts should coordinate.

Product	Hazard	Agency
Meat containing products from BSE at-risk countries, i.e.: soups, stews, gravies, sauces	Bovine Spongiform Encephalopathy (BSE)	U.S. Customs and Border Protection Agricultural Quarantine Inspection (CBP/AQI) (local) and Animal and Plant Health Inspection

		Service (APHIS), USDA, http://www.aphis.usda.gov/ (headquarters)
Meat, poultry and egg products (excluding shell eggs or egg product incorporated into another food	Any safety hazard	Food Safety and Inspection Service (FSIS), USDA, http://www.fsis.usda.gov/
Any product	Communicable diseases	Centers for Disease Control and Prevention (CDC), http://www.cdc.gov
"Bush Meat"/endangered species (African smoked meat) (Refer to Import Bulletin #17B-09, "CDC Embargo on African Rodents")	Bacteria, mold, parasites, viruses	U.S. Fish and Wildlife Service, http://www.fws.gov/
Any product	Bioterrorism	Department of Homeland Security, http://www.dhs.gov/dhspublic/
Alcoholic beverages (except wine beverages with less than 7% alcohol)	Safety hazard	Bureau of Alcohol, Tobacco, Firearms and Explosives, http://www.atf.gov/

PART III - INSPECTIONAL

INSPECTIONAL INSTRUCTIONS

A. *ENTRY REVIEW AND DOCUMENT REVIEW

The resources and instructions listed below should provide assistance in determining which products to select for examination and/or regulatory action. Also refer to IOM Subchapter 6.3–Review of Records, http://www.fda.gov/ora/inspect_ref/iom/ChapterText/6_3.html#SUB6.3.

1.) *The Public Health Security and Bioterrorism Preparedness and Response Act of 2002* (the Bioterrorism Act, http://www.fda.gov/oc/bioterrorism/PL107-188.pdf), which provides new requirements on the following:

a.) Registration

Refer to IOM 5.4.1.5 – Food Registration (http://www.fda.gov/ora/inspect_ref/iom/ChapterText/5_4.html#5.4.1.5) and http://www.cfsan.fda.gov/~dms/fsbtact.html.

b.) Prior Notice

Refer to http://www.cfsan.fda.gov/~dms/fsbtact.html. Prior notice review is handled by the Prior Notice Center (PNC) in Reston, Virginia. They will perform the initial review to determine if a firm is registered. If there are any problems, the PNC will contact the appropriate district for follow-up and possible examination.

2.) Import Alerts and Bulletins **#**

Refer to Import Alerts and Bulletins for further instruction covering specific products and problems.

3.) District Intelligence

4.) Program Priorities listed on pages 4-9.

5.) Foreign Establishment Inspection Reports

Results of the EIRs may be reflected in OASIS through Import Alerts, Bulletins and entry review rates.*

B. *FIELD EXAMINATIONS

Please refer to IOM (http://www.fda.gov/ora/inspect_ref/IOM/default.htm) subchapter 6.4, Field Examination, for general instruction. For FY 05 and beyond all import field exams are to routinely include: verification that the imported product is the same as that which was declared (reconciliation exam); an assessment of security concerns related to labeling and source country (including container integrity, signs of intentional adulteration, etc.); and traditional safety concerns. These activities are to be reported as a single import field exam under this compliance program and PAC. Only one exam should be reported per line entry. Only in the event of a pre-determined "for cause" food defense exam, or in the event food defense suspicions are raised conducting

routine work requiring follow-up, should an additional exam and time be reported under the food defense PAC (03R845). Specifically, the following is a listing of field examinations applicable to the products covered under this compliance program which are currently in use:

1.) Reconciliation Exam, a.k.a. PSI

A comparison of the import entry documents to the import entry to verify that the entry is consistent with declarations on product documents with regard to the type and quantity of product. Refer to IOM 5.4.1.4 (http://www.fda.gov/ora/inspect_ref/iom/ChapterText/5_4.html#5.4.1.4) for instruction.

2.) Sensory exams

a.) Visual (storage or in-transit damage, rodent or filth insect activity, water damage, spillage, foreign objects, physical color of product, tampering, dents, rust)
b.) Tactile (inadequate refrigeration)

3.) Physical tests

a.) Temperature probes (refrigerated/frozen products)

b.) Black light (rodent urine/droppings)

c.) Candling for mold detection in bottled fruit juices, (IOM 5.1.5.1)

d.) Measurement of pH

NOTE: CFSAN provides instruction in the Domestic and Imported NLEA, Nutrient Sample Analysis and General Food Labeling Requirements Compliance Program, CP 7321.005 regarding food products that may contain undeclared allergens or BSE at-risk foods and that bear a label in a foreign language as well as English. This will be done to ensure all ingredients noted in the foreign language are properly translated and declared in English.*

D. *SAMPLE COLLECTION

Detailed instruction for sample collection, including collection technique, aseptic sampling, field examination, selective sampling, and sampling of products susceptible to contamination with pathogenic organisms is contained in IOM Subchapter 4.3. See also IOM subchapter 6.5 Import Sample Collection and Bacteriological Analytical Manual (BAM) online Chapter 1, http://www.cfsan.fda.gov/~ebam/bam-1.html, for general instruction on collecting samples and IOM sample chart 1 and relevant DFI Inspectional Guidance Documents, http://web.ora.fda.gov/dfi/inspect_guid/food/default.htm for applicable sample size. (The DFI Inspectional Guidance Documents may be used as a resource if no specific instruction is available in the former references.) If you are unsure of the proper sample size, contact your supervisor or the IOM.

For additional information on sample collection for fresh produce, please follow the instruction in the current import produce assignment.

It is imperative that the District coordinate sample collection and shipment with their servicing laboratories in order to ensure that the sample will be analyzed expeditiously.

1.) Microbiology

Generally, refer to the IOM and applicable CPGs for instruction.

a.) Salmonella species

Refer to IOM Chapter 4, Sample Schedule Chart 1 - *Salmonella* Sampling Plan, if *Salmonella* is the pathogen of primary concern.

b.) Aerobic plate counts, total coliforms, fecal coliforms, *Escherichia coli* (including enteropathogenic strains), *Staphylococcus* spp., *Vibrio* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp., *Bacillus cereus*, and *Clostridium perfringens*

From any lot of food, collect ten 8-oz subsamples (or retail packages) at random. Do not break or cut larger retail packages to obtain an 8-oz subsample. Collect the intact retail unit as the subsample even if it is larger than 8 oz.

c.) Other species

Refer to the IOM and applicable CPGs for instruction.

2.) Filth

Refer to applicable CPGs, and Import Alerts and Bulletins for instruction. In the absence of specific instruction, collect ten (10), 2 lb subsamples at random. In the event that the District's Investigations Branch has contacted the laboratory and established that a smaller sample size would suffice for regulatory analysis, the established sample size may be collected.

3.) Decomposition

Refer to applicable CPGs, Import Alerts and Bulletins, and FDA Technical Bulletin No. 1 *Principles of Food Analysis for Filth, Decomposition and Foreign Matter* and FDA Technical Bulletin No. 5 *Macroanalytical Procedures Manual* (http://vm.cfsan.fda.gov/~dms/mpm-toc.html) for instruction. In the absence of specific instruction, collect ten (10), 2 lb subsamples at random.*

E. SAMPLE SHIPMENT

Refer to IOM (http://www.fda.gov/ora/inspect_ref/IOM/default.htm) Section 4.5.3.5 for sample handling of frozen samples or Section 4.5.3.6 for refrigerated samples as appropriate.

Samples will be submitted to the district's servicing laboratory as designated in Appendix III of the current ORA Field Workplan.

F. *FILER EVALUATION

Refer to the *Evaluation Procedures for Entry Filers Participating in the Electronic Entry Processing System (EEPS)*, http://web.ora.fda.gov/diop/policies/sops/eeps.htm*

G. *DATA CORRECTION

1.) Correcting Inaccurate Data

Inaccurate data found in entries to be assessed further, either through field examination, sample collection, or detention, should be updated with correct information. Evidence of inaccurate data transmission may be determined at several points in the life cycle of a line in OASIS before it is closed. Data entry errors may be determined during entry review if comparing entry documentation with the electronic transmission, during field examination, or during review by Compliance upon receipt of additional documentation regarding a shipment. If unusual errors or omissions are encountered during the evaluation of an import entry, this information should be forwarded to the PNC for a security review and consideration for a civil money penalty.

2.) Tracking Corrections of Inaccurate Data

OASIS retains a viewable record of the data changes through which corrections made by FDA personnel may be tracked. This new functionality provides ability to track changes made by FDA personnel to original data transmitted by Filers. Such updates are vital to ensure accurate screening/possible actions, accomplishment tracking, admissibility decisions, violation charges and Import Alert recommendations. The form developed to track these changes can be printed and used during Filer Evaluations.

When any of the data transmitted by filer is modified and saved, the field color will change and the data will display in *italics.* Track Data changes will record changes made to the entry information or entry/line summary.

There is a "View Update" button on both the Entry Details Screen and Line Details screen.

Selection of these buttons will display a pop-up screen which displays the original data transmitted on the left hand side and any data updates made by FDA on the right.

NOTE: Districts should keep track of chronic violators; filers with continuing problems would be good candidates for filer evaluations.*

H. *FOLLOW-UP TO REFUSAL

Districts should consider, under certain conditions (such as when refused goods are hazardous or if there is reason to suspect they may be diverted or have had other materials substituted), verifying that refused articles have been held intact pending exportation or destruction. Once the refusal is issued, the Importer of Record has 90 days to either re-export or destroy the merchandise under CBP supervision. CBP is responsible for the supervision of exportation of refused articles. If exportation or destruction has not occurred

within 90 days nor has an extension been granted by CBP, the case is referred to CBP for consideration of liquidated damages. The district Compliance Officer may, however, issue instructions (Refuse Request-Verify) to accompany CBP on an examination of outbound refused merchandise. This exam of outbound refused merchandise is performed by FDA to verify the identity and count of the re-exported merchandise. (These exams have revealed numerous problems, including substituted merchandise and short counts.)

NOTE: If FDA has not accompanied CBP to examine the exportation of the refused article and has not received notification of exportation or destruction of articles refused admission, the district should investigate the status of disposition.*

I. *PROBLEM IMPORTERS

Work with District Compliance Officers to identify problem importers who do not appropriately assure the products they import are in compliance. See Part V – Regulatory/Administrative Strategy.*

PART IV - ANALYTICAL

ANALYTICAL INSTRUCTIONS

*For additional information on pre-sample preparation, sub-sample rinse and analytical methods for fresh produce, please follow the instruction in the current import produce assignment.

Conduct and confirm analyses in field laboratories. Some confirmations or speciations must be performed at Headquarters. Use the methodology, special instruction or sample preparation cited below as necessary for each type of analysis.

1. Filth, Mold and Foreign Objects: Microscopic and Macroscopic

Methodology:	AOAC, 17th Ed. or most current Ed., Chapter 16 Extraneous Materials: Isolation JAOAC {Interim Official First Action Methods} Macroanalytical Procedures Manual (MPM), FDA Technical Bulletin, No. 5 Laboratory Information Bulletins (LIBs)
Comments:	*For Identification (as needed) submit to CFSAN's Division of Natural Products, Microanalytical Branch, HFS-345, Douglas L. Park, (301) 436-2401*

2. Decomposition

Methodology:	Organoleptic; AOAC, 17th Ed. or most current Ed., for chemical indices; mold counts, etc.
Field Laboratories:	Refer to the current ORA Field Workplan, Appendix III, "Servicing Laboratories".
Comments:	Consult ORO/DFS, HFS-140 for servicing laboratories, if necessary

3. Microbiology

Methodology:	Bacteriological Analytical Manual (BAM), most current Ed. (http://www.cfsan.fda.gov/~ebam/bam-toc.html); AOAC, 17th Ed. or most current Ed., Chapter 17 "Microbiological Methods".
Comments:	Final identification (if needed) submit to CFSAN/ Office of Plant and Dairy Foods/ Division of Microbiological Studies, HFS-515. Reginald Bennett at (301) 436 - 2009

- ***E. coli* and coliform detection** [See Ch. 4, eBAM Section I) (http://www.cfsan.fda.gov/~ebam/bam-toc.html)].

Alternatively, LST MUG method (Ch. 4, eBAM Section II) may be used to examine for coliforms when both *E. coli* and coliform analysis are required in **chilled and frozen foods ONLY and exclusive of bivalve molluscan shellfish**. The presumptive test for coliforms can be performed in conjunction with the test for *E. coli* by preparing tubes of LST – MUG medium with gas tubes (i.e., using the LST MUG for both *E. coli* and coliform determination).*

- **Enterotoxigenic *E. coli* (ETEC), Gene Probe**

  Do not perform ETEC analysis unless *E. coli* is greater than or equal to 10,000 CFU/gram.

  Method: eBAM, Ch. 24, "Identification of Foodborne Bacterial Pathogens by Gene Probe", "Enterotoxigenic E. coli", (http://www.cfsan.fda.gov/~ebam/bam-toc.html)

  PRL-SW will provide radioactive probes for ETEC. The laboratory contact is Michael Kawalek at 949-608-3505.

- ***Enterohemorrhagic *E coli* (*E coli* O157:H7, EHEC) – Individual Sub-samples.**

  Remove 25g from a sub-sample then add 225ml of EHEC Enrichment Broth (EEB) and place into a sterile blender. Blend (10,000 – 12,000 rpm for 60 seconds at a minimum). Include positive and negative controls. See eBAM, Ch. 4A Section M and N (http://www.cfsan.fda.gov/~ebam/bam-toc.html)

  Incubate with vigorous shaking for 24h (i.e., 130–140rpm on an orbital shaker at 37°C. Proceed with the method as described in eBAM, Ch. 4A Section O (http://www.cfsan.fda.gov/~ebam/bam-toc.html).

  Positive *E. coli* O157:H7

  * Submit *E. coli* O157:H7 isolates for Pulse Field Gel Electrophoresis (PFGE) assay to respective servicing laboratories *

- ***Shigella***

  PCR primers and the positive control will be supplied to each District. Contact Keith Lampel, CFSAN's scientific contact for *Shigella* at (301) 827-8617 for primers and positive control.

  ***Shigella* will be done on a composite basis (i.e., 2 composites per sample). Each composite for *Shigella* analysis will consist of 250mL.**

  Prepare each composite by removing 50mL from each of five (5) sub-sample rinses into a sterile beaker/flask and mix thoroughly.

  Remove 100mL (of each composite) and place it into centrifuge tubes and spin at 2000rpm for 3 minutes to pellet plant material. Then follow methodology as outlined in Attachment A, "Detection of *Shigella* by Polymerase Chain Reaction".

This method is to be used as presumptive evidence and confirmation for the presence of *Shigella*. Retain the PCR products in the event that DNA sequencing is required. In this case, send the remaining PCR product to Keith Lampel, the CFSAN Scientific contact for *Shigella*.*

- ***Listeria***

General Method

BAM 8th Ed., Revision A, or most current Ed., Ch. 10 "*Listeria monocytogenes*", pages 141 – 151 and Ch. 11, "Serodiagnosis of *Listeria monocytogenes*", pages 153 – 160 (http://www.cfsan.fda.gov/~ebam/bam-toc.html).

SAFETY PRECAUTIONS: Media preparation for *L. monocytogenes* directs the use of cycloheximide, which is an extremely toxic chemical, and acriflavine, which is a powerful mutagen (use caution).

Since the *L. monocytogenes* method gives the option of using α-naphthol, do not use α-naphthylamine. All analysts should take extreme safety precautions when handling these chemicals (e.g., weigh in a containment hood free of drafts; wear gloves and face masks). Those laboratories with pesticide capabilities should take additional precautions against possible contamination, as cycloheximide is a fungicide.

Compositing/ Sample Preparation Instructions

Listeria analysis will be done on ready-to-eat food products that require none or minimal processing (no adequate kill step by the preparer).

The analysis will be conducted on composite basis **ONLY** (e.g., analyze two (2) composites per sample).

This includes all follow-up samples collected based on an initial positive finding (if appropriate).

Use the following procedure for preparing each composite:

6-subs/ sample – Remove 83.3g from each of three (3) sub-samples. Each composite size is 250g.

10-subs/ sample – Remove 50g from each of five (5) sub-samples. Each composite size is 250g.

Once the two composites have been prepared, remove 25mL or g from each composite for analysis. Mix the 25mL or g with 225mL *Listeria* enrichment broth.

Incubate the enrichment broth (EB) mixture for a total of 48hrs at 30°C. Proceed with the method in the BAM 8th Ed., Revision A,

or most current Ed. Ch. 10, page 10.04, section D "Isolation Procedure" (http://www.cfsan.fda.gov/~ebam/bam-toc.html).

NOTE: IF THE SAMPLE IS TO BE ANALYZED FOR BOTH *LISTERIA* AND *SALMONELLA* THEN COMPOSITE SUB-SAMPLES FOR *SALMONELLA* AS OUTLINED IN BAM 8TH ED., REVISION A, OR MOST CURRENT ED. CHAPTER 1, PAGE 3, THEN RANDOMLY SELECT TEN (10) SUB-SAMPLES FROM THE ORIGINAL SAMPLE TO PREPARE THE TWO COMPOSITES FOR *LISTERIA* ANALYSIS AS OUTLINED ABOVE.

Isolation and Identification: BAM 8th Ed., Revision A, or most current Ed. Ch. 10 and 11 (http://www.cfsan.fda.gov/~ebam/bam-toc.html). Additionally, Rapid Test Kits mentioned in the July 9, 1998 memo, "Guidance for the Use of *Listeria* Rapid Methods for Food Microbiology", may be used. If the laboratory does not have a copy of the memo, they should request a copy from the Division of Field Science, HFC-140.*

Enumeration

Perform using the cultural method.

*Cultural Enumeration Method: This can be readily accomplished by the enumeration method in the current version of BAM 8th Ed., Revision A or most current Ed., Ch. 10, (http://www.cfsan.fda.gov/~ebam/bam-10.html). The Simultaneous Detection and Enumeration method is strongly recommended. Note: Use of BCM or ALOA media is preferred to use of Oxford and other esculin media because the former are more specific for *L. monocytogenes*. [Contact: A.D. Hitchins, CFSAN at (301) 436-1649]*

Positive *Listeria*

* Submit *Listeria* isolates for Pulse Field Gel Electrophoresis (PFGE) assay to respective servicing laboratories *

- ***Salmonella***

Isolation and Identification

Refer to the BAM online, April 2003, Ch. 5, *Salmonella* (http://www.cfsan.fda.gov/~ebam/bam-5.html). Additionally, Rapid Test Kits mentioned in the April 24, 1998 memo, "Guidance for the Use of Rapid Methods for Food Microbiology", may be used. If the laboratory does not have a copy of this memo, it should be requested from the Division of Field Science, HFC-140.

Speciation

If the sample is positive for *Salmonella*, prepare slants and provide hard copy information requested under BAM online, April 2003, Ch. 5, section E - 11, "Submission of cultures for serotyping" (http://www.cfsan.fda.gov/~ebam/bam-5.html). Send to Denver Laboratory (DEN) or designated lab by Division of Field Science (DFS) for speciation. Laboratories should submit appropriate form when sending isolates for Salmonella speciation.

Salmonella Positive Isolates

ARL will submit *one (1) positive *Salmonella* isolate of each somatic group recovered from each sub-sample* to Denver District Laboratory's (DEN-LAB) microbiology laboratory for antibiotic sensitivity assay.

Prepare cultures for shipment according to requirements for shipment of etiological agents. Submit cultures on brain heart infusion (BHI) agar slants in screw – cap tubes (13mm x 100mm) with caps securely tightened. Label each tube with the sample and sub-sample numbers, date and initials. Submit copies of the collection report and analytical worksheets. Place the cultures in a culture container with an official FDA seal. Place the accompanying record inside the shipping carton but not within the officially sealed culture container. Label the secondary shipping container according the service needed (see below). Send the container by the most rapid means available. Maintain duplicate cultures for all cases, which are under consideration for legal action.

Microbiology Field laboratories should follow the following instruction in sending Salmonella isolates for serotyping (for epidemiological purposes):

Isolates from NRL, WEAC, SRL and ARL will be serotyped in ARL:

Arkansas Regional Laboratory
3900 NCTR Road Building 26
Jefferson AR 72079
Attention: Gwendolyn Anderson
Tel # 870-543-4621
Fax# 870-543-4041

Isolates from SAN, PRL-NW, PRL-SW and DEN will be serotyped in DEN:

Denver District Laboratory
6th Avenue & Kipling Street
DFC Building 20
Denver Colorado 80225-0087
Attention: Doris Farmer
Tel # 303-236-9604
Fax # 303-236-9675

ARL submit *Salmonella* isolates for antibiotic sensitivity assays to:

*FDA/ ORA/ DEN
ATTN: Connie Kiessling
6th Ave & Kipling St,DFC,
Building 20, ENT-10
Denver, CO 80225-0087*

* Submit *Salmonella* isolates for Pulse Field Gel Electrophoresis (PFGE) assay to respective servicing laboratories *

- **Staphylococcal Enterotoxin**

General

Perform enterotoxin testing if:

1. Product abuse (e.g. temperature, outbreaks, etc.) is suspected

or

2. *Instructed by Compliance Program or Field Assignment to analyze the sample for *Staphylococcus*.*

If viable *Staphylococcus sp.* colonies are observed by:

- most probable number (MPN) when performed as directed per BAM Chapter 12 (http://www.cfsan.fda.gov/~ebam/bam-12.html)where the results are >11,000

and

- direct plate counts when performed as directed per BAM chapter 2 (http://www.cfsan.fda.gov/~ebam/bam-2.html)indicates a level of 10,000/ gram

Enterotoxin Analysis:

Follow the methodology outlined in BAM 8th Ed., Revision A, 1998 or most current Ed., Ch. 13, "Staphylococcal Enterotoxins" (http://www.cfsan.fda.gov/~ebam/bam-toc.html), beginning on page 13.01.

The laboratory will individually test each sub-sample using the TECRA™ ELISA with proper procedures followed accordingly.

NOTE: Under **no** circumstances should positive TECRA™ ELISA results be conveyed to a regulated firm or consumer without confirmation. The TECRA™ ELISA is intended as a screening method only.

NOTE: The total contents of each subsample should be retained until the original analyses are completed to ensure that a sufficient amount of product is available for subsequent additional and confirmation tests, if necessary.

TECRA™ ELISA Test Results

1. Negative result - the laboratory need not conduct further analysis for enterotoxin. The sample is considered "negative" and no other regulatory or follow-up action is warranted.

1. Positive result - the laboratory should analyze the original sample using the VIDAS method for confirmation refer to BAM 8th Ed., Revision A, 1998 or most current Ed., Ch. 13A, "Staphylococcal Enterotoxins: Micro-slide Double Diffusion and ELISA Based Methods"(i.e., VIDAS)". See

(http://www.cfsan.fda.gov/~ebam/bam-toc.html)

NOTE: If the District or Regional Laboratory cannot perform the VIDAS, contact Reginald Bennett, HFS-516 at (301) 436-2009 to arrange for shipment of portions of the actual sub-samples to CFSAN for confirmation.

*TECRA™ ELISA **and** the VIDAS Tests Results

1. When the TECRA™ ELISA **and** the VIDAS Tests are **positive**, the following **should be sent to CFSAN for re-confirmation analysis**:

   (a) The remaining portion of the original TECRA™ ELISA positive tested extract(s)

   (b) The remaining portion of the positive tested extract(s) from the VIDAS System

   (c) "all" of the remaining reserves portion of the positive subsample(s)

   (d) a copy of the analytical worksheets

   (e) a copy of the collection report

   to

   FDA/ CFSAN/ Microbiology Methods Research Branch
   Attention: Reginald Bennett, HFS-516
   5100 Paint Branch Parkway
   College Park, MD 20740.

   NOTE: Please notify the Division Director at 301.436.2007 that the sample is being sent to CFSAN for confirmation analyses.

   If additional information concerning sample preparation, handling or shipping to CFSAN is needed contact Reginald Bennett at 301.436.2009.

   The Microbiological Methods Development Branch will perform confirmation analyses on the extract and reserve subsample(s) as appropriate and provide sample(s) to Dr. A. Rasooly for SDS-PAGE immunoblot analyses as indicated.

   Districts should wait for Center confirmation before recommending any regulatory action.

2. When the result of the TECRA™ ELISA is **positive** and the VIDAS is **negative** then:

   Check for the presence of peroxidase.

   NOTE: Some foods contain peroxidase which can cause a false-positive reaction with the TECRA™ ELISA; therefore if this scenario presents itself the analyst should check for the presence of peroxidase and if present, inactivate the peroxidase and retest.

To determine peroxidase presence, refer to the method outlined in BAM Ch. 13A, "Staphylococcal Enterotoxins: Micro-slide Double Diffusion and ELISA Based Methods, Section "Extraction of Enterotoxins from Foods for ELISA Assays", A. General Precautions. If peroxidase is present, inactivate the peroxidase using the methods outlined in the General Precautions section and retest.

NOTE: Under **no** circumstances should positive TECRA™ ELISA results be conveyed to a regulated firm or consumer without confirmation. The TECRA™ ELISA is intended as a screening method only.

If the TECRA™ retest remains positive, send the extract used for the TECRA™ ELISA and the reserve portion for all of the original sub-samples to:

FDA/ CFSAN/ Microbiology Methods Research Branch
Attention: Reginald Bennett, HFS-516
5100 Paint Branch Parkway
College Park, MD 20740.

The Microbiological Methods Development Branch will consolidate CFSAN's analyses results and provide the finding to CFSAN/ Office of Compliance/ Division of Enforcement. The Division of Enforcement will contact the District's Compliance Branch with the results for appropriate follow-up.*

- ***V. cholerae***

General Instructions

Each sample will be examined on an individual sub-sample basis except for the analysis using the Polymerase Chain Reaction (PCR) method for *V. cholerae* Enterotoxigenic strains (see *V. cholerae* section below). When the PCR method is used, the sample will be analyzed on a composite basis (see below for instructions).

Methods

General Method:
BAM 8th Edition, most current Edition, Ch. 9, "*Vibrio cholerae, V. parahaemolyticus, V. vulnificus,* and Other *Vibrio* spp" (http://www.cfsan.fda.gov/~ebam/bam-toc.html)

PCR Methods for *V. cholerae*:
Attachment D "Polymerase Chain Reaction (PCR) for *V. cholerae* Enterotoxigenic Strains" of this program. Alternatively, BAM 8th Edition, most current Edition, Ch. 28 "Detection of Enterotoxigenic *Vibrio cholerae* in foods by the Polymerase Chain Reaction" may be used.

V. cholerae: "Isolation, identification, pathogenicity and PCR"

A. Each sample will be analyzed using the General Method and one of the PCR Methods for *V. cholerae* methods referenced above.

B. If the sample is found to be positive for *V. cholerae*, send one set of ALL isolates of *V. cholerae* 01 or non-01 for confirmation to:

FDA/ CFSAN/ Virulence Mechanisms Branch
ATTN: Mahendra Kothary (HFS – 025)
MOD 1
Laurel, MD 20708
(301) 827 - 8616

C. Alkaline Peptone Water (APW) Lysate Preparation for PCR analysis

NOTE: THE FOLLOWING INSTRUCTIONS ARE TO BE USED IN LIEU OF ATTACHMENT D (PAGE 3, STEP C).

1. Once the appropriate dilutions have been prepared for each of the individual ten (10) sub-samples using the BAM method, the laboratory will **prepare two (2) APW Lysate composites from the original 1:10 APW dilutions** (e.g., the blended solution) **PRIOR** to incubation.

   NOTE: APW Lysate composites will be prepared from the original 1:100 APW dilutions for products (e.g., products with possible high concentration of microflora) with a potential inhibitory effect to the PCR reaction.

2. One APW Lysate composite will be prepared by removing 1.0mL from each of the 1:10 or 1:100, as appropriate, for dilutions for sub-samples 1 thru 5 (e.g., composite #1A) and the second APW Lysate composite will reflect 1.0mL being removed from each of the 1:10 or 1:100, as appropriate, for dilutions for sub-samples 5 through 10 (e.g., composite #1B).

3. These APW Lysate composites will be **designated as zero (0) times lysates, (e.g., composites 1A and 1B).** Boil for 5 min, and then freeze.

   NOTE: THIS "0" TIME ALIQUOT WILL BE USED FOR PCR TESTING ONLY IF THE 6 – 8 HOUR OR 16 – 24 HOUR INCUBATED LYSATE SHOWS A POSITIVE REACTION ON THE PCR TEST.

4. A second set of APW Lysate composites will be prepared using step (2) above from the original 1:10 or 1:100 dilutions AFTER the 6 – 8 hour incubation period at 37°C.

   If the sample is a frozen food product, then the APW Lysate composites will be prepared using step (2) above from the original 1:10 or 1:100 dilution AFTER the 16 – 24 hour incubation.

   NOTE: THIS LYSATE WILL BE TESTED FIRST USING THE PCR TEST. IF THIS LYSATE CANNOT BE TESTED IMMEDIATELY, THEN FREEZE UNTIL THE PCR TEST CAN BE PERFORMED.

5. See the PCR Methods for *V. cholerae* referenced above for further instructions for PCR analysis.

- ***Yersinia enterocolitica***

  General Method

  Refer to the BAM online, http://www.cfsan.fda.gov/~ebam/bam-8.html.*

- ***Animal Drug Residue* in Milk**

  Refer to the Analytical Section of the National Drug Residue Milk Compliance Program (CP 7303.039) for instruction.

- ***Analytical Reporting***

  Report all analytical results (filth, decomposition, microbiological) into FACTS using the following PACs and Problem Area Flags (PAF):

PAC	PAF	Finding Screen, Prob(lem) Area	Sub-PAF	PAF Description
03819A (Filth)	FIL	ANI	--	Animal Filth
		ART	--	Arthropod Filth
		BIR	--	Bird Filth
		EXH	--	Filth Exhibit
		FOR	--	Foreign material
		OTH	--	Other, Not Listed, Identify in Remarks
03819B (Decomp)	FIL	DEC	--	Decomposition
		EXH	--	Filth Exhibit
		GEO	--	Geotrichum Mold
		HMC	--	Howard Mold Count
		MAC	--	Macroscopic Mold
		YEA	--	Yeast
		OTH	--	Other, Not Listed, Identify in Remarks

PAC	PAF	Problem	Sub-PAF	PAF Description
03819C (Micro)	MIC	MICROID	--	Microbiological analysis (included rapid test kits for micro analysis)
			SAL	Salmonella serotyping

	ABR	Antibiotic resistance testing
PHOS	--	Phosphatase analysis

PART V – REGULATORY/ADMINISTRATIVE STRATEGY

REGULATORY/ADMINISTRATIVE INSTRUCTIONS

*One of the goals of this program is to obtain sufficient evidence to support broad-based enforcement strategies. These would include Import Alerts for Detentions Without Physical Examination (DWPE) for importers, shippers, manufacturers, and countries. We request the field to be aware of detention patterns that could develop into broad-based DWPEs and notify CFSAN, Imports Branch Chief and/or the Regulatory Contact when these situations arise. For example, Districts should consider recommending more stringent enforcement action against problem importers when importers do not appropriately assure the products they import are in compliance. Chapter 9 of the Regulatory Procedures Manual (RPM) contains a section on Priority Enforcement Strategy for Problem Importers (http://www.fda.gov/ora/compliance_ref/rpm_new2/ch9strat.html). The Center intends to refocus its enforcement efforts on problem importers to ensure they assume appropriate responsibility for the commodities they import. The Center will routinely review import data to identify problem importers that may warrant increased observation and firm based enforcement. CFSAN will consider field assignments to conduct additional sampling and analyses to meet detention criteria for DWPE actions. See the RPM (http://www.fda.gov/ora/compliance_ref/rpm/) for DWPE criteria and procedures.

In general, refer to the Compliance Policy Guides (CPGs), (http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/default.htm) and Import Alerts **#**, which provide specific guidance on criteria for regulatory action. In addition to those resources, regulatory criteria and actions relevant to this compliance program are listed below.*

A. REGULATORY CONSIDERATIONS AND CRITERIA FOR RECOMMENDING ACTION

1.) Microbiological Findings

*If products are found to be positive for the pathogenic organisms listed below and at the noted levels where cited, refer to the Compliance Policy Guides and Import Alerts if applicable for regulatory guidance. In the absence of a relevant CPG or Import Alert, contact CFSAN/DE/Imports Branch, HFS-606, (301) 436-2413 immediately for further instruction.

In accordance with the guidance in applicable CPGs and Import Alerts, districts should submit recommendations for detentions and detentions without physical examination (DWPE) when pathogenic organisms in products are detected.

Recommendations must be accompanied by a complete regulatory package, consisting of all analytical worksheets (original and check, when required) and other appropriate documentation (i.e., entry paperwork, collection report, original labels, etc.) Districts may submit recommendations to the Center for consideration based on these criteria:*

a.) *Listeria monocytogenes* – **#**

b.) **Salmonella* – **#**

c.) *Staphylococcus aureus* – **#**

d.) **Enterohemorrhagic E. coli* (EHEC O157:H7) - **#**

e.) *E. coli* - **#**

f.) *V. cholerae* - **#**

g.) **Shigella* - **#**

h.) **Yersinia enterocolitica* - **#**

2.) Filth and Decomposition Findings

Apply the appropriate CPG criteria. In those instances when specific criteria for regulatory action are not available, submit analytical findings to CFSAN, Division of Enforcement, Chief, Imports Branch. Analytical findings may be submitted as described on page 1 of this program.

3.) *Other Considerations*

a.) Registration

Refer to Sec. 305, Registration of Food Facilities (http://www.cfsan.fda.gov/~dms/sec-ltr.html#sec305), Registration of Food Facilities Under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002; Interim Final Rule, (http://www.fda.gov/OHRMS/DOCKETS/98fr/03-25849.htm) and CPG Sec. 110.300 Registration of Food Facilities Under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (http://www.cfsan.fda.gov/~furls/cpgreg.html).

b.) Prior notice

Refer to Sec. 307, Prior Notice of Imported Food Shipments (http://www.cfsan.fda.gov/~dms/sec-ltr.html#sec307), Prior Notice of Imported Food Under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002; Interim Final Rule, (http://www.fda.gov/OHRMS/DOCKETS/98fr/03-25877.htm) and CPG Sec. 110.310 Prior Notice of Imported food Under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (http://www.cfsan.fda.gov/~pn/cpgpn.html).

B. *REGULATORY/ADMINISTRATIVE ACTIONS*

*The regulatory/administrative actions which may be considered under this compliance program are not mutually exclusive. In the event that a significant product defect, as previously described in this compliance program, is found, one or more of the following may be considered:

1.) Release with comment;

Refer to RPM, Chapter 9, Subchapter - Release Notices, (http://www.fda.gov/ora/compliance_ref/rpm_new2/ch9rel.html).

2.) Detention;

Refer to RPM, Chapter 9, Subchapter – Import Procedures, (http://www.fda.gov/ora/compliance_ref/rpm_new2/ch9det.html)

3.) Custom seizure and destruction

Refer to the Guidance Concerning Recommending Customs' Seizure and Destruction of Imported Human and Animal Food That Has Not Been Reconditioned, (http://www.cfsan.fda.gov/~dms/impquid.html)

4.) Reconditioning;

Refer to RPM, Chapter 9, Subchapter – Reconditioning, (http://www.fda.gov/ora/compliance_ref/rpm_new2/ch9recon.html)

5.) Relabeling;

Refer to RPM, Chapter 9, Subchapter – Reconditioning, (http://www.fda.gov/ora/compliance_ref/rpm_new2/ch9recon.html)

6.) Refusal;

Refer to RPM, Chapter 9, Subchapter – Notice of Refusal of Admission, (http://www.fda.gov/ora/compliance_ref/rpm_new2/ch9nora.html); for follow-up to refusal, refer to Part III – Inspectional, Section H.*

PART VI – ATTACHMENTS, REFERENCES, AND PROGRAM CONTACTS

ATTACHMENTS

1. Attachment A - DETECTION OF *SHIGELLA* BY POLYMERASE CHAIN REACTION

REFERENCES

1. *Office of Compliance Intranet website,* **#**

2. *Import Alerts and Bulletins,* **#**

3. *"High-Risk" (HR) definition,* **#**

4. *International Agreements,* http://www.fda.gov/oia/

5. *Compliance Policy Guides (CPGs),* (http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/default.htm)

6. *Regulatory Action Criteria for Filth and Other Extraneous Materials. V. Strategy for Evaluating Hazardous and Nonhazardous Filth*. AR Olsen, JS Gecan, GC Ziobro and JR Bryce. Journal of Regulatory Toxicology and Pharmacology 33: 363-392 (2001). Contact Douglas L. Park, CFSAN Filth and Decomposition Contact below for a copy.

7. *The Food Defect Action Levels,* http://vm.cfsan.fda.gov/~dms/dalbook.html

8. *The Public Health Security and Bioterrorism Preparedness and Response Act of 2002* (the Bioterrorism Act), http://www.fda.gov/oc/bioterrorism/PL107-188.pdf

9. *Investigations Operations Manual (IOM),* http://www.fda.gov/ora/inspect_ref/IOM/default.htm

10. CFSAN's website for specific information regarding the Bioterrorism Act, http://www.cfsan.fda.gov/~dms/fsbtact.html

11. *BAM online Chapter 1,* http://www.cfsan.fda.gov/~ebam/bam-1.html

12. DFI Inspectional Guidance Documents, **#**

13. FDA Technical Bulletin No. 1 *Principles of Food Analysis for Filth, Decomposition and Foreign Matter* and FDA Technical Bulletin No. 5 *Macroanalytical Procedures Manual,* http://vm.cfsan.fda.gov/~dms/mpm-toc.html

14. *Evaluation Procedures for Entry Filers Participating in the Electronic Entry Processing System (EEPS),* **#**

15. *Regulatory Procedures Manual (RPM),* http://www.fda.gov/ora/compliance_ref/rpm/

16. Registration and Prior Notice Links,

    http://www.cfsan.fda.gov/~dms/sec-ltr.html#sec305
    http://www.fda.gov/OHRMS/DOCKETS/98fr/03-25849.htm
    http://www.cfsan.fda.gov/~dms/sec-ltr.html#sec307
    http://www.fda.gov/OHRMS/DOCKETS/98fr/03-25877.htm

17. *Draft Guidance Concerning Recommending Customs' Seizure and Destruction of Imported Human and Animal Food That Has Not Been Reconditioned,* http://www.cfsan.fda.gov/~dms/impguid.html

PROGRAM CONTACTS

A. CFSAN CONTACTS

General Program Contact:
Carrie Lawlor, CFSAN, Office of Compliance, Division of Field Programs, Compliance Programs Branch, HFS-636, phone (301) 436-2068, fax (301)436-2657

Regulatory Contact:
Imports Branch Chief, CFSAN, Office of Compliance, Division of Enforcement, Imports Branch, HFS-606, phone (301) 436-2413.

Filth and Decomposition Contact:
* George C. Ziobro, CFSAN, OPDF, Division of Natural Products, Microanalytical Branch, HFS-315, phone (301) 436-1965*

Microbiology Contacts:

E. coli and Enterohemorrhagic E. coli O157:H7 (EHEC) and *ETEC*
Peter Feng, CFSAN, OPDF, Division of Microbiological Studies, Microbiology Methods Research Branch, HFS-516, phone (301) 436-1650.

Listeria monocytogene
Anthony Hitchins, CFSAN, OPDF, Division of Microbiological Studies, Microbiological Methods Development Branch, HFS-516, phone (301) 436-1649.

Staphylococcus aureus or Staphylococcal enterotoxin
Reginald W. Bennett, CFSAN, OPDF, Division of Microbiological Studies, Microbiological Methods Development Branch, HFS-516, phone (301) 436-2009.

Salmonella
Wallace H. Andrews, CFSAN, OPDF, Division of Microbiological Studies, Microbiological Methods Development Branch, HFS-516, phone (301) 436-2008.

Shigella
Keith Lampel, CFSAN, OPDF, Division of Microbiological Studies, HFS-515, phone (301) 436-2007.

V. cholerae
Mahendra Kothary, CFSAN, Division of Virulence Assessment, HFS-025, phone (301) 210-7873.

Phosphatase
George C. Ziobro, CFSAN, OPDF, Division of Natural Products, Microanalytical Branch, HFS-315, phone (301) 436-1965.

ORA CONTACTS

Analytical Contacts:

Filth
Larry D'Hoostelaere, ORA, Division of Field Science, HFC-141, phone (301) 827-1032, lawrence.dhoostelaere@fda.gov.

Decomposition
Donald Lech, ORA, Division of Field Science, HFC-140, phone (301) 827-4603, Donald.Lech@fda.gov

Microbiology
Marsha Hayden, ORA, Division of Field Science, HFC-141, phone 301.827.1039, MHayden@ora.fda.gov.

Import Alert and Import Procedures Contacts:
Doug Randes, ORA, Division of Import Operations and Policy, HFC-170, phone (301) 443-6553, fax (301) 594-0413 drandes@ora.fda.gov

Linda Wisniowski, ORA, Division of Import Operations and Policy, HFC-170, (301) 443-6553, fax (301) 594-0413, lwisniow@ora.fda.gov

PART VII - CENTER RESPONSIBILITY

*CFSAN aims to restructure agency responsibilities with regard to import enforcement of foods to more efficiently use resources by shifting decision making responsibility to those parties who have direct familiarity (District Offices) and authority (potential other Agencies). To this end, the Division of Enforcement, Imports Branch will initiate and direct the coordination of import enforcement activities in the following manner:

- Ensure that data developed by the Center to be used by the field is correct, available, timely, and beneficial.
- Target sampling efforts using all available information to develop sufficient evidence in support of broad based actions.
- Working with DIOP, assure that all import alerts and bulletins represent current Center priorities through continual review and updates while assuring consideration of the broad ramifications of such documents.
- Develop and maintain a very active surveillance/risk management system.
- Actively seek out additional direct reference opportunities.
- Solicit more direct reference requests from the field.
- Develop an enforcement strategy for addressing problem importers.
- Determine authorities of other Agencies over food products (APHIS, FSIS, etc.).
- Provide data to OPDF with which evaluation can be made.

The Office of Plant and Dairy Foods will periodically conduct evaluations of this compliance program. Among other accomplishments to be examined, the evaluations will examine the effectiveness of the program regarding field operations and program quality so that any necessary corrective action may be initiated.*

ATTACHMENT A - DETECTION OF *SHIGELLA* BY POLYMERASE CHAIN REACTION

NOTE: Previous steps are located in **Part IV - Analytical** of this program. This method is to be used for detection only.

1. Decant supernatant into another set of centrifuge tubes and spin at 8000 rpm for 10 minutes to pellet bacterial cells. Remove as much of the supernatant as possible (to avoid leaving behind any inhibitory compounds; aspiration may be a better alternative than decanting the supernatant).

2. Add 50-100 µl of 1 X phosphate buffer solution (PBS) (this will depend upon the size of the pellet). If the pellet is hardly visible, then add the smallest amount of 1 X PBS. Also, the total amount of PBS added should be 50-100 µl; therefore if two tubes were used for centrifugation, one tube should be suspended in 1 X PBS and this suspension added to the other tube(s).

3. For PCR template preparation: boil the cell suspension in a water bath for 5 minutes, cool on ice and centrifuge at 8000 rpm for 5 minutes. Transferring the supernatant to another tube is not necessary; use the supernatant as template without disturbing the pellet.

4. **PCR setup**

The following is a typical set up for PCR methodology.

dH2O	13 µl
Buffer	2.5 µl 10 X stock (*polymerase buffer)
dNTP	2.0 µl (1:10 dilution of 10 mM dNTP stock)
Primers	2.5 µl each primer (10 nmoles/ul stock) (will be provided)
Template	2.5µl (e.g., positive control or PCR template prep)

(*It is recommended to use the polymerase buffer that is supplied by the manufacturer of the Taq Polymerase; **NOTE**: not all buffers contain $MgCl_2$; if not provided, final concentration is 1.5 mM).

Add 1-2 drops of mineral oil (if necessary) and place in thermocycler.

Note: A strain of *S. flexneri* (2457M) is provided on a slant and should be streaked out onto nutrient agar plate (e.g., isolated colonies). This strain is a positive control, which can be used, with the PCR primers. However, this strain can be differentiated from any other *Shigella* isolates with another set of primers (607, 608) and its resistance to the antibiotic kanamycin [50 ug/ml]. PCR template is prepared by boiling a colony from an agar plate in 150 µl dH20 and using 1 µl in a PCR control tube. Another set of primers specific for this strain is available.

It is recommended that the positive control be used to ensure that the reagents are working properly.

5. Hot start PCR

This step can be eliminated if using Taq polymersases that require heat activation.

Set one file as follows: 80°C for 10 minutes (this amount of time can be increased if warranted). After thermocycler reached 80°C, 0.3 µl of Taq Polymerase (suggested manufacturer is Qiagen) can be added.

6. PCR Amplification cycles and steps

Each cycle consists of the following steps:

1. 94°C for 1 minute (denaturation)
2. 60°C for 1 minute (annealing)
3. 72°C for 1 minute (extension)

Total number of cycles is 30.

7. Agarose Gel Analysis of PCR Products

After amplification, transfer 10 µl of the PCR products to another microcentrifuge tube containing 2 µl of tracking dye and load on 1% agarose gel.

NOTE: Do not add dye directly to the PCR product.

A 100 base pair ladder is used as a molecular weight standard. A 620 base pair product is expected from the positive samples. When reactions are completed, keep PCR product at 4°C or stored at -20°C.

If a 620 bp amplicon is seen on the agarose gel, proceed to the nested PCR step below.

Note: PCR primers

PCR primers (ipaH-F and ipaH-R) are targeted to the ipaH genes; there are multiple copies of this gene residing in the chromosome and one copy in the virulence plasmid of *Shigella*.

Nested PCR protocol

Primers ipaH3 and ipaH4 are directed to internal sequences within the 620 bp amplicon generated from PCRs using primers ipaHF and ipaHR. Using primers ipaH3 and ipaH4, a 290 bp product should be amplified if the 620 bp fragment was generated from *Shigella* DNA. The objective of using the nested PCR assay is to confirm that the 620 bp fragment was amplified from *Shigella*.

The PCR assay is as follows:

1. Distilled water	16 µl	
2. 10 X buffer	2.5 µl	(see note I below)
3. dNTP	0.3 µl	(stock is 10 mM dNTP)
4. Primers		
	2.5 µl ipaH3	(stock is 10 pmol/µl)
	2.5 µl ipaH4	(stock is 10 pmol/µl)
5. Template	1.0 µl	(see explanation II below)
6. Mineral oil	2 drops	
7. Taq polymerase	0.25 µl	(see explanation III below)

PCR conditions (cycles and temperatures) are identical to amplification using PCR primers ipaHF and ipaHR.

After reactions are complete, run 8-10 µl through a 1% agarose gel in 0.5X Tris-acetate EDTA buffer, pH 8.3. Each gel should have a 100 bp ladder as molecular weight marker. A positive reaction generates a 290 bp fragment.

Notes:
I. Certain buffers, such as those from Qiagen, contain 1.5 mM $MgCl_2$; other buffers may not; therefore, add $MgCl_2$ to a final concentration in the reaction of 1.5 mM.

II. Usually if 1 µl of the PCR product is used directly from the reaction that amplified the 620 bp fragments, then 3 bands may be seen on the gel; the correct band at 290 bp and 2 other approximately 400 and 500 bp. The larger 2 bands are due to primers ipaHF and ipaHR being carried over from the first reaction. The larger amplified bands were generated from the combinations of ipaHF-ipaH4 and ipaHR-ipaHF. To avoid this, dilute the reactions that yielded a presumptive positive product 1:10 and 1:100 in separate tubes with dH_2O. Use 1 µl of the diluted products as template. In some cases, faint bands around 400 and 500 bp may be seen on agarose gels using the 1:10 diluted product as template; this is explained above. A band at 290 bp is confirmation for the presence of *Shigella*.

III. The DNA polymerase used to develop this assay was from Qiagen. Others are probably suitable.

Primer sequence
ipaH3: 5'-CCA CTG AGA GCT GTG AGG
ipaH4: 5'-TGT CAC TCC CGA CAC GCC